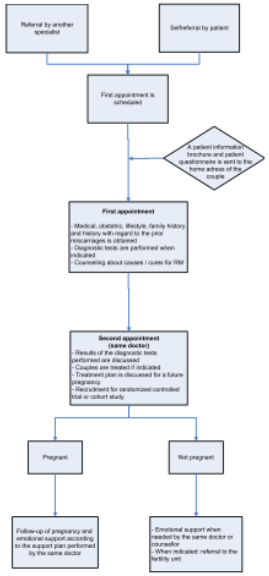
Annex 10: Evidence tables

1. How should care for the RM patient be organised? (18)

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Musters AM, et al. Hum Reprod. 2013;28(2): 398-405.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	All women who received diagnostic work-up for RMs from January 2010 to December 2010 were sent a questionnaire. 266 women were asked to participate in the study. In total, 174 women responded (65%) 171 questionnaires were analysed.		Women with RM preferred the following supportive care options for their next pregnancy: a plan with one doctor who shows understanding, takes them seriously, has knowledge of their obstetric history, listens to them, gives information about RM, shows empathy, informs on progress and enquires about emotional needs. Also, an ultrasound examination during symptoms, directly after a positive pregnancy test and every 2 weeks. Finally, if a miscarriage occurred, most women would prefer to talk to a medical or psychological professional afterwards. The majority of women expressed a low preference for admission to a hospital ward at the same gestational age as previous miscarriages and for bereavement therapy. The median preference, on a scale from 1 to 10, for supportive care was 8.0. Ethnicity, parity and pregnancy at the time of the survey were associated with different preferences, but female age, education level and time passed since the last miscarriage were not.		Women with RM preferred a plan for the first trimester that involved one doctor, ultrasounds and the exercise of soft skills, like showing understanding, listening skills, awareness of obstetrical history and respect towards the patient and their miscarriage, by the health care professionals. In the event of a miscarriage, women prefer aftercare.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Van den Berg MM, et al. Obstet Gynecol Clin North Am. 2014;41(1):145-55.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**			KEY POINTS ✓ A recurrent miscarriage (RM) clinic offers specialist investigation and treatment of women with recurrent first- and second-trimester miscarriage. ✓ RM care preferably should be provided by only one doctor per couple. ✓ A treatment strategy should be designed with the couple for a subsequent pregnancy. ✓ Evidence-based guidelines are necessary for the facilitation of evidence-based practice and to reduce practice variation between professionals. ✓ Guideline adherence can be achieved by implementation efforts.			Narrative review

Additional references included as background information
None

2. WHAT ARE THE KNOWN RISK FACTORS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Ajayi OO, et al. African health sciences 2012;12: 153-159.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	35 RPL patients 34 controls			serum zinc, copper, and vitamin E levels were significantly lower serum selenium, lead, and cadmium were significantly higher	heavy metals and a lack of micronutrients could cause pregnancy loss in RPL	
du Fossé NA et al., *Human reprod update* 2020;26: 650-669	Meta-analysis	Appropriate question? Yes Rigorous search? Yes Relevant studies included? Yes Quality of studies? Low to moderate Methodology? --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	9 included studies in the meta-analysis	association of advanced paternal age with spontaneous miscarriage during the first trimester of pregnancy	Miscarriage	there is an increased risk for miscarriage for male age categories 30-34, 35-39 and 40-44 and this risk was higher for the ≥45 age category	advanced paternal age is also associated with an increased risk of spontaneous miscarriage.	
Bhattacharya S, et al. Eur J Obstet Gynecol Reprod Biol. 2010;150(1): 24-7.	CS	X Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	excellent epidemiology 151,021				age > 30 signficant risk factor miscarriage	
Bouet PE,et al. Fertil Steril. 2016;105 (1):106-10.	observational	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	51 RPL patients	27% chronic endometritis not controls RIF				HIGH prevalence of endometritis in rm women

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Cauchi MN, et al. Am J Reprod Immunol . 1991; 26(2):[72-5 pp.].	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **x Acceptable (+)** ☐ **Unacceptable (-)**	119 couples white ell transfusion trial	age<30 compare to age >30	outcome	0.8	age >30 risk factoR for miscarriage in RM	
Cicinelli E, Reprod Sci. 2014;21(5):640-7.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	records of 360 women with unexplained RM were retrospectively analyzed.	Data from hysteroscopy, endometrial histology, endometrial culture, and PCR for chlamydia, performed before and after antibiotic treatment for chronic endometritis (CE), The occurrence of successful pregnancies within 1 year after treatment	Results showed that 208 (57.8%) women with RM showed CE at hysteroscopy; 190 (91.3%), positive at hysteroscopy, were also positive at histology, and 142 (68.3%) had positive cultures. Common bacteria were found in 110 (77.5%) patients. Mycoplasma and Ureaplasma were found in 36 (25.3%) patients and Chlamydia in 18 patients (12.7%). In 102 (71%) women, antibiogram-based antibiotic treatment normalized hysteroscopy, histology, and cultures (group 1); while in 40 (28.2%) patients, CE was still present at hysteroscopy (group 2). In 16 of the 66 patients positive at hysteroscopy, but not at cultures, the hysteroscopy becomes normal (group 3) after a Centers for Disease Control and Prevention-based therapy; while in 50 women, CE was still present (group 4). One year after treatment, group 1 showed a significantly higher number of pregnancies (78.4%) compared to group 2 (17.5%; P < .001) and group 4 (15.3%; P = .005).		CE is frequent in women with RM. Antibiotic treatment seems to be associated with an improved reproductive outcome.	
Gold EB, Tomich E. Occup Med. 1994;9(3):435-69. (7831592)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	good review	notes serious bias in all reported studies	video display terminals magnetic field organic solvents heavy metals	Conflicting results Conflicting results consistent association	not conclusive not conclusive causal associations not conclusive	blighted by poor studies
Grande M, Borrell A, et al. Hum Reprod. 2012;27(10):	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias	retrospective cohort of 353 miscarriages successfully karyotyped Among the 353 women, 153		Sporadic and recurrent miscarriage did not show significantly different chromosomal anomaly rates (68 versus 60%) and maternal age was the only statistically significant predictor of the chromosomal anomaly risk we identified. Some trends were observed in the chromosomal anomaly spectrum when			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
3109-17. (22888165)		☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	were below 35 years (73 with sporadic, 48 with two and 32 with recurrent miscarriage) and 200 were 35 years or more (81 with sporadic, 55 with two and 64 with recurrent miscarriage).		sporadic was compared with recurrent miscarriage: recurrent miscarriage exhibited a decrease in viable trisomies (37 versus 11%) and an increase in non-viable trisomies (38 versus 57%) in women >35 years, together with an increase in unbalanced structural anomalies (4.9 versus 29%) in younger women.			
Guirguis SS, Pelmear PL, et al. Br J Ind Med. 1990;47(7):490-7. (2383519)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x Detection bias ☐ No bias detected --- ☐ **High quality (++)** **x Acceptable (+)** ☐ **Unacceptable (-)**	anesthetic gases theatre staff 8032 exposed 2525 not exposed	questionaire history only	female exposure male exposure	1.98 2.30	anesthetic agss exposure increases miscarriage	history only not prospective large bias
Kitaya K. Fertil Steril 2011;95: 1156-1158.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	58 women with three or more consecutive losses of intrauterine pregnancies before the 22nd gestational week	Chronic endometritis		Chronic endometritis was identified immunohistochemically in 9.3% of patients with recurrent miscarriages (in 12.9% of patients with miscarriages of unknown etiology).	Chronic endometritis is not negligible in patients with recurrent miscarriages.	
Kolte AM, et al. Hum Reprod 2015;30: 777-782.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	301 RPL patients 1813 women trying to conceive naturally	Assessment of stress and depression		A high stress level, defined as ≥19 on the PSS scale, was more prevalent in RPL patients (41.2%) as compared to controls (23.2%). the odds of moderate to severe depression was more than five times higher in RPL patients		
Li W, Newell-Price J, et al. Reprod Biomed Online.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---	45 RPL WOMEN CONTROLS 40 WOMENS		stress questionnaires	ADJUSTED OR 1.1 STRESS SCALES	stress risk factor for RM but moderate stress better pregnancy outcome	small effect size

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
2012;25(2):180-9. (22687324)		☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**						
Lo W, Rai R, et al. J Family Community Med. 2012;19(3):167-71. (23230382)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x Detection bias ☐ No bias detected --- ☐ **High quality (++)** **x Acceptable (+)** ☐ **Unacceptable (-)**	696 history of RM Pregnancy outcome	miscarriage underweight overweight obese	miscarriage	adjusted OR 0.12 underweight 1.27 overweight 1.73 obese women	obesity independent risk factor for miscarriage	prospective in RM Patients
Lucas ES,et al. Stem Cells 2016;34: 346-356.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Menstruation drives cyclic activation of endometrial progenitor cells, tissue regeneration, and maturation of stromal cells, which differentiate into specialized decidual cells prior to and during pregnancy. Aberrant responsiveness of human endometrial stromal cells (HESCs) to deciduogenic cues is strongly associated with recurrent pregnancy loss (RPL), suggesting a defect in cellular maturation. MeDIP-seq analysis of HESCs did not reveal gross perturbations in CpG methylation in RPL cultures, although quantitative differences were observed in or near genes that are frequently deregulated in vivo. However, RPL was associated with a marked reduction in methylation of defined CA-rich motifs located throughout the genome but enriched near telomeres. Non-CpG methylation is a hallmark of cellular multipotency. Congruently, we demonstrate that RPL is associated with a deficiency in endometrial clonogenic cell populations. Loss of epigenetic stemness features also correlated with intragenic CpG hypomethylation and reduced expression of HMGB2, coding high mobility group protein 2. We show that knockdown of this sequence-independent chromatin protein in HESCs promotes senescence and impairs decidualization, exemplified by blunted time-dependent secretome changes. Our findings indicate that stem cell deficiency and accelerated stromal senescence limit the differentiation capacity of the endometrium and predispose for pregnancy failure.				
Lund M, Kamper-Jorgensen M, et al. Obstet Gynecol. 2012;119(1):37-43. (22183209)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	excellent study 987 RPL	5 year follow up			Decreased chance of live births with increasing maternal age	definitive paper

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
McQueen DB, et al Fertil Steril. 2014;101(4): 1026-30.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	395 women with a history of two or more pregnancy losses of less than 10 weeks' size or a fetal demise of 10 or more weeks' size	endometrial biopsy. Chronic endometritis was treated with antibiotics, and a second endometrial biopsy was recommended as a "test of cure."	The overall prevalence of chronic endometritis was 9% (35/395) in this cohort; 7% (21/285) in the REPL group, 14% (8/57) in the FD group, and 11% (6/53) in the combined REPL/FD group. The cure rate was 100% after a course(s) of antibiotics. The subsequent cumulative LBR was 88% (21/24) for the treated chronic endometritis group versus 74% (180/244) for the group without chronic endometritis. The per-pregnancy LBR for the treated chronic endometritis group was 7% (7/98) before treatment versus 56% (28/50) after treatment		There was a high prevalence of chronic endometritis. The test of cure with antibiotics was 100%. Subsequent LBRs after treatment were encouraging.	
McQueen DB, et al. Fertil Steril. 2015;104(4): 927-31.	observational	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	285 RPL patients	21/285 chronic endometritis 7%	all has antibiotics	21/24 -81% livebirths post treatment not chronic endometritis 71% 180/244		high prevalence endometritid in rpl antibiotic encouraging
Nelson DB, Grisso JA, et al. Ann Epidemiol. 2003;13(4):223-9. (12684187)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	326 women in early pregnancy 228 live births 98 miscarriages case control	stress scores in both groups	no differnece		stress does not cause miscarriage	
Nepomnaschy PA, Welch KB, et al. Proc Natl Acad Sci U S A. 2006;103(10):3938-42.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)**	22 pregnancies 9 miscarriages		cortisol levels	highER in miscarried pregnancies	association between maternal stress and miscarriage	small study

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
(16495411)		☐ **Acceptable (+)** ☐ **Unacceptable (-)**						
Pathak R, Mustafa M, et al. Clin Biochem. 2010;43(1-2):131-5. (19804770)	Other	xabout bv and Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	orgnocholorine pesticides serum of in RPL and controls case 30 control 30		high levels of OCP in RM cf controls	p values only	OCP may cause miscarriage	too small study retrospective
Russell P, Pathology. 2013;45(4):393-401.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	1767 cases	same assessment parameters of the earlier study.	This updated analysis of 1989 endometrial biopsies provides reference ranges for CD8+, CD163+, CD56+ and CD57+ cells for individual 'days' of a normalised menstrual cycle. CD8+ T-cells displayed a modest (50%) increase in numbers in the luteal phase and periglandular aggregation was a useful indicator of a subtle focal endometritis, possibly of infective origin, and generally not identified in H&E sections. A rapid accumulation of CD163+ macrophages occurs in the superficial stroma after day 22 of the cycle, while a significant number of cases displayed single or clustered macrophages within glandular lumens of the superficial endometrium in luteal phase, especially after day 20 of the cycle. The significance of this change is unclear but may relate to a macrophage response to abnormal glandular secretion or to bleeding occurring at the time of ovulation. CD56+ uterine natural killer (uNK) cells show such a dramatic rise in both absolute numbers and percentage of stromal cells from day 22 of the standardised 28 day cycle that this needs to be taken into account in all clinical studies or individual assessments of endometrial biopsies. CD57+ NK cells are seen in small numbers in most cases and cell counts of greater than 10 per mm2 are regarded as abnormal.		
Sauer MV. Fertil Steril 2015;103: 1136-1143.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)**			Advanced age is a risk factor for female infertility, pregnancy loss, fetal anomalies, stillbirth, and obstetric complications. These concerns are based on centuries-old observations, yet women are delaying childbearing to pursue educational and career goals in greater numbers than ever before. As a result, reproductive medicine specialists are treating more patients with age-related infertility and recurrent pregnancy loss, while obstetricians are faced with managing pregnancies often complicated by both age and comorbidities.		

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
		☐ Acceptable (+) ☐ Unacceptable (-)			Doctors should also actively educate both patients and the public that there is a real danger of childlessness if individuals choose to delay reproduction.			

Additional references included as background information

Habbema JD, Eijkemans MJ, Leridon H, te Velde ER. Realizing a desired family size: when should couples start? Hum Reprod 2015;30: 2215-2221.

Plana-Ripoll O, Parner E, Olsen J, Li J. Severe stress following bereavement during pregnancy and risk of pregnancy loss: results from a population-based cohort study. J Epidemiol Community Health 2015.

Sharma R, Agarwal A, Rohra VK, Assidi M, Abu-Elmagd M, Turki RF. Effects of increased paternal age on sperm quality, reproductive outcome and associated epigenetic risks to offspring. Reprod Biol Endocrinol 2015;13: 35.

van den Berg MM, van Maarle MC, van Wely M, Goddijn M. Genetics of early miscarriage. Biochim Biophys Acta 2012;1822: 1951-1959.

Brighton PJ, Maruyama Y, Fishwick K, Vrljicak P, Tewary S, Fujihara R, Muter J, Lucas ES, Yamada T, Woods L et al. Clearance of senescent decidual cells by uterine natural killer cells in cycling human endometrium. eLife 2017;6.

Diniz-da-Costa M, Kong CS, Fishwick KJ, Rawlings T, Brighton PJ, Hawkes A, Odendaal J, Quenby S, Ott S, Lucas ES et al. Characterization of highly proliferative decidual precursor cells during the window of implantation in human endometrium. Stem cells (Dayton, Ohio) 2021.

Fukui A, Funamizu A, Fukuhara R, Shibahara H. Expression of natural cytotoxicity receptors and cytokine production on endometrial natural killer cells in women with recurrent pregnancy loss or implantation failure, and the expression of natural cytotoxicity receptors on peripheral blood natural killer cells in pregnant women with a history of recurrent pregnancy loss. The journal of obstetrics and gynaecology research 2017;43: 1678-1686.

Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocrine reviews 2014;35: 851-905.

Katano K, Suzuki S, Ozaki Y, Suzumori N, Kitaori T, Sugiura-Ogasawara M. Peripheral natural killer cell activity as a predictor of recurrent pregnancy loss: a large cohort study. Fertility and sterility 2013;100: 1629-1634.

Kong CS, Ordoñez AA, Turner S, Tremaine T, Muter J, Lucas ES, Salisbury E, Vassena R, Tiscornia G, Fouladi-Nashta AA et al. Embryo biosensing by uterine natural killer cells determines endometrial fate decisions at implantation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2021;35: e21336.

Lucas ES, Dyer NP, Murakami K, Lee YH, Chan YW, Grimaldi G, Muter J, Brighton PJ, Moore JD, Patel G et al. Loss of Endometrial Plasticity in Recurrent Pregnancy Loss. Stem cells (Dayton, Ohio) 2016;34: 346-356.

Lucas ES, Vrljicak P, Muter J, Diniz-da-Costa MM, Brighton PJ, Kong CS, Lipecki J, Fishwick KJ, Odendaal J, Ewington LJ et al. Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. Communications biology 2020;3: 37.

3. ARE HEALTH BEHAVIOUR MODIFICATIONS RELEVANT FOR REDUCING THE RISK OF MISCARRIAGE IN WOMEN WITH A HISTORY OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Du Fossé NA et al., Fert. Steril. 2022;117: 144-152	Meta-analysis	Appropriate question? Yes Rigorous search? Yes Relevant studies included? Yes Quality of studies? Low to moderate Methodology? --- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	11 studies included. Six case-control studies, 4 prospective cohort studies and 1 retrospective study.	Six studies evaluated the association between preconceptional paternal smoking behavior and pregnancy loss, 2 studies focused on paternal alcohol consumption and pregnancy loss, and 3 studies addressed both exposures.	Pregnancy loss	Risk estimate of pregnancy loss: 1–10 cigarettes per day: 1.01; 95% CI 0.97–1.06 11–19 cigarettes per day: 1.12; 95% CI 1.08–1.16 R20 cigarettes per day: 1.23; 95% CI 1.17–1.29. No clear association was found between paternal alcohol consumption and pregnancy loss, based on 5 available studies.	Paternal smoking of>10 cigarettes per day in the preconception period was found to be associated with an increased risk of pregnancy loss, after adjustment for maternal smoking status	
Bellver J, Rossal LP, et al. Fertil Steril. 2003;79(5):1136-40. (12738508)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	360 egg donation IVF pregnancies risk of miscarriage	miscarriage in overweight , obese	miscarriage	OR 1.45 underweight 1.21 overweight 4.02 obese women	obesity independent risk factor for miscarriage	prospective convincing data
Boots C, Stephenson MD. Semin Reprod Med. 2011;29(6):507-13. (22161463)	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	systematic review 2011 28,00 women 6 studies miscarriage in spontaneous conception	bmi <25 25-30 >30	one or more miscarriage	overweight 1.11 obese 1.31	obesity associated with miscarriage but need prospective studies	heterogenous data

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Boots CE, Bernardi LA, et al. Fertil Steril. 2014;102(2):455-9. (24907916)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	117 miscarriages with karyotypes	percentage euploid miscarriages 58% obese 37% non obese		OR 1.63 of obese women having euploid miscarriages	obesity associated with euploid miscarriage	interesting study
Brandes M, Verzijden JC, et al. Reprod Biomed Online. 2011;22(2):192-9. (21195668)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	1809 pregnancies 286 miscarried	miscarriage history of alcohol use confounding factor in whether ART increased miscarriage	female alcohol no effect	male alchohol yes 18.9% no 14.6% p 0.01	study found male alcohol use related to miscarriage s a confounding factor in study	not major point of study
Lashen H, Fear K, et al. Hum Reprod. 2004;19(7):1644-6. (15142995)	Other	x Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	retrospective case control 4932 : 3288 controls 1644 obese	miscarriages early, late and recurrent (>2) miscarriages cases v controls		OR 1.2 Early miscarriage 3.51 recurrent miscarriage	obesity associated with one and recurrent miscarriage	case control study
Lo W, Rai R, et al. J Family Community Med. 2012;19(3):167-71. (23230382)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias x Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	696 history of RM Pregnancy outcome	miscarriage underweight overweight obese	miscarriage	adjusted OR 0.12 underweight 1.27 overweight 1.73 obese women	obesity independent risk factor for miscarriage	prospective in RM Patients

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Metwally M, Saravelos SH, et al. Fertil Steril. 2010;94(1):290-5. (19439294)	CS	X Selection bias ☐Performance bias X Attrition bias ☐ Detection bias ☐ No bias detected ---------- **x High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	471 pregnancies to women with RM	1ST Pregnancy all pregnancies in clinic	miscarriage	1st pregnancy underweight OR 2.58 overweight OR 0.89, obese OR 1.12 all pregnancies underweight OR 3.98 overweight OR 1.02 obese OR 1.71	obese and underweight increases risk of miscarriage	retrospective study
Pandey S, Pandey S, et al. J Hum Reprod Sci. 2010;3(2):62-7. (21209748)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **x High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	review good review of metanalyssis			adjusted OR underweight overweight 1.33. 5.11 obese 1.51, 1.52	increase risk miscarriage if obese after spontaneous and ART	
Sata F, Yamada H, et al. Mol Hum Reprod. 2005;11(5):357-60. (15849225)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	case control 58 2 or more miscarriage's 147 controls caffine consumption mild <100mg a day moderate 100-300mgs a day hight>300gs a day	caffine consumption and CYP1A2 polymorphism mild	Rm versus not	CYP1A2 heterozygous OR for RM with caffeine consumption mild 1.0 moderate 1.03 high 1.03 homozygous OR for RM with caffeine consumption mild 1.0 moderate 31.94 high 5.23	caffine effect only in women CYP1a2 Allells	interesting but small numebrs
Stefanidou EM, Caramellino L, et al. Eur J Obstet Gynecol Reprod Biol. 2011;158(2):220-4. (21636205)	CS	x Selection bias xPerformance bias ☐ Attrition bias x Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x Acceptable (+)** **☐ Unacceptable (-)**	retrospective case control 250 women 52 RM (>3 miscarriages) caffine consumption mild <150mg a day moderate 150-300mgs a day hight >300gs a day	caffeine consumption Rm v controls		OR for RM with caffeine consumption mild 1.0 moderate 3.0 high 16.0	caffeine may be a risk factor for Rm but prospective studies needed	retrospective case control

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Venners SA, Wang X, et al. Am J Epidemiol. 2004;159(10): 993-1001. (15128612)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x Detection bias ☐ No bias detected ---------- **x High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	526 couples women did not smoke 216 husbands non smoker 239 <20cigs a day 72 > 20 cigs s say based on self reported histories	paternal smoking risk of first, second, third conception miscarrying		adjusted OR of miscarriage after 1st 1.17, 2nd 1.22, 3rd 1.39 or conceptions 1.45	paternal smoking associated with recurrent miscarriage	important paper
Wilcox AJ, Weinberg CR, et al. Epidemiology. 1990;1(5):382 -5. (2078614)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x Acceptable (+)** **☐ Unacceptable (-)**	128 pregnancies 43 miscarried	smoking, mother, father alcohol caffine	miscarriage	RR 1.5 moderate, caffeine 2.4 high caffeine mother smoking 1.5 fathers smoking minimal Alcohol mother minimal	study too small to make definitive conclusions	small study
Winter E, Wang J, et al. Hum Reprod. 2002;17(12):3 220-3. (12456627)	Other	x Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x Acceptable (+)** **☐ Unacceptable (-)**	1196 IVF pregnancies 195 miscarried	smokers versus non		adjust OR 2.0	smoking increases miscarriage	ivf conceptiosn but relevant
Zhang BY, Wei YS, et al. Int J Gynaecol Obstet. 2010;108(2):1 35-8. (19897189)	Other	x Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x Acceptable (+)** **☐ Unacceptable (-)**	326 cases Rm 3-6 miscarriages 400 Controls one live birth retrospective	smoking <9, 9-19, >20 exposure never, <1 hour,> 1hour day alcohol never, <5 units, 5 units a week caffine 99mgs, 99-300, >300mgs	Rm compare to controls	adjusted OR Smoking, 1.41, 1.62,2.11 exposure 2.30, 4.75 alcohol 0.83, 0.84 caffine 2.55, 2.39, 2.76	smoking, exposure to tobacco smoke, associated with miscarriage but need prospective studies to confirm this	case control but well done

Additional references included as background information
Andersen AM, Andersen PK, Olsen J, Gronbaek M, Strandberg-Larsen K. Moderate alcohol intake during pregnancy and risk of fetal death. Int J Epidemiol 2012;41: 405-413.
Avalos LA, Roberts SC, Kaskutas LA, Block G, Li DK. Volume and type of alcohol during early pregnancy and the risk of miscarriage. Subst Use Misuse 2014;49: 1437-1445.
Brent RL. Protection of the gametes embryo/fetus from prenatal radiation exposure. Health Phys 2015;108: 242-274.
Greenwood DC, Alwan N, Boylan S, Cade JE, Charvill J, Chipps KC, Cooke MS, Dolby VA, Hay AW, Kassam S et al. Caffeine intake during pregnancy, late miscarriage and stillbirth. Eur J Epidemiol 2010;25: 275-280.
Jensen TK, Gottschau M, Madsen JO, Andersson AM, Lassen TH, Skakkebaek NE, Swan SH, Priskorn L, Juul A, Jorgensen N. Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. BMJ Open 2014;4: e005462.
Leung LW, Davies GA. Smoking Cessation Strategies in Pregnancy. J Obstet Gynaecol Can 2015;37: 791-797.
Maconochie N, Doyle P, Prior S, Simmons R. Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. Bjog 2007;114: 170-186.
Metwally M, Ong KJ, Ledger WL, Li TC. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. Fertil Steril 2008;90: 714-726
Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, Joshi SR, Sadikot S, Gupta R, Gulati S et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. J Assoc Physicians India 2009;57: 163-170.
Moscrop A. Can sex during pregnancy cause a miscarriage? A concise history of not knowing. Br J Gen Pract 2012;62: e308-310.
Schlussel MM, Souza EB, Reichenheim ME, Kac G. Physical activity during pregnancy and maternal-child health outcomes: a systematic literature review. Cad Saude Publica 2008;24 Suppl 4: s531-544.

4. WHAT IS THE VALUE OF MEDICAL AND FAMILY HISTORY TAKING IN ESTABLISHING THE PROGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Alexander SA et al in " Early Pregnancy Loss: Mechanisms and Treatment" eds: Beard and Sharp	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **x Acceptable (+)** ☐**Unacceptable (-)**	100 unselected women with primary RPL (≥3 consecutive losses) and 100 control women Study of immunisation with paternal lymphocytes Setting: University hospital, Belgium Period: ?	Obstetric + family history	7% of the patients' mothers or sisters had experienced RPL.			If the familial trait can be confirmed, it might point toward a genetic component.	
Bhattacharya S, et al. Eur J Obstet Gynecol Reprod Biol. 2010;150(1): 24-7.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **+ Acceptable (+)** ☐ **Unacceptable (-)**	women with a history of miscarriages in previous pregnancies, 143,595 pregnancies with none, 6,577 with one, 700 with two, 115 with three and 24 with four consecutive previous miscarriages. Setting: University hospital, Ireland Study period: 1950 – 2000.	risk of further miscarriage or preterm delivery in adjusting for maternal age and smoking.	The odds of miscarriage were greater in pregnancies following one previous miscarriage than none {adj.O.R. 1.94 (95% C.I. 1.80, 2.09)}. The risk of miscarriage following two miscarriages was greater than in pregnancies following one {adj.O.R. 1.56 (95% C.I. 1.28, 1.90)}. However, there was no further significant increase in odds of miscarriage for pregnancies following three {adj.O.R. 1.37 (95% C.I. 0.86, 2.17)} previous consecutive miscarriages. Age and smoking was strongly related to miscarriage risk. Odds of spontaneous preterm delivery were greater following one miscarriage than none {adj.O.R. 1.52 (95% C.I. 1.36, 1.69)} but no further increases in risk were seen.			After adjusting for age and smoking, the risk of a further miscarriage increased sequentially in women who had one and two miscarriages. Three miscarriages did not increase the odds any further.	Only 139 women had 3-4 miscarriages before the next pregnancy.
Brigham S.A. et al Hum Reprod 1999	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **+ Acceptable (+)** ☐**Unacceptable (-)**	79 women with 2 unexplained cons pl and 246 women with ≥3 unexplained cons PL followed in next pregnancy. Setting: University hospital Period: 10 years	Successful outcome: Viability after 24 wks	226 became pregnant after referral, 2 ectopics, 2TOP, 167 LB. No difference btw primary and secondary RPL.			Previous miscarriage history and age of the patient significantly affected the chances of a successful outcome. Fetal cardiac activity was a positive	Viability after 24 weeks, not live birth was the successful outcome

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
								prognostic factor	
Cauchi MN, et al Am J Reprod Immuinol 1995;33:165-170		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected ---------- ☐ **High quality (++)** **+ Acceptable (+)** ☐**Unacceptable (-)**	Data from 777 couples with unexplained RPL from independent studies at 7 centers	logistic regression analysis The covariates: - age - number of previous misc - length of previous abortions history - sub-fertility index - primary or secondary RPL - received leukocyte immunotherapy.	highly significant difference between the 7 centers in success rates in the subsequent pregnancy and a highly significant association between success rate and each of the following covariates: the number of previous abortions, the length of the previous abortion history and the sub-fertility index. Little evidence of an association between the success rate in the subsequent pregnancy and age, parity, or immunization with cells from the husband.			The sub-fertility index may be a useful measure of likelihood of success in a subsequent pregnancy.	
Christiansen OB et al Acta Obstet Gynecol Scand 1990;69:597-601	case/control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected ---------- ☐ **High quality (++)** **x Acceptable (+)** ☐**Unacceptable (-)**	90 couples with unexplained RPL (63 primary, 27 secondary), 631 randomly selected Danish women with at least one live birth Setting: Danish women, University Hospital Period: 1986 - 1989	Obstetric and family history	25.3% of RPL patients' sisters and 18.8% of patients' brothers' wives had experienced pregnancy losses, 12.6% of the controls. The difference was statistically significant for the sisters, p<0.001.			There is a familial disposition to RPL	
Egerup P, et al. Hum Reprod 2016;31: 2428-2434.	CS retrosp	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected ---------- ☐ **High quality (++)** **+ Acceptable (+)** ☐**Unacceptable (-)**	127 sec RPL with live birth or PL after informed consent	Prognostic impact of : - age, - the number of early PLs before and after the last birth, - a second trim PL before or after the last birth The outcome variable: unexplained loss in the index pregnancy.	In patients with secondary RPL, both a late and each early loss before the last birth did not significantly influence the risk of a new pregnancy loss in the index pregnancy: incidence rate ratio (IRR) 1.31 (95% CI 0.62-2.77) and IRR 0.88 (95% CI 0.70-1.11), respectively. In contrast, the impact on risk of pregnancy loss conferred by a late and by each early pregnancy loss occurring after the birth was significant: IRR 2.15 (95% CI 1.57-2.94, P < 0.0001) and IRR 1.14 (95% CI 1.04-1.24, P = 0.002), respectively.				

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Greenberg et al. J Matern Fetal Neonatal Med, 2015; 28(1): 63–67	CS	x Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **x Acceptable (+)** **☐Unacceptable (-)**	262 women with ≥2 prior PL. Outcome in index pregnancy (IP) and post-index pregnancy (PIP) Setting: University hospital, Israel Period: 2002 – 2010	Parents' ages, occupation, ethnicity, chronic diseases, medications, and obstetric history (number of prior pregnancies/births, number of miscarriages, previous pregnancy complications), as well as results of all evaluations for RPL (genetic, endocrine, anatomic, autoimmune, etc.).	Only variable significantly associated with chance of live birth was the number of pregnancy losses prior to IP; OR for live birth 0.68 (95% CI 0.51 – 0.92)				
Ho HN et al Am J Obstet Gynecol 1991;165(2): 461-466	Case/control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- **☐ High quality (++)** **x Acceptable (+)** **☐Unacceptable (-)**	218 couples with RPL and 934 first degree relatives. 406 controls and 2519 first degree relatives Setting: University hospital, Taiwan Period: ?	Family history	Among RPL patients relatives 13 had experienced 3 or more pregnancy losses, whereas 4 of the controls' relatives had experienced RPL, p<0.0001			major histocompatibility complex—linked genes are involved in the pathogenesis of RPL	
Johnson PM et al Disease Markers 1988;6:163-171	Case/control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- **☐ High quality (++)** **x Acceptable (+)** **☐Unacceptable (-)**	80 couples with primary RPL and 33 with secondary RPL. 68 control women Setting: University hospital, UK Period: ?	Family history of RPL and number of siblings	16% of women with primary RPL had a family history of RPL and a smaller number of siblings, compared with controls.			In primary RPL there may be a familial aggregation	
Kaandorp SP, et al. Hum Reprod. 2014;29(6):1146-52.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x No bias detected --- **x High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	251 unexplained recurrent miscarriage (RM), 2 PL. Median time to conception: 21 weeks (interquartile range (IQR) 8-55 weeks), with a cumulative incidence of conception of 74% after 12 months of trying to conceive.	**1) What is <u>time</u> to conception (weeks) after referral for RPL?** **2) Time to live birth** Putative prognostic factors: - Maternal age - N prior PL - Interventions in ALIFE	**Factor V Leiden mutation** → shorter median time to conception:11 weeks for carriers versus 23 weeks for non-carriers (HR 1.94, 95%CI 1.03-3.65). The cumulative incidence of a live birth of the subsequent pregnancy was 0% after 6 months, 23% after 12 months and 50% after 24 months. The median time to a live birth of the subsequent pregnancy			Time to conception is comparable for women with RPL and other women N prior miscarriages are	Censored at 24 months Only outcome of the pregnancy in the ALIFE study Study of **<u>time</u>** not chance.

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
			Setting: nested prospective cohort study (ALIFE) Period, the Netherlands Period: 2004 - 2009	- +/- late miscarriage - Prior live birth - Factor V Leiden	was 102 weeks (IQR 82-115 weeks). The number of previous miscarriages was the only prognostic factor (HR 0.83, 95% CI 0.74-0.94) significantly associated with time to a live birth of the subsequent pregnancy. **Not confirmed as prognostic factors for time to pregnancy; female age, the number of preceding miscarriages, interventions within the trial and the presence or absence of a preceding late miscarriage, a previous live birth**			significantly correlated to time to live birth	
Kling C, et al. Arch Gynecol Obstet. 2016;293: 1113-1123.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x No bias detected --- **x High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	Observational trial, tertiary immunological center, Germany 228 couples : maternal ages 20-39 years after 3 or more spontaneously conceived first trimester miscarriages. 25% of the original cohort was lost to follow-up. Setting: University Hospital, Germany Period: 1996-2003 Follow-up 2006	Correlation btw obstetric history and 2-year pregnancy- and LBR.	Pregnancy rate: 90.4% LBR: 76.4% **Duration of infertility** was associated with lower CPR (up to 3/>3 years, $p < 0.01$), whereas age and **number of preceding losses** inversely correlated with CDR (<35 years/35-39 years, $p < 0.002$; 3/>3 miscarriages, $p < 0.002$). **Detection of an embryonic heart beat in 2-3 of the first three miscarriages** resulted in favourable outcome (CPR: $p < 0.02$, CDR: $p < 0.002$). Prognosis was excellent in younger fertile women after 3 miscarriages where vital signs had been detected; under less favourable conditions not only risks for further miscarriage, but also for secondary infertility were elevated.			N PL was correlated with LBR. Surprisingly, maternal age was not a prognostic factor.	Only primary RPL
Knudsen UB, et al. Eur J Obstet Gynecol Reprod Biol. 1991;39(1):31-6.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **x Acceptable (+)** **☐ Unacceptable (-)**	Outcome of pregnancy following 0 to 4 consecutive spontaneous abortions. including approximately 300,500 pregnancies. Setting: Register-based, Denmark Period: 1977 - 1984	risk for a clinical spontaneous abortion	overall risk for spontaneous abortion was 11% and the risk for a spontaneous abortion was 16, 25, 45 and 54% after 1 to 4 **previous consecutive spontaneous abortions**, respectively. For women over 35 years, the risk for spontaneous abortion was significantly increased, but the almost **identical abortion rates after repeated abortions in both young and old women indicate a risk factor which is not age-related.**			Increasing numbers of miscarriages → poorer prognosis.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Kolte AM , et al. Hum Reprod. 2014;29(5):931-7.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **+ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	Retrospective study of 587 women with unexplained RPL. Data on the outcome of the first pregnancy after referral were analysed for 499 women. All: ≥3 PL after spontaneous conception or IUI-H. Setting: University hospital, Denmark Period: 2000 - 2010	Prognostic impact of miscarriages and NVPL on chance of live birth in first pregnancy after referral.	RR for live birth - NVPL: 0.90 (95% CI 0.83; 0.97) - Miscarriage: , 0.87 (95% CI 0.80; 0.94) Women with ≥2 miscarriages: RR for live birth: - NVPL: 0.89 (95% CI 0.80; 0.98) - Miscarriage: 0.82 (95% CI 0.74; 0.92 EP: More common if no confirmed miscarriages vs ≥1 mis: (22 versus 6%, difference 16% (95% CI 9.1%; 28.7%); RR for ectopic pregnancy was 4.0 (95% CI 1.92; 8.20).			NVPL have similar prognostic impact as miscarriages on chance of live birth.	
Kolte AM et al Mol Hum Reprod; 2011:17(6):379-385.	CS	x Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐No bias detected --- **☐ High quality (++)** **x Acceptable (+)** **☐Unacceptable (-)**	244 patients with unexplained RPL and 268 siblings. Per pregnancy loss rate compared with register data Setting: Danish women, University Hospital Period: 1986 – 2010	Family history + Genome-wide linkage study of 38 affected sib-pairs	23.3% of patients' siblings had experienced pregnancy loss, significantly more than in the general population, where 13.5% of pregnancies did not end as a live birth			There may be a familial disposition to RPL	Recruitment of siblings was dependent on patients, may have led to selection bias
Kolte AM et al Hum Reprod; 2021: ;36: 1065-1073	Cohort study	☐Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **x High quality (++)** **☐ Acceptable (+)** **☐Unacceptable (-)**	Ntaionwide, registry-based cohort study of 1 285 230 women with a total of 2 722 441 pregnancies Setting: all women living in Denmark with at least one pregnancy in either the Danish Medical birth Registry or the Danish National Patient Registry Period: from 1977 to 2017.	Prognostic impact of the prior pregnancy outcomes and their sequences and maternal age on chance of live birth in next pregnancy	Obstetric complications, still birth, ectopic pregnancies and pregnancy losses had a negative effect on the chance of live birth in the next pregnancy. Consecutive, identical pregnancy outcomes (pregnancy losses, live births or ectopic pregnancies) immediately preceding the next pregnancy had a larger impact than the total number of any outcome.			The study showed that the estimate of chance of live birth should be based on the exact pregnancy history	
Li J, et al. Eur J Obstet Gynecol Reprod Biol. 2014;176:55-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected	Retrospective CS. 138 women w/ primary RPL and 170 women with secondary RPL. All unexplained Setting: University Hospital, UK	birth sex ratio before and after pregnancy losses	Secondary RPL (i) The male:female sex ratio of the first stillbirth was 10:2, significantly (OR=4.76) higher than the male:female sex ratio of 1.05 among all births in UK. (ii) When the first born was a male, the male:female sex ratio of the subsequent birth was 21:35, significantly (OR=0.57) lower than			There was a subtle relationship between the sex of the first and subsequent	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
		---------- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	Period: 1992- 2010, follow-up until 31-03-13		the sex ratio among the general population. (iii) A m**ale firstborn did not affect the chance of a subsequent live-birth.** The findings did not apply to sex ratio in primary RM.			births and secondary recurrent miscarriage, but not primary recurrent miscarriage.	
Lund M, et al. Obstet Gynecol. 2012;119(1): 37-43.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	987 women with primary or secondary RPL All: ≥3 PL Setting: University hospital, Denmark with register-based follow-up Period: 1985 – 2008, follow-up in 2010	age-specific and miscarriage-specific proportions of women with a live birth after the first consultation and similar hazard ratios compared with the prognosis in women aged 30-34 years with three miscarriages before the first consultation.	LBR 5 years after referral: 66.7% (95% CI 63.7-69.7) LBR 15 years after referral: 71.1% (95% CI 68.0-74.2). Negative prognostic factors: high **maternal age** at referral (log-rank P<.01) and increasing **number of miscarriages** (log-rank P<.01) at first consultation.			Maternal age and number of PL are significantly associated with chance of live birth.	
Nielsen HS, et al.. Hum Reprod 2010;25: 1543-1552.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	358 women with sec RPL - 213 gave birth after the diagnosis Controls (Danish National Birth Registry): all women with singleton birth of parity 0, 1982-2005 (n = 608,068) and parity 1, 1986-2008 (n =510,264).	relations between maternal carriage of H-Y-restricting HLA, fetal sex, obstetric complications and prognosis	The sex ratio was 1.49 in births prior to Sec RPL and 0.76 in birth after Sec RPL (P < 0.0001). For Sec RPL patients with only late miscarriages (>10 weeks gestation), the corresponding sex ratios were 2.31 and 0.21. Compared with the control groups, obstetric complications were more frequent both before (39% versus 24% P <or= 0.01) and after (19% versus 14%, P = 0.01) Sec RPL diagnosis. Births were more frequently complicated when the child was a boy (44% versus 31%, P = 0.02) before and a girl (24% versus 13%, P = 0.04) after sec RPL diagnosis. Sec RPL patients with H-Y-restricting HLA class II alleles and a firstborn boy gave birth to children who weighed on average 381 g less (P = 0.006) and were born 0.9 weeks earlier (P = 0.06) and their births had more obstetric complications (P = 0.05) than patients with the same HLA alleles but a firstborn girl.			Obstetric complications, sex ratios in births prior and subsequent to SRM and maternal carriage of H-Y-restricting HLA class II alleles are associated parameters.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Ooi PV, et al. J Reprod Immunol. 2011;88(1):38-41.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **+ Acceptable (+)** ☐ **Unacceptable (-)**	retrospective cohort study of 85 cases of secondary RPL All: ≥3 PL Setting: Univeristy hospital, Ireland Period: 2008 – 2009. Follow-up: 1-2 years	RM was associated with (i) gender of previous child, maternal age, or duration of miscarriage history, and (ii) increased risk of pregnancy complications.	Significantly more had a boy < PL: 62.0%; 53/85) (p=0.002). The majority (91.7%; 78/85) had uncomplicated, term deliveries and normal birth weight neonates, with one quarter of the women previously delivered by Caesarean section. All had routine RM investigations and 19.0% (16/85) had an abnormal result. 57 (67%) women conceived again and 33.3% (19/57) miscarried, but there was **no significant difference in failure rates between those with a previous male or female child** (13/32 vs. 6/25, p=0.2).			A previous male birth may be associated with an increased risk of secondary RM but numbers preclude concluding whether this increases recurrence risk.	Short follow-up period Small study
Parazzini F, et al. Br J Obstet Gynaecol. 1988;95(7):654-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	95 couples with unexplained primary RPL Setting: University hospital, Italy Period: 1980 - 1986		The actuarial overall 3-year livebirth delivery rate was 64%, increasing constantly with time. The reproductive success rate decreased with the **number of previous miscarriages** from 80% in women with two, to 60% with three and 46% with four or more miscarriages. **No effect of age and socio-economic status emerged.** There was a positive association between the number of previous miscarriages and the risk of miscarriage in the next pregnancy. Compared with women with two miscarriages the relative risk of another miscarriage was 2.3 for those with three previous miscarriages and 5.0 for those with four or more (chi 2 1 for trend adjusted for age = 5.2, P = 0.02).			N previous PL was the most important determinant of future outcomes. Follow-up truncated at 3 years.	
Quenby SM, Farquharson RG. Obstet Gynecol. 1993;82(1):132-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected --- ☐ **High quality (++)** **+ Acceptable (+)** ☐ **Unacceptable (-)**	203 consecutive couples Setting: University hospital Period: 1989-1992. Follow-up 4 yrs	125 conceived	A successful pregnancy outcome was most likely in the presence of the following features: **menstrual regularity, fewer than four previous miscarriages, maternal age of less than 30 years, absence of antiphospholipid antibodies, and a previous live birth.** Oligomenorrhea was a considerably more significant feature than any other in predicting a subsequent miscarriage. These high-risk oligomenorrheic women were found to have low luteal phase estradiol levels, but normal luteal phase progesterone profiles and normal LH profiles throughout the menstrual cycle.			differing risk categories. Women at high risk of a subsequent miscarriage had oligomenorrhea and an isolated deficiency of estradiol in the luteal phase of the menstrual cycle	Oligomenorrhea, N pl >4, older age → lower chance of live birth

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
ZhangB-Y et al Int j gynecol obstet 2010;108:135-138	case/control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias + No bias detected ---------- ☐ **High quality (++)** **x Acceptable (+)** ☐**Unacceptable (-)**	326 women with ≥3 pregnancy losses compared with 400 randomly selected controls who had at least one live born child or ongoing pregnancy after 20 weeks' gestation. Setting: Han Chinese, Guangdong Province Period: 2007 - 2009	BMI, family history, smoking, environmental smoke exposure, alcohol consumption, coffee intake	16% of RPL patients' had a family history of pregnancy loss, compared with 8.5% of controls, p=0.003. OR for family history was 1.90 (95% CI 1.074 – 3.36) among the patients with 3 pregnancy losses and 3.09 (1.51 – 6.33) among patients with ≥ pregnancy losses			There may be a genetic component to RPL in South Chinese populations	

Additional references included as background information
None

5. WHAT IS THE VALUE OF SCREENING FOR GENETIC FACTORS IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Barber JC, Cockwell AE, et al. Bjog. 2010;117(7): 885-8. (20482539)	CS	x☐ Selection bias ☐Performance bias ☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **x☐ Acceptable (+)** ☐ **Unacceptable (-)**	20432 RM patients	G banded karyotype	1.9% balanced translocations		UK	Karyotyping couples expensive given pick up rate with G banding. Consider using different techniques CGH	
Bernardi LA, et al. Fertil Steril 2012;98:156 -161.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **x☐ Acceptable (+)** ☐ **Unacceptable (-)**	second miscarriage (< 10 weeks)	Selective versus universal RPL evaluation	The estimated cost of selective RPL evaluation after the second miscarriage was $3,352, versus $4,507 for universal RPL evaluation, resulting in a cost savings of $1,155. With stratification by maternal age groups, selective RPL evaluation resulted in increased cost savings with advancing maternal age groups.			Selective RPL evaluation is cost saving	
Colley E et al. Hum Reprod Update 2019;25: 452-472.	SR	Appropriate question? Yes Rigorous search? Yes Relevant studies included? Yes Quality of studies? Low to moderate Methodology? ---------- ☐**High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	50 studies included published between 2009 and 2018	whole-exome sequencing; copy number variation; and other studies related to pregnancy loss including recurrent molar pregnancies, epigenetics, and mitochondrial DNA aberrations.	Putatively causative variants were found in a range of genes, including CHRNA1 (cholinergic receptor, nicotinic, alpha polypeptide 1), DYNC2H1 (dynein, cytoplasmic 2, heavy chain 1), and RYR1 (ryanodine receptor 1), which were identified in multiple studies. Copy number variants were also identified to have a causal or associated link with recurrent miscarriage.			monogenic aetiologies could contribute to a proportion of pregnancy losses.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Flynn H, Yan J, et al. J Obstet Gynaecol Res. 2014;40(1):109-16. (24033546)	CS	x☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	795 couples Not clear if primary or secondary RPL 2 or more misc	Parental karyotype		3.5% of couples had a chromosomal abnormality	Miscarriage rate in carrier couples significantly higher and low birth rate significantly lower than in non carrier control group but cumulative live birth rate was 64% NB 17% decided not to try again		
Foyouzi N, Cedars MI, et al. Fertil Steril. 2012;98(1):151-5. (22748232)	CS	x☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	Monte Carlo simulation of 1000 patients	Economic modelling of karyotyping after 2nd miscarriage and further investigations only if euploid loss Ability of process to give definitive diagnosis	Aneploidy rates fo 52-75%	Sensitivity analysis dependent on rate of aneuploidy or method of miscarriage management - no diffrenece to outcome		Cost beneift providing aneuploidy rates greater than 51%	
Franssen MT, Korevaar JC, et al. Bmj. 2006;332(7544):759-63.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	278 carrier, 427 non carrier couples	2 yrs reproductive outcome			Dutch	More misc if carrier recip>inverison > robersonian	
Franssen MT, et al. Bmj. 2005;331:137-141	Nested case-control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	Couples referred for chromosome analysis after two or more miscarriages in 1992-2000; 279 carrier couples were marked as cases, and 428 non-carrier couples served as controls.	Independent factors influencing the probability of carrier status	4 factors influencing probability of carrier status: - maternal age at 2nd miscarriage - a history of 3 or more miscarriages - a history of 2 or more miscarriages in a brother or sister of either partner - a history of 2 or more miscarriages in the parents of either partner. The calculated probability of carrier status in couples referred for chromosome analysis after 2 or more miscarriages varied between 0.5% and 10.2%.			Selective chromosome analysis would result in a more appropriate referral policy, could decrease the number of analyses, and lower costs.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Hogge WA, Byrnes AL, et al. Am J Obstet Gynecol. 2003;189(2): 397-400; discussion - 2.	CS	x☐ Selection bias x☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	517 (20 weeks or less) POC miscarriages (subgroup analysis 370 less than 13 weeks)	Karyotype	69% aneuploidy (<13 wks subgroup) 6% inherited 82% aneuploidy >35			Should karyotype POC and only if euploid proceed with rest of testing.	
Kudesia R, Li M, et al. Reprod Biol Endocrinol. 2014;12:19.	CS	x☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	20 specimens of preserved miscarriage tissue from 17 women	array CGH		40% aneuploid	yes	Array CGH clinically useful and better than conventional karyotyping	
Mathur N, Triplett L, et al. Fertil Steril. 2014;101(5): 1349-52.	CS	x☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Patients with 2 or more miscarriages at <10 weeks and at least one preserved miscarriage specimen 58 women, 77 miscarriage specimens	CGH - if euploid XX then MSA ? fetal or maternal		22/77 aneuploid 23% maternal contamination in 46XX specimens Informative in 79% of patients	Yes	Clinically useful test	*Added from search 2* *Suggests strategy of genetic analysis after 2nd miscarraige*
Ozawa N, et al SpringerPlus 2016;5: 874.	**CS**	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	15 spontaneously discharged POC	karyotypes by array-based comparative genomic hybridization (array-CGH	All specimens were successfully analyzed and 10 cases had abnormal results: gain in copy number (n = 7) and loss in copy number (n = 3). Most of them were estimated to be whole chromosome aneuploidy, whereas one case was compatible with microdeletion. Two cases were suspected to be male diploid contaminated by maternal DNA or triploid because of the unsatisfactory signal patterns on X/Y chromosomes. Two of three cases with normal female DNA pattern were identified to be contaminated with maternal DNA by the additional analysis of short tandem repeats				

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Petracchi F et al. Prenatal diagnosis 2017;37: 282-288	**Other**	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**		compare the cost-effectiveness of performing chorionic villus sampling (CVS) of products of conception (POC) in the evaluation of recurrent miscarriage versus standard evidence-based work-up (EBW) of the couple	The expected cost-effectiveness of CVS and karyotype of POC in recurrent miscarriage was: $US769.79 versus $US 1361.8 for the standard EBW of the couple. When stratified by maternal age the results remained cost-effective for this strategy. The arrayCGH strategy has a higher diagnostic yield, but still expensive in our setting to be considered cost-effective.			Chorionic villus sampling and karyotype analysis of products of conception in a 3rd miscarriage proved a more cost-effective strategy than standard EBW of the couple	
Popescu F et al. Hum reprod) 2018;33: 579-587	Cohort study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	100 patients with two or more pregnancy losses, a complete evaluation for RPL as defined by the ASRM, and miscarriage tissue evaluated by 24-chromosome microarray analysis after their second or subsequent miscarriage. Settings: in a private RPL clinic Period: from 2014 to 2017.						
Quintero-Ronderos and Laissue 2020	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**		Sanger sequencing for description of variants potentially related to RPL pathogenesis.				most studies have described sequence variants only having statistical associations with the phenotype, suggesting an increased risk of RPL	Genes having already published conclusive functional tests (eg, FOXD1, ALPP) may represent promising RPL diagnostic biomarkers since their missense mutations have been related to harmful effects.

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Rajcan-Separovic 2020	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**		NGS studies that included inheritance analysis in families with RPL, NGS studies on only the partners, or only the miscarriages	Mutations in candidate genes responsible for recurrent embryonic/fetal loss were found in up to 60% of cases			Genome qsequencing of the couple with RPL with follow up of candidate parental mutations in liscarriages appears to be a promising avenue when miscarriage DNA amounts or quality are suboptimal for genome studies	
Robberecht C, et al. Genet Med 2009;11:646-654	Comparative study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	103 miscarriages	T-banding and 1-Mb array comparative genomic hybridization.	an overall abnormality rate of 35% (34 of 96)	In a comparison of 70 samples that were successfully analyzed by both techniques, 54 (77%) had identical karyotypes (42 normal, 12 abnormal) and 16 (23%) cases showed discrepancies. Most of these differences were due to maternal contamination during cell culture, which resulted erroneously in a normal female karyotype.		improved diagnostic yield of array CGH	
Sahoo T, et al Genetics in medicine 2017;19: 83-89.2017		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	Over a 44-month period, 8,118 consecutive samples were received by our laboratory for CMA analysis. This included both fresh (76.4%) and FFPE samples (22.4%), mostly RPL and/or spontaneous abortion (83%).	The majority of samples were evaluated by a whole-genome single-nucleotide polymorphism (SNP)-based array (81.6%); the remaining samples were evaluated by array-comparative genomic hybridization (CGH).	A successful result was obtained in 7,396 of 8,118 (91.1%), with 92.4% of fresh tissue samples and 86.4% of FFPE samples successfully analyzed. Clinically significant abnormalities were identified in 53.7% of specimens (3,975 of 7,396), 94% of which were considered causative.			Analysis of POC specimens by karyotyping fails in 20-40% of cases. SNP-based CMA is a robust platform, with successful results obtained in >90% of cases. SNP-based CMA can identify aneuploidy, polyploidy, whole-genome homozygosity, segmental genomic imbalances, and maternal cell contamination, thus maximizing sensitivity and decreasing false-negative results.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Shamseldin HE, Swaid A, et al. Genet Med. 2013;15(4):307-9. (23037934)	Other	☐x Selection bias ☐Performance bias ☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **☐ Acceptable (+)** **x☐ Unacceptable (-)**	1 patient - case report	NGS - for autosomal recessive cause of NIFH	Unknown	Not known	Not known	NGS may be useful for NIFH	
Stephenson MD, Sierra S. Hum Reprod. 2006;21(4):1076-82. (16396938)	CS	☐ Selection bias ☐Performance bias x☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	1893 RPL couples	reproductive outcomes		2.7% structural chromosomal arrangement		36% misc unbalanced 71% livebirth rate prognosis better if robertsonian, worst if inversion	
Sugiura-Ogasawara M, Aoki K, et al. J Hum Genet. 2008;53(7):622-8. (18414779)	CS	x☐ Selection bias ☐Performance bias x☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	2,382 couples 1207 controls	Karyotypes			Multicenter Japan	5.4% karyotypical abnormality 63% live birth afterwards, significantly lower than controls	
Sugiura-Ogasawara M, Ozaki Y, et al. Fertil Steril. 2004;81(2):367-73. (14967375)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	1284 couples 102 recip translocation 1184 normal			4.5% chromosomal aberration		Increased risk of further misc (61% pat or 73% mat) reciprocal translocation lower rate normal karyotypes in misc (14% vs 48.9%	

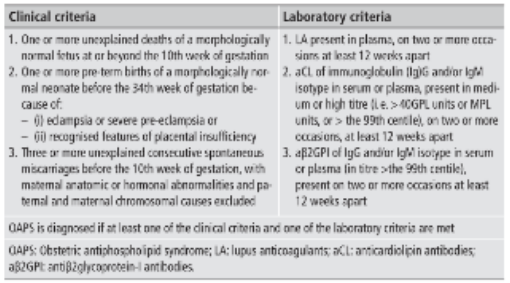
Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
van den Berg MM, van Maarle MC, et al. Biochim Biophys Acta. 2012;1822(12):1951-9.	Other	x☐ Selection bias ☐Performance bias ☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **x☐ Acceptable (+)** ☐ **Unacceptable (-)**	Literature review	Comparison of karyotyping vs whole genome CGH, array CGH, FISH, MLPA, QF- PCR			Yes	Other techniques useful to complement karyotyping especially in case of culture failure	
Vansenne F, et al. Reprod Biomed Online 2011;23: 525-533.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **x☐ Acceptable (+)** ☐ **Unacceptable (-)**	about knowledge of genetic testing only						**Used as additional information**

Additional references included as background information

Philipp T, Philipp K, Reiner A, Beer F, Kalousek DK. Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies. *Human reproduction (Oxford, England)* 2003;18: 1724-1732.

Freeman JL, Perry GH, Feuk L, Redon R, McCarroll SA, Altshuler DM, Aburatani H, Jones KW, Tyler-Smith C, Hurles ME *et al.* Copy number variation: new insights in genome diversity. *Genome Res* 2006;16: 949-961.

6. WHAT IS THE VALUE OF THROMBOPHILIA SCREENING IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Aoki K, Hayashi Y, Hirao Y, Yagami Y. Am J Reprod Immunol 1993;29(2):82-7.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	334 RM (≥ 2 PL) without AI disease 38 RM + aPL + no treatment 280 healthy women	At least 1 aPL PA, Phosphatidic acid *IgG* PG, phosphatidylglycerol PI, phosphatidylinositol PS, phosphatidylserine CL, cardiolipin PE, phosp.-ethanolamine IgG and IgM	14% 9 7 7 7 8 8%	Pregnancy outcome in 38 RM patients (aPl pos) Fetal loss in 82% of IgG aPL vs 40% of IgM aPL (n=5) (sign) Fl = 100% in 21 patients with ≥ 2 IgG aPLs	These results suggest the possibility that 2 or more lgG APL-pos value against PE, PI, PS, or CL, may be more accurate as a predictive variable than that of only 1 IgG APL-posin patients with RPL		
Arachchillage DR, et al. Thromb Haemost 2015;113:13-19.		SR	**Clinical criteria for diagnosis of obstetric APS :**  **Antiphospholipid antibodies (aPL) : associated with an increased risk of recurrent and late PL** - **approximately 15 % of RM women have aPL (Rai 1995 + Robertson 2006).** - **- Meta- analysis: overall frequency of aPL in pregnancy morbidity to be 6 % (interquartile range 2–13 %) (Andreoli 2013).** - **LA associated with late recurrent pregnancy loss ([OR] 7.79, 95 % CI 2.30–26.45),/ data were insufficient for early PL (Galli 2003)** - **IgG aCL, both low and moderate to high antibody levels, were associated with both early (OR 3.56, 95 % CI 1.48–8.59) and late rRPL (OR 3.57, 95 % CI 2.26–5.65). subanalysis; moderate to high aPL levels (> 99th centile) increased the strength of the association (OR 4.68, 95 % CI 2.96–7.40). (Galli 2003)** **IgM aCL were also associated with late recurrent fetal loss (OR 5.61, 95 % CI 1.26–25.03). No association was found between early RPL and aβ2GPI (OR 2.12, 95 % CI 0.69–6.53) (Opatrny2006).**					**International consensus criteria**

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Bizzaro N, et al. Archives of pathology & laboratory medicine. 2005;129(1): 61-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	25 aCL+ primary APS (pAPS) 89 SLE, ⇨ 23 of whom had thrombotic complications (SLE/APS) ⇨ 66 no thrombosis 77 uRM 120 healthy subjects matched for age and sex	Is aPL (aBeta2GPI, prothrombin (PT), AnxV, not aCL) a risk factor for miscarriage in RM patients? IgG and/or IgM aCL, aAPL, anti-beta(2)GPI, anti-PT, IgG anti-Anx V All negative A risk factor for thrombosis in SLE patients (data not added to table)	In RM: 6% 12% 6% 16% 17% 51/77	IgG anti-AnnexinV = only antibody significantly associated with miscarriage (P = .02).		neither aCL nor anti-β2GPI proved to be related to miscarriages in patients with SLE and women with uRM anti-Anx V antibodies may play an important role in recurrent pregnancy loss.	Included in Shovman prognostic
Bouvier S, et al. Blood. 2014;123(3): 404-13.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	NOH-APS observational study obstetric antiphospholipid syndrome = without a history of thrombosis + 3 consecutive spontaneous abortions before the 10th week of gestation or 1 fetal loss at or beyond the 10th week. (n=513) aPL negative RM controls (n=791)	LMWH + LDA (APS) No treatment (controls)		Among APS women, prior fetal loss was a risk factor for fetal loss, preeclampsia (PE), premature birth, and the occurrence of any placenta-mediated complication. Being positive for anticardiolipin IgM was a risk factor for any placenta-mediated complication. Among RM women, APS women were at a higher risk than other women of PE, placenta-mediated complications, and neonatal mortality. Among women with prior fetal loss, LMWH+LDA–treated APS women had lower pregnancy loss rates but higher PE rates than other women.			Not "treatment vs not treated". Relevant control group for assessment of treatment?? If relevant, add further details
Bradley LA, et al. Genetics in medicine 2012;14(1):39-50.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- X **High quality (++)** ☐ **Acceptable (+)**	the association of inherited thrombophilia with RPL, focusing on tests for two genetic variants that are frequently ordered: Factor V Leiden ("F5") and prothrombin G20210A ("F2"). - Analytic validity: (adequate Quality): F5 sens 98.8%, spec 99.3% - F2: sens 98.3%, spec 99.6% (3 studies – Hertzberg, Jennings, ACCE) - Clinical validity: => association between F5 variants and RPL: OR 2.02 (1.60-2.55; p<0.001, based on 33 Case-control) => Assoc between F2 and RPL : OR 2.07 (1.59-2.70; p<0.001, based on 29 Case-control) => F5 and risk for next PL in RPL patients: Summary OR 1.93 (1.21–3.09; p=0.006, 4 prospective CS)				++ studies included up to April 2011	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
		☐ **Unacceptable (-)**	=> F2 and risk for next PL : OR 3.29 ; p=0.17, 1 study) => occurrence rate of PL among F5 carriers: summary OR 2.03 (1.29-3.17; p=0.002, 8CS) => Occurrence rate of PL in F2 carriers: summary OR 1.77 (0.87-3.61; p=0.11, 4CS) (consistent and adequate evidence) - Clinical utility (change clinical management, improve outcomes, benefits>harms) => Treatment (Aspirine, LMWH+aspirin, placebo) : no difference in 2 RCTs + 3 Meta-anal (adequate evidence for lack of treatment) => non-health-related benefits of F5/F2: identifying a "cause" : no studies => risk of VTE in pregnancy: no evidence => harms of testing; anticoagulant-related maternal risks, costs; false-positive result => Overall harm of testing > benefit					
Chen H, Yang X, Lu M.. Arch Gynecol Obstet 2016;293: 283-290.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☒**High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss: a systematic review and meta-analysis 16 articles involving 1420 RPL cases and 1408 controls MTHFR C677T polymorphism was significantly associated with RPL risk under dominant (TT + CT vs. CC; OR 2.10, 95 % CI 1.76–2.50), recessive (TT vs. CC + CT; OR 2.36, 95 % CI 1.92–2.90), heterozygote (CT vs. CC; OR 1.77, 95 % CI 1.32–2.37), homozygote (TT vs. CC; OR 3.55,95 % CI 2.76–4.56), and additive (T vs. C; OR 1.83, 95 % CI 1.64–2.05) model. MTHFR A1298C mutation, no significant association Identification of MTHFR C677T mutation would have some implication for primary prevention of RPL and screening of high-risk individuals in China.					
Galli M, et al. Blood. 2007;110(4): 1178-83.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	WAPS study : 462 patients with persistent LAs and/or moderate to high positive aCL Study population 112 patients	Association between Ab and 1-diagnosis APS; 2-thrombosis; 3-future thrombosis; 4-abortions before recruitment	annexin AV IgG antibodies were associated with a 9-fold higher risk of abortion, aβ2GPI IgG antibodies10-fold higher risk of abortion no association with IgM different AB combinations have different impact on risk of abortion		APS criteria, Include aβ2GPI, further investigate Annexin AV Ab, only include IgG	Relevance unclear PL, not RPL
Gao H, Tao FB. Thromb Res 2015;135: 339-346.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	37 case-control studies (5400 patients vs. 4640 controls) showed an overall 2-fold increased risk of RPL in women with G20210A (pooled OR: 1.81, 95% CI: 1.26-2.60) a positive association between G20210A and RPL was found in European studies (OR: 1.80 with 95% CI: 1.35-2.41), but not in the studies in the Middle-East (OR: 2.39 with 95% CI: 0.96-5.92). (prevalence + sample size) approximate 1-fold increased risk of RPL among women older than 29 years (OR: 1.91with 95% CI: 1.36-2.66). However, the positive relationship was missing among women aged 25-29 years (OR: 1.74 with 95% CI: 0.90-3.38) and younger than 25 years (OR: 4.80 with 95% CI: 0.39-4.25). Significant associations were observed in two-losses RPL (OR: 2.51, 95% CI: 1.36-4.63), and RPL scenario of three losses or more (OR:					

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			1.75, 95% CI: 1.09-2.83). In different types of RPL, the OR for embryonic loss was 0.82 (95% CI: 0.35- 1.92), while the OR for fetal loss was 3.14 (95% CI: 1.61-6.11). The OR for primary RPL was 2.85 (95% CI: 1.58-5.14),while the OR for secondary RPL was 3.97 (95% CI: 1.17-13.45). Comment to REVIEW BRADLEY 2012: included 29 case-control studies that defined RPL as more than two losses in the first or second trimester, or more than one stillbirth or intrauterine fetal demise in the third trimester; which was different from the present meta-analysis, which defined RPL as no less than 2 miscarriages. Interestingly, they reported a remarkable finding (OR=2.07, 95% CI: 1.59-2.70) that different diagnosis criteria did not substantially alter the risk of RPL conferred by G20210A.					
Govindaiah V et al; Clin Biochem 2009;42: 380-386.	case-control study	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	140 RPL (≥3Pls) 140 couples with normal reprod history	total plasma homocysteine, C677T MTHFR polymorphism and DNA damage The 95 percentiles of homocysteine levels in male and female controls were 19.6 µmol/L and 14.0 µmol/L- used as threshold for HHcy	Maternal [mean: 11.6+/-5.0 versus 8.6+/-4.2 micromol/L, OR 4.48] and paternal [mean: 19.6+/-9.5 versus 14.2+/-7.4 micromol/L, OR: 6.92] HHCycysteinemia, paternal age [OR: 1.16], paternal MTHFR 677T allele [OR: 2.30] and DNA damage were found to increase the risk for RPL. DNA damage showed positive correlation with plasma homocysteine and MTHFR 677T allele. Mean maternal homocysteine levels and mean paternal homocysteine levels were higher in cases than controls with 4.48 (95% CI: 2.30–8.70) and 6.92 (95% CI: 3.90–12.29) fold increased risk for RPL (p<0.0001). There was a correlation between maternal and paternal HCY levels with a correlation coefficient of 0.19.		Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss.	Mentioned in Hickey 2013
Hickey SE, et al. Genetics in medicine. 2013;15(2):153-6. PMID: 23288205	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**		MTHFR polymorphism testing	A modest positive association has been found between the MTHFR "thermolabile" polymorphism and many different medical complications, including, but not limited to recurrent pregnancy loss,(Nelen 200 + Govindaiah V2009). Conversely, many other studies looking at similar complications found no statistical association.45–52 The c.1286A→C variant has been studied less, but current evidence suggests that it is milder than the "thermolabile" variant. Preliminary findings in combined genotypes have found that they are not significantly different from controls.57,58		MTHFR polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss	GUIDELINE

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Matsukawa Y et al. European journal of obstetrics, gynecology, and reproductive biology 2017;211: 90-97	CS	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	355 Japanese women with two or more consecutive pregnancy losses and 101 parous women.	The frequency of PS-Tokushima and the subsequent live birth rate in relation to a PS deficiency defined as low PS-specific activity (total PS activity/total PS antigen) and the carriage of PS-Tokushima	There was no significant difference in the frequency of PS-Tokushima between patients and controls. The 8 patients carriers of PS-Tokushima variant were capable of a subsequent live birth without the use of heparin. There was no significant difference in subsequent live birth rates between patients with low or normal PS-specific activity/PS activity without heparin prophylaxis after excluding miscarriages caused by an abnormal embryonic karyotype using multivariate logistic regression analysis. There was no association between PS-Tokushima and RPL and a PS deficiency or low PS activity was shown not to serve as a reliable clinical predictor of subsequent miscarriage.			
Nelen WL, et al .. Fertil Steril 2000;74: 1196-1199.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	REPL : two or more spontaneous miscarriages within 16 weeks' menstrual age 10case-control studies Risk estimation for hyperhomocysteinemia and the MTHFR 677C→T mutation in recurrent early pregnancy loss. Definition REPL: Number of pregnancy losses; Menstrual age (wk). Homocysteine metabolism: Cut-off point (μmol/L); Cases/ total cases; Control/ total controls. Author and year (references); OR (95% CI) **Fasting tHcy** Wouters et al. 1993 (6, 24) — ≥2 — ≤16 — >15[b] — 22/180 — 3/46 — 2.0 (0.6–7.0) Quere et al. 1998 (7)[a] — ≥3 — ? — >10[c] — 12/100 — 5/100 — 2.6 (0.9–7.7) Nelen et al. 2000 (9) — ≥2 — ≤16 — >18.3[c] — 19/123 — 5/103 — 3.6 (1.3–10.0) Overall — 2.7 (1.4–5.2) **Afterload tHcy** Steegers-Theunissen et al. 1992 (5, 25) — ≥2 — ≤16 — >38[b] — 4/14 — 1/15 — 5.6 (0.5–57.9) Wouters et al. 1993 (6, 24) — ≥2 — ≤16 — >51[b] — 29/180 — 1/46 — 8.6 (1.1–65.2) Coumans et al. 1999 (8) — ≥2 — ≤16 — >51[b] — 6/35 — 3/67[d] — 4.4 (1.0–18.9) Nelen et al. 2000 (9) — ≥2 — ≤16 — >61.5[c] — 15/122 — 5/101 — 2.7 (0.9–7.7) Overall — 4.2 (2.0–8.8) **MTHFR 677C→T** Nelen et al. 1997 (10) — ≥2 — ≤16 — T/T — 29/185 — 6/113 — 3.3 (1.3–8.3) Quere et al. 1998 (7)[a] — ≥3 — ? — T/T — 20/100 — 14/100 — 1.5 (0.7–3.2) Grandone et al. 1998 (11) — ≥2 — <17 — T/T — 17/94 — 28/150 — 1.0 (0.5–1.9) Holmes et al. 1999 (13) — ≥3 — ≤12 — T/T — 11/129 — 6/67 — 0.9 (0.3–2.7) Kutteh et al. 1998 (12) — ≥3 — first-trimester — T/T — 4/50 — 2/50 — 2.1 (0.4–11.9) Lissak et al. 1999 (14) — ≥2 — ≤16 — T/T — 4/41 — 4/18 — 0.4 (0.1–1.7) Overall — 1.4 (1.0–2.0) calculated for fasting and afterload plasma homocysteine concentrations, respectively. For the MTHFR T/T genotype a pooled risk estimate of 1.4 (1.0 to 2.0) was found.		Pooled risk estimates of 2.7 (1.4 to 5.2) and 4.2 (2.0 to 8.8) were		hyperhomocysteinemia as a risk factor for REPL	
Opatrny et al. J Rheumatol 2006; 33:2214-21	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	**25 case control studies** **Early PL = prior to 13 weeks' gestation** **Late PL = prior to 24 weeks' gestation**	LA :::::::::::::::::::::::::::::::::::::: ACA	EPL : no data LPL: strong, consistent, and significant association with LAC (OR 7.79, 95% CI 2.30–26.45) 9 studies (n = 2195) :: **IgG:** EPL: OR 3.56, 95% CI 1.48–8.59; 2 studies, n=907, all titers LPL: OR 3.57, 95% CI 2.26–5.65; 10 studies, n=3631 Only moderate and high IgG aCL titers (6 studies, n = 2724), OR 4.68, 95% CI 2.96–7.40 light increase in the strength of association			**Added based on paper Arachchillage 2015**

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				::::::::::::::::::::::::::::::::::::::: aβ2GPI	**IgM** EPl: no studies LPL : OR 5.61, 95% CI 1.26–25.03 ; 4 studies, n = 1822) all titers Only moderate to high titers (3 studies, n = 1579) : OR 4.03, 95% CI 0.84–19.34 Not all positive exclusively for IgM aCL. **IgG and IgM combined** LRPL: 15 studies (n = 4567)), too heterogeneous restricted to studies using our a priori definition for moderate to high antibody titers, 10 studies ; n = 3534; OR 5.39, 95% CI 3.72–7.82) ::::::::::::::::::::::::::::::::::::::: EPl: No association was found between early RPL and aβ2GPI (OR 2.12, 95 % CI 0.69–6.53, 5 studies, n=1788)			
Rey E, et al. Lancet 2003; 361: 901–908.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	13 studies recurrent fetal loss as two or more losses that occurred during the period of pregnancy studied by the investigators	FVL and recurrent fetal loss before 13 weeks MTHFR and recurrent fetal loss prothrombin G20210A (PTm) and recurrent fetal loss PTm and recurrent fetal loss before 13 weeks Activated protein C resistance and recurrent fetal loss before 13 weeks Protein C deficiency and RPL		OR 2.01 (1.13-3.58) (7 studies) OR 0.98 (0.55-1.72) (8 studies) OR 2,05 (1,18–3,54) (9 studies) OR 2,32 (1,12–4,79) (4 studies) Sign for ≥2 RPL, but not for ≥3 RPL Association 3.48 (1.58-7.69), no OR due to heterogeneity (2 studies) OR 1.57 (0.23-10.54) (2studies) OR 14.72 (0.99-217.01, p=0.05)		assessment of women with early recurrent fetal loss should include screening for factor V Leiden, activated protein C resistance, PTm, and protein S deficiency,	

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				Protein S deficiency and RPL Antithrombin deficiency and RPL		(2studies) OR 0.88(0.17-4.48) (1 study)			
Robertson L, et al. Br J Haematol 2006;132: 171-196.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	79 studies	risk of VTE and adverse pregnancy outcomes associated with thrombophilia in pregnancy	The risks for individual thrombophilic defects: - for VTE (ORs, 0.74-34.40); - early pregnancy loss (ORs, 1.40-6.25); - late pregnancy loss (ORs, 1.31-20.09); - pre-eclampsia (ORs, 1.37-3.49); - placental abruption (ORs, 1.42-7.71) - IUGR (ORs, 1.24-2.92). Low-dose aspirin plus heparin was the most effective in preventing pregnancy loss in thrombophilic women (OR, 1.62).			Thrombophilia in pregnancy: a systematic review.
Santos TDS et al., Journal Reprod. Immunol. 2017;123: 78-87	Meta-analysis	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? Heterogeneity ---------- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	9 case-control studies	monitoring APS among women with recurrent miscarriage	association between antiphospholipid antibodies (aPLs) and/or APS compared to the patients with RM (OR: 0.279; 95% CI: 0.212-0.366) and APS cases compared to the patients with RM (OR: 0.083; 95% CI: 0.036-0.189).			Positive association was reported of Lupus anticoagulant (LA) with late RPL	
Sater J et al. J Reprod Immunol 2011;89: 78-83.	Case control	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	244 women with idiopathic RPL, and 283 multi-parous control women	anti-annexin IgM and IgG (ELISA)	RPL: - - significant elevation in anti-annexin V IgM and IgG - increased prevalence of elevated anti-annexin V IgM (to a lesser extent anti-annexin V IgG) - ROC analysis indicated that the area under the curve for anti-annexin V IgM was 0.916, and for anti-annexin V IgG was 0.725. - A systematic shift in anti-annexin V IgM and IgG distributions toward higher values occurred in RPL women, which was confirmed by percentile analysis.			anti-annexin V IgM and IgG antibody positivity are independent risk factors for RPL	

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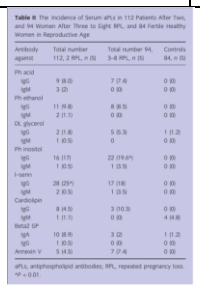
					For each of the anti-annexin V isotypes, the adjusted odds ratio increased as the percentile value increased; the strongest risk was for anti-annexin V IgM, in which the 99th percentile (P99) was associated with a 165-fold higher risk than P50, and for anti-annexin V IgG where P99 was associated with a 38-fold higher risk than P50. In addition, a higher prevalence of elevated anti-annexin V IgM and anti-annexin V IgG was seen in RPL cases than in control women.				
Song Y, et al. Chin Med J 2017;130: 267-272.	CS	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	123 patients with RM and APS pretreated with a low dose of prednisone plus aspirin before pregnancy, and heparin was added after conception.	levels of antiphospholipid antibodies	All patients were positive for anti-beta2-GP1 IgM.	99 of 123 patients became pregnant, and 87 of those pregnancies resulted in live births, 12 resulted in miscarriage ,(success rate of 87.9%). In live birth group, levels of anti-beta2-GP1 were 56.8 +/- 49.0 RU/ml before the pretreatment regimen, 32.1 +/- 26.0 RU/ml after 2 months of pretreatment, and 24.1 +/- 23.1 RU/ml during early pregnancy ($P < 0.05$). In the miscarriage group, titers were 52.8 +/- 30.7 RU/ml before, 38.5 +/- 34.2 RU/ml after, and 33.9 +/- 24.7 RU/ml during early pregnancy; the decrease in antibodies was lower in the miscarriage group than in the live birth group ($P < 0.05$). Of the 24 infertile patients, the average antibody titer did not decline after pretreatment ($P = 0.802$).		The decreases in antiphospholipid antibody titers correlated with better pregnancy outcomes. The shorter treatment regimen was effective and economical.	
Subrt I, et al. Am J Reprod Immunol 2008;59(3):193-200. PMID: 18275512	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	206 unexplained RPL *112 with 2 RPL* *94 with ≥3 RPL* 2-8 RPLs without live birth Exclusion of chromosomal aberrations, reproductive organs malformations,	8 aPL ph-serine, ph-ethanolamine, ph-inositol, DL-glycerol, phosphatidic acid, anti-annexin V, cardiolipin, beta2-GPI.				aPL and genetic thrombophilic factors are important risk factors in the pathogenesis of RPL. Both autoantibodies against various	**Included in review Bradley**

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
			infectious and endocrine disease 84 healthy controls (*≥ 1 live birth)*	4 genetic thrombophilic factors FV 1691G>A (Leiden mutation), FII 20210G>A mutation, MTHFR 677C>T MTHFR 1298A>C variant	Significantly increased prevalence of aPLs against ph-inositol (17-19.6% dependent on nr of PLs) and against ph-serine (18-25%). In 96%, at least one risk factor was found *≥3 RPLs*: strong positive correlation of aPLs positivity and thrombophilic risk factors			kinds of phospholipides and genetic thrombophilic factors must be studied together in diagnosis of RPL for appropriate treatment.	
Tebo AE, et al. Clin Exp Immunol . 2008;154(3): 332-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	62 patients with APS 66 women with RPL 50 healthy blood donors 24 women with a history of successful pregnancies	aPL other than aCL and abeta2GPI IgG /IgM: IgM and IgG Ab to: phosphatidic acid, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol phosphatidyl serine with and without beta2GPI aCL abeta2GPI antibodies		See paper for numbers, none clinically relevant		overall combined sensitivity of the non-recommended aPL assays was not significantly higher than that of aCL and aB2GPI	. Multiple aPL specificities in RPL group is not significantly different from controls and therefore of no clinical significance.
van den Boogaard E, et al. Fertility and sterility. 2013;99(1):188-92.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	312 women with RM + APS 1407 women with unexplained RM. Similar age and number of previous PL RM clinic: Retrospective	Association between the number and sequence of preceding miscarriages and antiphospholipid syndrome (APS).		No differences between groups number of preceding miscarriages and live births, consecutive miscarriages:	There is no increased diagnostic yield for APS after 3 miscarriages rather than after 2 miscarriages and no increased diagnostic yield for APS after consecutive miscarriages rather than after nonconsecutive miscarriages. Therefore, APS testing should be considered for all women with 2 or more miscarriages.		
Vora S, et al. The National medical	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias	381 unexplained RPL women (early and late PL) 100 age-matched fertile	Coagulation test LA ACA IgG / IgM B2GP1	Data for EARLY PL (n=136) OR 11.4 (1.9-68.4; p=0.003 OR 20.4 (5.3-78.4; p<0.001 OR 2.6 (0.6-11.6; p=0.3)			Thrombophilia is an important factor in both early and late	

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journal of India. 2008;21(3):116-9.		☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	controls (≥1 child)	Annexin V protein C, protein S and AT III Genetic markers factor V Leiden (FVL), PT gene G20210A, MTHFR C677T, EPCR 23 bp insertion PAI 4G/5G	OR 14.4 (2.4-86.7; p= 0.001) no significant differences in the mean levels For both early and late PL: 3.4% RM vs 1% controls 0% vs 0% 2.6% 5.8% 21.5% vs 10% ≥2 genetic factors : 41 (10.8%) of cases genetic + acquired risk factor : 79 (20.7%) No more than one risk factor was observed in any of the controls. 176 (46.2%) patients had at least 1 acquired thrombophilia - 143 (37.5%) had at least 1 genetic thrombophilia marker. 288 patients (75.6%) had either an acquired, genetic or both markers of thrombophilia.			pregnancy losses.	

Additional references included as background information

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO, American College of Chest P. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012;141: e691S-736S.

Bates SM, Middeldorp S, Rodger M, James AH, Greer I. Guidance for the treatment and prevention of obstetric-associated venous thromboembolism. J Thromb Thrombolysis 2016;41: 92-128.

Levin BL, Varga E. MTHFR: Addressing Genetic Counseling Dilemmas Using Evidence-Based Literature. J Genet Couns 2016.

Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, PG DEG, Koike T, Meroni PL et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4: 295-306.

7. WHAT IS THE VALUE OF IMMUNOLOGICAL SCREENING IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Al-Hussein K, Al-Mukhalafi Z, et al. Am J Reprod Immunol. 2002;47(1):31-7.	CS	**acceptable**	24 couples with RM and 6 fertile control couples	Undetermined maternal antibodies detected by flowcytometry against husbands lymphoycytes and semn	No sign associations detected				Study too small for any conclusions
Amani D, Dehaghani AS, et al. J Reprod Immunol. 2005;68(1-2):91-103.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☒ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	111 RM patients (3+ misc) 110 ethnically matched controls (2+ births)	TGFB1 polymorphism in 10 SNPs investigated		No differences in SNP frequencies			OK
Aoki K, Kajiura S, et al. Lancet. 1995;345(8961):1340-2.	CC Pros COH	**High quality**	68 RM pts (2+ misc) and 47 healthy controls (no prev misc)	Peripheral blood investigated for NK toxc in standard test. No CD information. Subseq pregnancy achieved within 9 months after NK tests.		NK tox 39.4% in RM pts vs 29.0% in contr (p =?) Pts with NK-tox > 41%: 71% subseq. misc. rate; pts with NK tox < 41%: 20% misc rate.		RR for misc 3.5 (1.8-6.5) in pts with high NK toxc.	Good study but no inform about CD day
Aruna M, Nagaraja T, et al. Hum Reprod. 2011;26(4):765-74.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **☒ Acceptable (+)** **☐ Unacceptable (-)**	143 RM pats (2+ misc) 139 controls with one child	HLA-DR,-DQ typing		No different sharing in DQA, DQB and DRB between patient and control couples DQB1*03:03:02 associated with RM (OR = 2.66; 1.47-4.84), pc 0.02			Patients and controls ethnically heterogeneous

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Bao SH, Shuai W, et al. Eur J Obstet Gynecol Reprod Biol. 2012;165(2):326-30.	CS	**Acceptable**	32 RM pts 35 women with induced abortion	Investigation of NK toxicity tests in NK cells isolated from decidual tissue from miscarriage/induced abortion				Higher NK cytotox in RM	Flawed since cells from necrotic and vital tissue are compared
Bartel G, Walch K, et al. Hum Immunol. 2011;72(2):187-92.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x☐ No bias detected ---------- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	167 RM pts 96 multiparous controls an no misc.	Testing for HLA antibodies in sera obtained 2-13 months after last birth/miscar	Anti HLA class I or II abs: 19% in pts and 49% in controls ($p < 0.0001$). Abs pos: 17% with idiopath and 22% with known cause of RM			No link between anti-HLA ab and RM	Good and reliable study
Beydoun H, Saftlas AF. Tissue Antigens. 2005;65(2):123-35. (15713211)	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	12 case-control studies. Patients with 3+ miscarriages	HLA-A,-B, -C or –DR sharing in patients and control couples		No difference in HLA-A,-B and –C allele sharing between patients and controls. HLA-DR sharing sign increased in RM couples OR 1.33 (1.01-1.75). p = 0.04			Serological testing used in most studies
Bustos D, Moret A, et al. Am J Reprod Immunol. 2006;55(3):201-7.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	118 RM pts (3+ misc) 125 cont (2+ LB) Same age	Invest of ANA, ACA antigliadin		Pts 13.5% cont 11.2% ANA pos (NS) IgG ACA 15.3% pts vs 3.2% in cont ($p < 0.01$)			

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Calleja-Agius J, et al. Clin Dev Immunol 2012;2012: 175041.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Villous (n = 38) and venous blood samples (n = 26) were obtained from women with missed miscarriage. Tissue chromosome analysis indicated 23 abnormal and 15 normal karyotypes.	TNFalpha TNF-R1 TNF-R2, interleukin (IL)-10	Plasma TNFalpha/IL-10 ratios were significantly ($P < 0.05$) lower in miscarriages with abnormal karyotype. In abnormal karyotype group, there were significantly higher levels of TNFalpha ($P < 0.01$), IL-10 ($P < 0.01$), TNF-R1 ($P < 0.001$), and TNF-R2 ($P < 0.001$) in the villous extracts and culture-conditioned medium compared to normal karyotype group.			In miscarriage with abnormal karyotype, there is an exacerbated placental inflammatory response, in contrast to miscarriage of normal karyotype where maternal systemic response is increased.	
Carbone J, Gallego A, et al. J Rheumatol. 2009;36(6):1217-25.	CC	**High quality**	36 RM pts with antiphosph abs (APS) and 36 RM pts without APS 73 control women, 36 of these parous	Blood samples for FACS taken outside of pregnancy but no specific CD.CD56,16+ NK cells measured		APS neg pts: 14% NK cells APS pos pts: 8-11% NK cells Controls: 13% NK cells		No sign difference of NK cells been APS neg. pts and controls	Nice, informative study
Cavalcante MB, Costa FD, et al. J Matern Fetal Neonatal Med. 2014:1-5.	Retrospective cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X☐ **Acceptable (+)** ☐ **Unacceptable (-)**	106 RM patients treated with lymphocyte injection therapy (LIT) 82 had subseq. LB 24 miscarried	14 risk factors for RM investigated and related to outcome		In pts with new miscarriage ANA pos (29.2%) and Tgb-Ab pos (29.2%) were sign ($p < 0.001$) increased compared with those with birth (3.9%, 4.9%)			LIT treatment of all pts will flaw study results

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Chao KH, Yang YS, et al. Am J Reprod Immunol. 1995;34(5):274-80.	CC	**Acceptable**	10 RM pts (3+ misc), 21 pts with anembryonal pregn and 21 multiparous with induced abortion	Blood samples and endometrial tissue investigated for CD16,56 and NK toxicity at time of miscarriage/abortion. Tissue homogenized without enzymatic digestion		No sign. differences in periph blood or decidual CD16+ or CD56+ or NK toxicity in peripheral or decidual blood between RM pts and controls		In normal pregnancy is dec. NK toxicity sign lower than periph blood NK toxc which is not the case in RM or anembr loss NK toxc not related to NK count in the same decid. sample	Small study but some infomative value
Chen et al., Semin Arthritis Rheum, 2020. 50(4): p. 534-543.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☒ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Twenty-one studies with 5038 participants (including 2683 RPL patients and 2355 controls)	assess whether ANA was positively associated with increased RPL risk	RPL patients had a significantly higher ANA positive rate than controls (OR = 2.97, 95%CI 1.91-4.64, P<0.00001; I2 = 75%), and a significant association between positive ANA and unexplained RPL was also observed (OR = 3.27, 95%CI 2.01-5.31, P<0.00001; I2 = 70%).			*ANA positivity was positively associated with increased RPL risk. ANA positivity is an important risk factor for RPL which needed to be screened among women with RPL.*	
Choi YK, et al.. Am J Reprod Immunol 2008;60: 91-110.	SR	**Acceptable**	RPL	Cytokine gene polymorphism	Either allele and/or genotype frequencies of the following polymorphisms were reported to be significantly different between women with RSA and controls: IFN-gamma +874A-->T, TA (P = 0.01), AA (P = 0.04); IL-6, -634C-->G CG/GG (P = 0.026); IL-10, -592C-->A CC (P = 0.016); IL-1B -511C (P = 0.035), -31T (P = 0.029); IL-1RA, IL1RN*2 (P = 0.002), and IL1RN*3 (P = 0.002). None of these studies was repeatedly reported by others to be significantly different. Among these, four cytokine polymorphisms (IFN-gamma, +874A-->T; IL-1B -511C; IL-1RA, IL1RN*2, IL1RN*3) were refuted by others and rest of them were studied once.				

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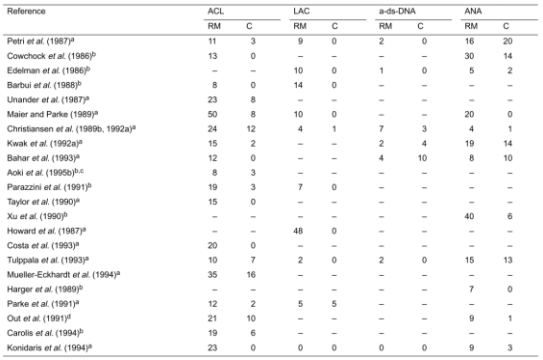
Christiansen OB. Hum Reprod Update 1996;2: 271-293.	SR	**Acceptable**			**Table III. Case-control studies of the prevalence (%) of various autoantibodies in non-SLE women with RM and controls** 				Narrative with a good overview of case-control studies
Christiansen OB, et al. Hum Reprod. 1998;13:3326-3331			123 Danish and Czech women with RPL	- 6 APL antibodies : - ACL antibody. - ANA, - anti-zona pellucida antibodies and - anti-sperm antibodies HLA-DR and -DQ typed by DNA-based methods.	The frequency of HLA-DR phenotypes did not differ significantly between APL antibody positive RPL and APL antibody negative RPL or healthy controls. Among ACL antibody positive RPL, significantly more were positive for the HLA-DR3 phenotype and negative for the HLA-DR2 phenotypes compared with healthy controls ($P < 0.05$). Among ANA positive RPL, 55% carried the HLA-DR3 phenotype compared with 28% of ANA negative patients ($P < 0.05$) and 21% of healthy controls ($P < 0.002$).			the HLA-DR3 phenotypes seem to predispose to formation of ACL antibodies and ANA.	
Clifford K, Flanagan AM, et al. Hum Reprod. 1999;14(11): 2727-30.	CC	High quality	29 RM pts (3+ misc) and 10 parous controls	Endometrial biopsies taken in luteal phase. CD56 cells investigated by IHC		Sign. ($p < 0.001$) higher density per high powered field of CD56 pos cells in RM pts vs controls			Nice but small study

Reference	ACL		LAC		a-ds-DNA		ANA	
	RM	C	RM	C	RM	C	RM	C
Petri *et al.* (1987)[a]	11	3	9	0	2	0	16	20
Cowchock *et al.* (1986)[b]	13	0	–	–	–	–	30	14
Edelman *et al.* (1986)[b]	–	–	10	0	1	0	5	2
Barbui *et al.* (1988)[b]	8	0	14	0	–	–	–	–
Unander *et al.* (1987)[a]	23	8	–	–	–	–	–	–
Maier and Parke (1989)[a]	50	8	10	0	–	–	20	0
Christiansen *et al.* (1989b, 1992a)[a]	24	12	4	1	7	3	4	1
Kwak *et al.* (1992a)[a]	15	2	–	–	2	4	19	14
Bahar *et al.* (1993)[a]	12	0	–	–	4	10	8	10
Aoki *et al.* (1995b)[b,c]	8	3	–	–	–	–	–	–
Parazzini *et al.* (1991)[b]	19	3	7	0	–	–	–	–
Taylor *et al.* (1990)[a]	15	0	–	–	–	–	–	–
Xu *et al.* (1990)[b]	–	–	–	–	–	–	40	6
Howard *et al.* (1987)[a]	–	–	48	0	–	–	–	–
Costa *et al.* (1993)[a]	20	0	–	–	–	–	–	–
Tulppala *et al.* (1993)[a]	10	7	2	0	2	0	15	13
Mueller-Eckhardt *et al.* (1994)[a]	35	16	–	–	–	–	–	–
Harger *et al.* (1989)[b]	–	–	–	–	–	–	7	0
Parke *et al.* (1991)[a]	12	2	5	5	–	–	–	–
Out *et al.* (1991)[d]	21	10	–	–	–	–	9	1
Carolis *et al.* (1994)[b]	19	6	–	–	–	–	–	–
Konidaris *et al.* (1994)[a]	23	0	0	0	0	0	9	3

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Emmer PM, Nelen WL, et al. Hum Reprod. 2000;15(5):1163-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	43 RM 37 non-preg controls 39 controls pregnant after IVF	CD56% and NK toxicity tests in per. blood measured in pts and non-preg controls and related to subsequent outcome in pts		CD56+16+ cells similar in pts and non-preg controls In pts 8/8 (100%) with CD56+ < 12% gave birth compared with 7/14 (50%) with CD56+ > 12% (p <0.05)		High CD56+16+ % before pregnancy predictive for increased risk of miscarriage	OK study but multiple tests and comparison Pregnant control group invalid
Emmer PM, Veerhoek M, et al. Transplant Proc. 1999;31(4):1838-40.	CC and prosp COH	**High quality**	142 RM pts (2+ misc) 26 with subsequent unexplain misc and some with subsequent birth. Controls 40 successful IVF pts and 42 parous controls.	Peripheral blood taken before pregnancy investigated for CD56,16 by FACS and NK toxicity by standard tests		NK toxc in RM with subs. misc. 390 LU vs 420 LU in RM pts with LB (nonsign). CD56,16 NK cells sign. higher in RM with subs mis: 22%; vs RM with subs. LB:. 8%. In parous controls 13%			Interesting and large and good study
Fan W, et al . J Assist Reprod Genet 2014;31: 173-184.	SR	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	17 studies were included, representing 1786 cases and 1574 controls two or more miscarriages	HLA-G 14-bp polymorphism.	HLA-G 14-bp polymorphism was not associated with RPL risk in all genetic models and allele contrast(+14 bp vs. −14 bp:OR=1.13; 95% CI, 0.96,1.32; +14 bp/+14 bp vs. −14 bp/−14 bp: OR=1.16, 95%CI, 0.85, 1.59; +14 bp/−14 bp vs. −14 bp/−14 bp: OR=1.21, 95 % CI, 0.92,1.58; dominant model: OR=1.33; 95 % CI, 0.99,1.78; recessive model: OR=1.06; 95 % CI, 0.79,1.43). (significant heterogeneity across studies) Subgroup analysis: significant association between HLA-G 14-bp polymorphism and patients with three or more miscarriages(+14 bp vs. −14 bp: OR=1.27; 95 % CI, 1.04, 1.55; dominant model: OR=1.52; 95 % CI, 1.16, 1.99; and model +14 bp/−14 bp versus −14 bp/−14 bp: OR=1.51; 95% CI, 1.15, 1.97;).				

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Faridi RM, Agrawal S. Hum Reprod. 2011;26(2):491-7.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	177 prim RM pts Cont: 200 women with 2+ LB	Maternal KIR genotyping and parental HLAC typing		Inhib comb: 2DL1/C2,C2,C2,C2 OR 0.28 (highly sign. Rarer in pts) Activat comb: 2DS2/C1,C1,C1,C1 OR 2.83 high sign more freq in pts)		Activating mat KIR: parental HLA-C combinations predispose to RM	Nice and large study
Giasuddin AS, Mazhar I, et al. Bangladesh Med Res Counc Bull. 2010;36(1):10-3.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X☐ Acceptable (+)** **☐ Unacceptable (-)**	35 RM pts (3+ misc) 37 control women (1+ LB)	ANA antibodies invest		No significant difference in ANA pos between groups			Small study
Hadinedoushan H, Mirahmadian M, et al. Am J Reprod Immunol. 2007;58(5):409-14.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X ☐ Acceptable (+)** **☐ Unacceptable**	21 RM pts (3+ misc) and 32 normal pregnant parous women	PB samples taken in pts within 24 hour of latest miscarriage and in controls at matched time points. NK cytotoxicity against K562 cells investigated by FACS?		At all three effector:target ratios NK cytoxicity was signif higher in RM pts vs controls		Increased NK cytotoxicity is a risk factor for RM	The higher NK cytox. in pts may be a result of miscarriage, evacuation or anaestesia
Harger JH, Archer DF, et al. Obstet Gynecol. 1983;62(5):574-81.	Prospective	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X☐ Acceptable (+)** **☐ Unacceptable (-)**	155 women with RM (2+ misc) followed in next pregnancies			7.5% were ANA positive Miscar rate 3/7 (43%) in ANA pos pts. Overall misc rate 29/106 = 27%			Small numbers of ANA pos Outcome data not completely clear

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Hefler-Frischmuth K, et al. Am J Reprod Immunol 2017;77.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable**	114 women with RPL 107 healthy controls	ANA IgG Ab histone, IgG Ab nucleosomes, IgG Ab against double-stranded (ds) DNA	No difference in prevalence Ab in RPL versus controls No associations were found between serum levels and clinical characteristics of affected women.			serologic parameters of autoimmunity are not elevated in women with RPL and are not associated with clinical characteristics of affected women.	
Hiby SE, Regan L, et al. Hum Reprod. 2008;23(4):972-6.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable**	95 RM pts (3+ misc) , 67 of their male partners and 269 parous control women	KIR and HLA-C genotyping		KIR AA found sign more frequent in RM women than controls (OR = 1.80; 1.11-2.94) Paternal HLA-C2 sign increased in male partners comp with contr (OR = 1.62; 1.10-2.40). KIR2DS1 decreased in RM women (24%) vs control women (44%) (p 0.00035)		Maternal.-paternal KIR/HLA-C combinations in theory associated with NK cell inactivation sign associated with RM	Good study, however no HLA-C typing of control male partners
Hviid TV, Christiansen OB.. Hum Immunol 2005;66:688-699.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable**	Women with RPL and their partners (n = 103) control women + partners (n = 92)	linkage disequilibrium between HLA class II genes, primarily HLA-DRB1 alleles, and HLA-G alleles	We found a significant linkage disequilibrium between HLA-DR3 and HLA-G*010102 in RPL and controls. For all 4 studied HLA loci, the alleles in haplotype HLA-DRB1*03.DQA1*05.DQB1*02.G*010102 was in clear linkage disequilibrium. This HLA haplotype has repeatedly been associated with different AI diseases but also with RPL. The G*010102 allele includes a 14-bp sequence polymorphism in the 3' untranslated region of the gene, which has been associated with differences in HLA-G mRNA alternative splicing and stability. This 14-bp polymorphism has also been associated with RPL, pre-eclampsia, and outcome of IVF.				

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Jablonowska B, Palfi M, et al. Am J Reprod Immunol. 2001;45(4):226-31. (11327549)	CS and cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x☐ No bias detected --- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	31 RM pts included in a RCT of IvIg 10 controls	Antibodies blocking MLR (BA) before and in pregnancy	19.7% in pts 30% in controls			No increased BA% in RM pts and presence of BA not predictive of outcome	Small but good
Kaider AS, Kaider BD, et al. Am J Reprod Immunol. 1999;42(6):335-46. (10622463)	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X☐ Acceptable (+)** **☐ Unacceptable (-)**	302 RM pts (3+ misc) 112 population contr (men/women) 20 fertile contr	Investg. of ANA (comb. of ssDNA, dsDNA, s-SM, SSB, anti-histone)		35.1% ANA pos pts 1.8% GP cont ANA pos (p < 0.001) 10% ANA pos in fertile contr			Small fertile group
Karami N, Boroujerdnia MG, et al. J Reprod Immunol. 2012;95(1-2):87-92. (22854126)	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X☐ Acceptable (+)** **☐ Unacceptable**	23 RM pts 20 RIF pts 43 non-pregnant women (36 multiparae)	Peripheral blood luteal phase CD56+ and NK toxicity		12.9% CD56dim and NK tox 32.1 in RM 5.4% CD56dim and NK tox 10.7 in controls P = 0.001			Informative study
Katano K, Suzuki S, et al. Fertil Steril. 2013;100(6):1629-34.	Prospective cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias X☐ No bias detected --- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	552 RM pts with no treatment and subsequent LB or clinical miscarriage	Peripheral blood NK activity in the luteal phase		In multivariate regression adjust for age, no. of prev. misc., previous births etc increased NK cell activity had no relationship to outcome (p = 0.37) Miscarriage rate was highest in pts with lowest NK activity		No association between peripheral blood NK cell activity and risk of new miscarriage in RM pts	Very informative and large study

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King K, Smith S, et al. Hum Reprod. 2010;25(1):52-8. (19819893)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x☐ No bias detected ---------- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	104 RM pts 33 controls	CD56 and CD16 cells in per. blood in luteal phase		NK% > 18% highy specifiv for RM	Pts: 12.5% NK cells > 18% Cont: 3% NL cells > 18%	Peripheral NK% in midluteal phase can discriminate between women with RM and controls.	
Kruse C, et al. Hum Reprod 2003;18: 2465-2472.	Case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x☐ No bias detected ---------- **☐ High quality (++)** **x ☐ Acceptable (+)** **☐ Unacceptable (-)**	14 pregnant women with RM (≥ 3 previous consecutive miscarriages) during the first 14 weeks of pregnancy (4 LB, 10 miscarried) 15 control women in gestational weeks 7-8.	Lymphocytes were in-vitro-stimulated by mitogens, allogeneic cells and microbial antigens, and the production of a series of cytokines, the proliferative responses and lymphocytic expression of CD62L (which may be a marker of T-helper type 2 lymphocytes) were measured.	Repeated measurements of cytokine production were reproducible during the first trimester. The proliferative responses to herpes simplex and tetanus antigens were increased, and the ratio of CD62L-/CD62L+ expressing CD4+CD45RO+ lymphocytes was decreased in patients compared with controls (P = 0.01, P < 0.01 and P < 0.01 respectively).			The importance of CD62L expression on lymphocytes for RPL and the relevance of the maternal response to microbial antigens during pregnancy should be further explored.	
Kruse C, Steffensen R, et al. Hum Reprod. 2004;19(5):1215-21.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	354 and 234 women with RM 202 and 360 controls All Caucasians	HLA-DRB1; DQA1 and DQB1 patients vs controls		OR for RM In DR3 pos women: 1.4 (1.1-1.9,.p = 0.01 Stronger association in patients with 4+ miscarriages or secondary RM		Maternal HLADR3 predisposes to RM and especially secondary RM	
Kwak JY, Beaman KD, et al. Am J Reprod Immunol. 1995;34(2):93-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)**	81 non-pregn and 26 preg RM pts Control: 17 non-preg and 22 pregn women (no other inform)	Meaurement of CD56/CD16 and B cells- no cycle information All pts got heparin/aspirin in pregn		CD56% approx 14% and 9% in pts and controls (p < 0.0005) No differences in CD56 in pts who miscarried or gave birth			Mixture of pregnant and non-pregnant pts and controls

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(8526995)		**x☐ Acceptable (+)** **☐ Unacceptable (-)**							
Lachapelle MH, Miron P, et al. J Immunol. 1996;156(10):4027-34. (8621945)	CC	High quality	20 RM pts (3+ misc) and 15 parous women	Endometrial biopsies taken CD 18-25, homogenized and investigated by FACS for CD56,16 and 45		RM pts: 14,5% CD56bright vs 21% in controls ($p < 0.05$) RM pts CD56dim 8% vs 8% in controls RM pts: 11% CD56+16+ vs 6% in control ($p < 0.001$)		Sign higher CD16 expression in RM pts. No difference in NK parameters between prim and secondary RM and between those who subsequently miscarried or gave birth	Nice but small study. Analysis of homogenized tissue may be a flaw.
Lashley EE, et al. Am J Reprod Immunol 2013;70: 87-103.	SR	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	the effect of antipaternal antibodies on pregnancy complications		risk ratio for HLA class I and class II antibodies on pregnancy complications. risk for first- and third-trimester complications	meta-analysis (17 studies) : No significant effect of HLA class I or class II antibodies on pregnancy outcome. (high level of statistical and clinical heterogeneity)	No consistent conclusions can be drawn from the meta-analysis. Discrepancies in the meta-analysis are the result of different screening techniques, varying time points of screening, and use of incorrect control groups.		Beneficial or harmful effect of antipaternal human leukocyte antibodies on pregnancy outcome? A systematic review and meta-analysis.
Lee SK, Na BJ, et al. Am J Reprod Immunol. 2013;70(5):398-411.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)**	95 RM women (42 unexpl) and 29 age matched fertile controls	Investigation of TNF-and other cytokines and Th1 and Th2 cells in periph blood in the follicular phase		% TNF-a + Th1 cells and TNF-a/IL10 produc Th1/Th2 ratio signif increased in RM pts. vs controls In log regr. analysis: TNF-a/IL10 prod T cells associated with			No inform about interval from last pregnancy to time of blood samples

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		X **Acceptable (+)** ☐ **Unacceptable (-)**				OR 4.78 (1.3-17.6) for RM			
Liang P, Mo M, et al. Am J Reprod Immunol. 2012;68(2):164-74.	Pros coh	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable**	76 RPL pts 29 had subseq LB 5 had subseq euploid misc	Invetsigation of CD56 markers an dNK-toxicity in luteal phase smaples		No sign differences in CD56, CD56dim, CD56bright or NK tox between pts who had LB or miscar			All pts had lymphocyte immunisation before pregnancy. Miscarriage group very small
Makhseed M, et al. Hum Reprod. 2001;16(10):2219-26.	CC, prospec	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	39 preg. RPL women (3+ mis) who gave birth 24 preg. RPL women who miscarried 54 normal pregn (3 prev. births) during labour; 24 of these also tested in week 12	Lymphocytes mitogen stimulated and cytokine production measured.		Production in PHA stimul lymph: IL4, IL6, IL10 were sign increased in 1st trimester preg. controls vs RM women who miscarried IL2 sign incr. in RM comp with cont. Higher Th2 cytokines in RM women with subs. birth than new misc			Flaws: many samples were taken at the time of miscarriage or birth which may affect results
Matsubayashi H, Sugi T, et al. Am J Reprod Immunol. 2001;46(5):323-9.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X☐ **Acceptable (+)** ☐ **Unacceptable (-)**	273 RPL pts (2+ misc) 200 healthy, age-matched women	Test for LAC, ACA and ANA		ANA pos: Pts 2+ mis: 23.4% Pts 3+ mis: 24.1% Contr 13.0% ($p < 0.05$)			

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Medica I, et al. Reprod Biomed Online 2009;19: 406-414	SR	**Acceptable**	RPL	Investigations of a single polymorphism/gene involvement in RM reported more than five times were selected.	308/TNF-alpha polymorphism, OR for RPL: 1.1 (0.87-1.39) if the polymorphism was considered under a dominant genetic model (7 case-control) 1082/ IL-10 polymorphism, the OR under a dominant model was 0.76 (0.58-0.99), and under a recessive model the OR was 0.90 (0.71-1.15) (6 studies). 174/IL-6 polymorphism, the OR for RM under a recessive model was 1.29 (0.69-2.40) (5 studies).			The results show a statistically significant association with RM for the -1082/IL-10 genotype.	Association between genetic polymorphisms in cytokine genes and recurrent miscarriage--a meta-analysis.
Michimata T, et al. Am J Reprod Immunol. 2002;47(4):196-202.	Prospective COH	**Acceptable**	17 RM pts (2+ misc), 11 had subsequent LB and 6 had euploid miscarriage. Controls: 15 women with male factor infertility who all had a subsequent LB	Endometrial biopsy in luteal phase investigated for CD56,16 by immunohistochemistry		Lymphocyte subsets including NK cell did not diverge between pts and controls and between pts with subsequent LB or miscarriage			Informative but small study
Molazadeh M, et al. Iran J Reprod Med. 2014;12(3):221-6.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	560 RM pts (2+ misc) 560 age-matched control women	ANA invest on Hep-2 cells Titres ≥ 1:40		RM pts: 74/560 (13.2%) ANA pos Contr: 5/560 (0.9%) pos (< 0.001)			Very large study, unknown fertility status of controls
Morikawa M, et al. Gynecol Obstet Invest. 2001;52(3):163-7.	Prospective COH	High quality	56 RM patients who had a subsequent pregnancy, 39 had LB, 10 had euploid miscarriage and 7 had aneuploid miscarriage	Peripheral blood taken before pregnancy (no CD indicated) investigated for NK toxicity and CD56,16 by FACS.		Similar CD56+CD16- and CD56+CD16+ count in LB, aneuploid and euploid misc. In euploid miscarriage NK toxicity tended to be increased compared with LB group (p = 0.01)			Nice, informative but small study.
Motak-Pochrzest H, Malinowski A. Neuro	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias	155 primary RM pts (3+ misc) 50 control women (1 LB)	8 serum immune biomarkers and 2 cytokines invest in-vitro after PHA stimul. of PBL taken before pregn.		ACA, LAC, antisperm abs, INF-g and TNF-a sign increased in pts ANA 18.7% in pts and			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Endocrinol Lett. 2013;34(7):701-7.		☐ No bias detected ---------- ☐ **High quality (++)** ☒☐ **Acceptable (+)** ☐ **Unacceptable (-)**		All pts neg. for anti-HLA and blocking abs.		10.0% in controls (NS)			
Mueller-Eckhardt G, et al. J Reprod Immunol. 1994;27(2):95-109. (7884745)	Prospective	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☒ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	32 RM patients with subs. birth and 19 RM pats with subs misc	TNF-alpha in periph blood before and during index pregnancy HLA-A,B,DR,DQ typing		Pts with LB: 25% had > 6.54 pg TNF-a Pts with misc: 81.8% had > 6.54 pg TNF-a (p = 0.015). In RM couples sign increased sharing of two HLA alleles			
Nielsen HS, et al. Fertil Steril. 2008;89(4):907-11.	Prosp	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☒☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Two cohorts of 175 and 130 patients with secondary RM	Chance of birth in next pregnancy		Multivariate analysis: OR for live birth in pts with a firstborn boy : 0.37 (95% CI 0.2-0.7),p = 0.01		A firstborn boys before sec. RM reduces the prognosis significantly	Indirect evidence for a role for anti-HY immunity in RM
Nielsen HS, et al. Hum Mol Genet. 2009;18(9):1684-91.	prospective	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☒☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	358 patients with secondary RM and 203 of their firstborn children	Live birth rate in next pregnancy according to maternal carriage/non-carriage of class II HY-restrict. HLA Miscarriage rate in next pregnancy according to maternal HLA	Carriage of one HY restrict class II HLA associated with OR for LB: 0.46 (0.2-0.9) Carriage of two HYrHLA: OR = 0.21 (0.1-0.7)			Mat. HY-restric-HLA predisposes to new misc. in sec. RM	Indirect evidence for a role of anti-HY immunity in RM Proof that HYresticting HLA play a role in sec RM

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Nielsen HS, et al. Hum Reprod. 2010;25(11): 2745-52.	Case-contr and prospective	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	84 pts with sec. RM, 12 with prim RM and 37 female controls	ELISA testing antibodies against 5 recombinant HY proteins		Anti-HY pos: 46% sec RM, 19% cont 8% prim RM P = 0.01 for diff Prospective preg: Anti-HY pos: 12% boys Anti-HY neg: 49% boys P = 0.03 for diff		Anti-HY antibodies more frequent in sec RM after a firstborn boy than in other women Anti-HY antibodies associated with low male:female birth ratio	Direct evidence for a role of anti-HY antibodies in sec RM
Ozcimen EE, Kiyici H, et al. Arch Gynecol Obstet. 2009;279(4): 493-7.	Prospective cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☒ **Unacceptable (-)**	23 RM pts andwomen 23 withinduced ab	CD57+ uterine NK cells			No difference in CD57+ cells in two groups		Not informative since necrotic tissue is compared with vital
Perricone C, De Carolis C, et al. Rheumatology (Oxford). 2007;46(10): 1574-8.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable**	77 idiopat RM (3+ misc) and 42 healthy control women of reproductive age	PB samples collected in second phase of menstr cycle. FACS analysis for CD56 and cD16		71/77 pts (92.2%) vs 3/42 (7.1%) had NK% > 15 (significant)			Originally 218 RM pts were excluded but very many were excluded due to various reasons
Piosik ZM, etal . Am J Reprod Immunol 2013;70: 347-358.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)**	47 RPL patients, Plasma was repeatedly sampled in the first trimester	concentrations of 5 cytokines including TNF-alpha TNF-alpha levels were correlated to carriage of five TNFA promoter polymorphisms	TNF-alpha levels increased (P = 0.014) with progressing pregnancy, with higher levels in secondary than primary RM (P = 0.042) but with no significant impact on outcome. Carriage of TNFA -863C and TNFA -1031T was associated with higher TNF-alpha levels, and the former was found more often in secondary than primary RM (P < 0.02).			Plasma TNF-alpha levels increase during early pregnancy in RM women regardless of outcome, but	Plasma TNF-alpha levels are higher in early pregnancy in patients with secondary compared with primary recurrent

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
		X ☐ **Acceptable (+)** ☐ **Unacceptable**						are higher in secondary than primary RM, which may be partly genetically determined.	miscarriage.
Prado-Drayer A, Teppa J, et al. Am J Reprod Immunol. 2008;60(1):66-74.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable**	18 pts with 2 or more misc. and 10 parous controls	FACS analysis of PB taken CD 17.-26		CD56,16+: 13.9% in pts vs 6.0% in controls (p = 0.002) CD56dim 6.7% in pts and 0.5% in controls (p= 0.003) CD56,16+ > 12%: 11/18 of pts vs 0/10 of cont (p = 0.001)		NK cell subsets increased in PB of RM pts vs controls	Very small study Large variation of CD of sample taking
Quenby S, Kalumbi C, et al. Fertil Steril. 2005;84(4):980-4.	CS	High quality	75 RM pts (3+ misc) and 18 cont with 2+ LBs	Endometrial biopsies from CD 21+/- 2 days investigated by IHC for CD56 and CD16		Sign. higher NK% in pts vs controls (p = 0.008) 43% of pts vs 2/18 controls had NK% > 5% Sens of low (<5%) NK% for RM: 43% and spec 89%			Good study
Quinn PA, Petric M. Am J Obstet Gynecol. 1988;158(2):368-72.	CS	☐ Selection bias x☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable**	67 RM pts 32 normal pregnant women	Anticomplementary activity	Anticomp act: pos: 41.8% RM pts and 12.9% in controls (p < 0.01)				Anticompl activity poorly defined test Pregn controls compared to some non-pregn pts

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Sater MS, Finan RR, et al. Am J Reprod Immunol. 2011;65(5):526-31.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- **☐ High quality (++)** **x☐ Acceptable (+)** **☐ Unacceptable (-)**	265 RM pts and 283 age-matched controls	Anti-proteinZ IgM and IgG	OR for RM: 1.10 (1.06-1.14) for pos IgM a-PZ OR for RM: 1.08 (1.05-1.12) for IgG a-PZ			Presence of anti-PZ is a risk factor for RM	RM pats more obese than controls. Same controls and pts as in previous study. Testing of multiple biomarkers and selective reporting?
Shakhar K, Ben-Eliyahu S, et al. Fertil Steril. 2003;80(2):368-75.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	38 primary RM pts and 29 secondary RM pts. 25 control women of these 13 parous	Peripheral blood taken on unspecified CD. Lymphocytes investigated by two techniques for NK toxicity and FACS for CD56 and CD16		NK%, NK numb, stand. NK tox, whole blood NK tox.: prim RM: 13.2, 302, 44.8; 73.4; Sec RM: 11.0, 239, 31.5, 38.7 Nullipar con: 8.4, 178, 14.6, 20.0; parous con.: 8.6, 164, 7.8, 15.4		In prim RM were all NK biomarkers sign higher than in all controls. In sec. RM, NK biomarkers not increased.	Very informative study but lack of CD information
Shakhar K, Rosenne E, et al. Hum Reprod. 2006;21(9):2421-5.	CS	High quality	38 RM pts (3+ misc) 14 with prim RM; and 22 controls (11 nullip + 11 multipar)	NK% and NK cytotoxicity invest. in peripheral blood in two samples taken with 20 minuttes intervals. No inform about CD of blood sampling. All NK test investigated on fresh samples	In first blood samples sign higher NK% and NK tox in pts vs controls. In second blood sample signif. decline in all NK indices in primary RM but not sec RM or controls. In second blood sample NK% and NK toxc not different between sec. RM and controls			RM have exaggerated transient stress response at time of blood sampling	Good and exciting but small study
Sharshiner R, Romero ST et al. J Reprod Immunol 2013; 100	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	116 RPL and 116 controls with 2 or more births	Invest. of tissue transglutaminase and endomysial antibodies assocaied with celiac disease in pts and controls	Same very low frequencies of both antibodies in patients and controls			Screening for celiac disease markers not recommended in RM	

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Shimada S, Kato EH, et al. Hum Reprod. 2004;19(4):1018-24.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	20 pts with primary RM and 17 women with one previous birth	Endometrial biopsies taken in luteal phase (5-9 days after tp rise) Biopsies homogenized and CD56,16 analysed by FACS		CD56+: 18.3% in pts and 15.9% in controls (NS). Comparisons of CD16+ and CD16- cells did neither show differences		No difference in NK cell subsets in non-pregnant RM and controls	FACS analysis of homogenized biopsies yields questionable results. Small study.
Souza SS, Ferriani RA, et al. J Reprod Immunol. 2002;56(1-2):111-21. (12106887)	CC	Acceptable	9 RM pts and 9 control pts with 2+ LBs	Peripheral blood taken in luteal phase. Investigated for CD56,16 and NK cytotox in fresh blood		CD16+, CD56+, NK tox at ratio 320:1 and NK act 40% LU: RM pts 142, 169, 46%,6.3 and controls: 192, 230, 54% and 13.7. NK tox sign lower in RM pts than controls (p = 0.04)		NK activity reduced in RM pts when expressed in LU	Nice study using fresh cells, exciting results but small
Stern C, Chamley L, et al. Fertil Steril. 1998;70(5):938-44. (9806580)	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	97 RM pts (3+ misc) 106 control women (1 + LB) 38% of pts and 55% of controls pregnant when sampled	Invest. Of ANA, LAC, beta2-GPI various APLs	Pts 22.7% ANA pos vs 9.4% cont ($p < 0.05$) Anti-beta2GPL IgM and ANA positivity significantly increased in RM pts compared with controls				Very nice and large study
Ticconi C, Rotondi F, et al. Am J Reprod Immunol. 2010;64(6):384-92. (20482520)	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	194 RM pts (2+ misc) 100 contr (2+ LB) age matched	ANA antibodies measured		Pts: 50% ANA pos vs 16% of contr. ($p < 0.001$) Titre 1:80 33.5% pts vs 16% con; 1:160 11.8% vs 11.8% ($p < 0.001$			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Thomsen CK et al., J Reprod. Immunol. 2021;145: 1033082021	Case-control study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	1078 Caucasian women with RPL 2066 controls	HLA typed by DNA-based low and high-resolution techniques		the HLA-DRB1*07 allele was significantly associated to RPL (OR 1.29; 95%CI 1.09-1.52 in heterozygous RPL patients and OR 2.27; 95%CI 1.31-3.93 in homozygous patients)		an association to HLA-DRB1*07 was detected for the first time	
Toth B, et al., Reprod Biol Endocrinol, 2019. 17(1): p. 72.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	575 RM patients; n = 248 idiopathic RM patients (iRM, n = 167 primary iRM (ipRM), n = 81 secondary iRM (isRM)).	Peripheral blood levels of CD45+CD3−CD56+CD16+ NK cells were determined by flow cytometry and uterine CD56+ NK cells by immunohistochemistry in mid-luteal non-pregnant RM patients		pNK level: pRM/ipRM vs sRM/isRM, mean ± SD /µl: 239.1 ± 118.7/244.9 ± 112.9 vs 205.1 ± 107.9/206.0 ± 105.6, p =0.004/ p = 0.009; mean ± SD %: 12.4 ± 5.5/12.8 ± 5.4 vs 11.1 ± 4.6/11.1 ± 4.3, p =0.001; p =0.002). uNK levels isRM vs ipRM: mean ± SD /mm2 288.4 ± 239.3 vs 218.2 ± 184.5, p =0.044).		differences in NK cell recruitment and potentially different underlying immune disorders between pRM and sRM.	
Tuckerman E, Laird SM, et al. Hum Reprod. 2007;22(8):2208-13. (17656418)	CS and COH	High quality	87 RM pts (3+ misc) 32 with subs LB and 19 with subs misc. Controls: 10 cont women (7 proven fert)	Endometrial biopsies collected in midluteal phase, CD56 invest by IHC		Mean CD56+% were 11.2 vs 6.2 in controls (p = 0.01). Mean CD56+% was 13.3 in LB pts vs 9.6 in misc. pts (p 0 0.44).		Uterine NK cells higher in RM than controls. uNK cells not predictive of outcome in next pregnancy	Good and informative study

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Vargas RG, Bompeixe EP, et al. Am J Reprod Immunol. 2009;62(1):34-43.	CS	High quality	63 RM pts with 3+ miscarriages 68 parous women	KIR genotype investigation		25/68 pts vs 12/68 (17.6%) of controls carry activating KIR genes		OR for RM is 2.71 (1.23-6.01) for RM	Nice study but many comparisons and findings may be due to multiple testing
Varla-Leftherioti M, , et al. Am J Reprod Immunol. 2003;49(3):183-91.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	26 primary RM couples (2+ mis) and 26 control couples with 2+LB	Genotyping for 3 inhib and 2 act. KIRS		Carriage of all 3 inh KIRs: RM pts 30.8% vs 69.2% of control women (p = 0.01)		Less NK inhibition in RM women than controls	Nice but small study
Vassiliadou N, Bulmer JN. Hum Reprod. 1996;11(7):1569-74. (8671506)	CC	**Acceptable**	40 pts with sporadic misc and 19 with induced abortion	Endometrial tissue from evacuation investigated by IHC for CD57		CD57 sign increased in RM			Flawed due to comparison of necrotic and vital tissue. Not RM pts and not relevant to PICO questsion
Wang Q, Li TC, et al. Reprod Biomed Online. 2008;17(6):814-9. (19079966)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	85 pts with 2 or more misc 27 control with one or more births	Blood samples taken CD 2-5 FACS analyses for CD56 and CD16		CD56+: 20.0% pts vs 20.4% controls CD56+,16+: 16.5% pts vs 16.6% cont. CD56+,16-: 4.4% pts vs 3.8% cont		No sign differences between NK cell number in pts and control and in pts relating to number of miscarriages	Good study but blood samples taken CD2-5!
Wang X, et al. Tissue Antigens. 2013, pp. 108-115.	SR	**Acceptable**	Unexplained RPL 14 studies with 1464 cases and 1247 controls	human leukocyte antigen-G (HLA-G) 14bp insertion (ins)/deletion (del) polymorphism	Significant associations between 14bp ins/del polymorphism and risk of URSA were observed in both dominant [random effect model (REM) OR=1.469, 95% CI=1.127-1.914] and codominant (REM OR=1.195, 95% CI=1.005-1.420) models. After excluding two articles that deviated from Hardy-			This meta-analysis suggests that the 14bp ins HLA-G allele is associated with	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
					Weinberg equilibrium in cases and sensitivity analysis, significant associations were also observed in dominant [fixed effect model (FEM) OR=1.224, 95% CI=1.020-1.470] and codominant (FEM OR=1.158, 95% CI=1.028-1.305) models.			increased risk of URPL	
Wilson R, Moore J, et al. Hum Reprod. 2003;18(7):1529-30.	Cand pros cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias x☐ No bias detected --- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	49 non-preg. RM pts and 22 cont. with no misc	IL2 receptor levels	IL-r = 1589 in pts and 1082 in cont ($p < 0.05$) Same Il2-r level in 21 pts who subs. gave birth or misc.				Small but nice study
Witt CS, Goodridge J, et al. Hum Reprod. 2004;19(11):2653-7.	CC	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **x☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable**	51 patients with RM (3+ misc) and 55 women with 2+ LBs	Genotyped for KIR alleles		No difference in frequencies of any KIR gene between patients and controls. No difference between total number of activating or inhibitory KIRs between pts and controls. No differences in % of pts and controls with A or B genotypes.			Very good study although small and no HLA-C data
Yamada H, Morikawa M, et al. Am J Reprod Immunol. 2003;50(4):351-4.	Prospective COH	High quality	85 pts with RM (2 + misc) 11 had subsequent euploid misc., 6 had biochem. pregn. And 59 had LB	Blood samples taken before conception, no CD indicated. Investigated for NK cytotoxicity and NK subsets by FACS.		Pts with LB: NK toxc 33%; pts with euploid mis 48% NK toxc and pts with aneuploid misc. 28% NK toxc ($p < 0.05$). No sign difference between CD56+ cells in pts with LB or miscarriage.			Nice and unique study; however small and lack of cycle day inf.
Yoo JH, Kwak-Kim J, et al. Am J Reprod Immunol. 2012;68(1):38-46.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)**	48 RM pts 15 parous controls	Investigation of CD56 and NK tox in peripheral blood before pregn.. No cycle day indicated		CD56+ higher in pts (15.6%) than controls (10.1%); $p < 0.001$. NK tox sign. ($p < 0.05$) higher in all dilutions in pts than cont			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
		x☐ **Acceptable (+)** ☐ **Unacceptable**							
Zhang B, Liu T, et al. Hum Immunol. 2012;73(5):574-9.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- X **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	12 case-control studies of the prevalence of –two TNF-alpha promoter polymorphisms in RM	Genotyping of the TNF-alpha 308G/A or -238 G/A promoter polymorphisms		All studies: combined -308 polymorphism OR 1.04 (0.86-1.26) for RM. Asian studies: OR 1.33 (0.95-1.86) All studies no association between -238 polymorphism and RM		No association between the most important promoter genes in the TNF-alpha gene and RM	

Additional references included as background information
Chen, et al., Measurement of uterine natural killer cell percentage in the periimplantation endometrium from fertile women and women with recurrent reproductive failure: establishment of a reference range. Am J Obstet Gynecol, 2017. 217(6): p. 680.e1-680.e6.

8. WHAT IS THE VALUE OF SCREENING FOR METABOLIC/ENDOCRINOLOGICAL ABNORMALITIES IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Alonso A, et al. Am J Obstet Gynecol 2002;187: 1337-1342.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	75 women with >/=1 unexplained fetal loss, and 75 control subjects with at least 1 healthy term infant and without gestational complications.	mutations of factor V Leiden, MTHFR, and prothrombin gene; deficiencies of antithrombin-III, protein C, and protein S; antiphospholipid antibodies fasting homocysteine concentration. A placental histologic study	35% of the patients had thrombophilia (control subjects, 16%; P =.008; odds ratio, 2.78). increase of intrauterine fetal death in patients with thrombophilia (P =.01) and early pregnancy loss in patients without thrombophilia (P =.02). Hyperhomocysteinemia with low folate acid : 1.3% of patients (n=1) and 0 controls Hyperhomocysteinemia, without C677T-MTHFR mutation: 2% of patients (n=3) and 0 controls				
Atasever M,: Fertil Steril. 2016;105(5): 1236-40.	cohort study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	71 recurrent miscarriage 70 sequentially selected age-matched fertile women	ovarian reserve Serum levels of FSH, LH, E2, and antimullerian hormone (AMH); FSH/LH ratio; ovarian volumes; and antral follicle count (AFC)	The levels of FSH were 8.6 +/- 3.7 U/L in the RM group and 7.1 +/- 3.9 U/L in the control group; this difference was statistically significant. The levels of AMH were significantly lower in the RM group than in the control group (2.9 +/- 1.7 ng/mL vs. 3.6 +/- 1.7 ng/mL). The percentage of women with levels of FSH >/=11 U/L was significantly higher in the RM group than in the control group (18.3% vs. 4.3%). In the RM group, the percentage of women with levels of AMH </=1 ng/mL was significantly higher than in the control group (19.7% vs. 5.7%).				
Badawy SZ, Westpfal EM. Early Pregnancy. 2000;4(4):253-60.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)** **no controls**	90 patient charts	hysterosalpingogram, endometrial biopsy, cervical cultures for Chlamydia and ureaplasma, and chromosomal karyotyping luteal phase defect; measured by endometrial biopsy	32/83 38,6%	highest positive findings were hysterosalpingogram, endometrial biopsy, cervical cultures, and immunologic studies.			Frequency of etiologic factors, costs

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Balasch J, Creus M, et al. Hum Reprod. 1986;1(3):145-7.	CCS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	60 RPL ≥2 AB 1rst trimester Unexplained: no abnormalities karyotype, endocrinology, toxoplasmose, uterine 25 control women with previous pregnancy	Luteal phase deficiency By endometrial biopsy			17/60 (28.3%) patients vs 1/25 controls (4%) : significant difference		Study not conducted for RPL but infertility. RPL subgroup of infertility.
Bernardi LA, Cohen RN, et al. Fertil Steril. 2013;100(5):1326-31.	CS	X Selection bias XXPerformance bias X Attrition bias – Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** X **Unacceptable (-)**	N=286 women History ≥2 pregnancy losses < 10 weeks 2004-2007 controls 2008 intervention with levothyroxine Abnormal karyotype was excluded	No controls Subclinical hypothyreoid: TSH>2.5 mIU/L fT4 and fT3/fT4 index normal	55/28619% subcl. Hypo 30/286 10.5% hypo 8/286 3% hyper	Not calculated	No info	Study to be included as intervention study not applicable for prevalence or incidence estimation RQ11	
Bussen S, Sutterlin M, et al. Hum Reprod. 1999;14(1):18-20.	CCS	X Selection bias (controls were infertility patients) - Assesment X Confouding - Statistical issue---------- ---------- ☐ High quality (++) ☐ Acceptable (+) x Unacceptable (-)	N=42 ≥ 3 RPL N=42 no PL but male or tubal infertility Exclusion: chromosomal or uterine abnormalities	TSH TSH<0.3 TSH >4	= 1.2 vs 1.3 = =		REPL is not associated with abnormal TSH secretion REPL is associated with abnormal PRL secretion suggesting an endocrine aetiology for REPL REPL is associated with abnormal androstenedion secretion suggesting an endocrine aetiology for REPL		
				PRL (follicular phase) PRL >16 ng/ml	↑ 14.2 vs 10.5 15 vs 2				
				FSH >8 FSH	4/42 vs. 9/42 NS 6.2 +- 1.7 vs. 6.5 +- 1.9 NS				
				NS differences in Progesterone measurements		0.6 +- 0.2 vs. 0.5 +- 0.2 NS			

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				Testosterone DHEA-S Androstenedion androstenedion>3.1	= = ↑ 2.3 vs 1.7 ↑ 6 vs 0				
				Early follicular serum FSH LH E2	= = =	6.2 +- 1.7 vs. 6.5 +- 1.9 3.9 +- 1.9 vs. 5.1 +- 2.5 66.6 +- 49.8 vs. 75.3 +- 34.2			
Carp HJ, Hass Y, et al. Hum Reprod. 1995;10(7):1702-5.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-) no controls** **Prognostic study**	N=153 RPL ≥3 No abnormalities in karyotyping, glucose, thyroid, prolactin metabolism, luteal phase, uterine, toxoplasmosis, APS Study is conducted to investigate the treatment of antipaternal cytotoxic antibodies, therefore for prognostic value only non-immunized women included	Serum LH>10IU/L LH/FSH ratio > 3 In non-immunized women LBR LH normal LBR LH elevated	56/153 (36.6%) 22/153 (14%) 9/23 (39%) 6/14 (42%) NS			No significant relationship between pregnancy outcome and LH concentrations	
Chakraborty P, Goswami SK, et al. PLoS One. 2013;8(5):e64446.	CCS	**- Selection bias** **-Assessment** **- Confounding** **+ Statistical analysis----------** ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-) study population and comparison is not suited**	Patients and controls are all REPL patients (≥2 first trimester) with no reason for REPL due to uterus or chromosomal abnormalities, hypothyroid, DM, APS, infections (toxopl. CMV, HSV) Retrospective design N=126 cases PCOS (R'dam criteria)	Insulin resistance = HOMA2-IR>2.1 HOMA2-IR = fasting insulin x fasting glucose/ 22.5	71/126 (56.3%) 8/117 (6.8%)	Sensitivity 80% specificity 62% ROC-AUC 0.62	significantly higher BMI, LH/FSH ratio, post-prandial blood sugar, HOMA-IR and homocysteine levels in women with PCOS compared to	In REPL and PCOS patients REPL IR and HHC mediated	India

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		for the scope of this guideline	N=117 non-PCOS controls matched for age				those without PCOS. There was no difference in prolactin, TSH, or fasting blood sugar		
					Incidence of HHcy and IR was 70.63% (n=89) and 56.34% (n=71), respectively, in RPL-affected PCOS population which was significantly higher ($p<0.04$; $p<0.0001$) when compared to the non-PCOS set (HHcy: 57.26%; IR: 6.83%). A probabilistic causal model evaluated HHcy as the strongest plausible factor for diagnosis of RPL.			
Chakraborty P, et al.. PloS one 2013;8: e74155.	prospective observational study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	women with history of RPL, who were treated with low dose acetylsalicylacid (ASA) during their last spontaneous pregnancy. the patients were stratified: presence or absence of PCOS was the initial dividing criteria, while subsequent stratification was based on plasma levels of homocysteine (Hcy), IR, and body mass index (BMI).	187 women finally received LMWH at a prophylactic dose of 2500 IU sc everyday in concomitant with ASA 5 mg/day since foetal cardiac activity was observed by USG and continuing up to 12 weeks of gestation. all patients also received luteal support in the form of intravaginal micronised progesterone (100 mg, twice daily), vitamin B12 and folic acid (10 mg/day) as a part of antenatal care, and metformin (500 mg/twice a day), for those diagnosed with IR, continuing until term.	In Aspirin treated women: pregnancy salvage (uneventfull pregnancy to 36 week) was 6.17% in women with HHCy (n=81) (>12µmol/L), compared to 54.9% in women with no HHcy (n=255) (OR 0.27(0.08-0.80) In LMWH Aspirin treated women: pregnancy salvage was 84.21 % in women with HHCy (>12µmol/L) (n=76), compared to 54.9% in women with no HHcy (n=111) (OR 1.55 (1.29-1.88)		Aspirin and low-molecular weight heparin combination therapy effectively prevents recurrent miscarriage in hyperhomocysteinemic women	Treatment study, multiple treatments

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Cocksedge KA, Saravelos SH, et al. Hum Reprod. 2008;23(4):797-802. (18263637)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** **X Unacceptable (-) no controls** **Study relevant for prognostic value**	Total cohort N=571 Relevant for this prognostic study N=437 RPL≥3 No abnormalities APS, uterine, karyotype N=263 new pregnancy and known data on androgens	Free androgen index (T/SHBG)*100 Elevated > 5 Normal ≤ 5 Misc. Rate in FAI elevated vs. normal	49/437 (11%) 23/34 (68%) vs. 91/229 (40%)			In women with RPL an elevated FAI a prognostic factor for a subsequent miscarriage. Even a stronger predictor than maternal age> 40 y or ≥6 previous RPL	
Craig LB, Ke RW, et al. Fertil Steril. 2002;78(3):487-90. (12215322)	CCS	? Selection bias - Assesment - Confouding - Statistical issues ---------- ---------- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	N=74 women history REPL ≥2 <20wks Exclusie: abnormalities in hysteroscopy/HSG, thyroid function, karyotyping, progesteron, LAC, AC, APS, bacterial vaginosis N=74 Parous women with no REPL Matching on: age, BMI and race	FI FG IR = FI>20uU/mL or FG/FI<4.5 HOMA-IR	↑ = 20/74 (27%) 7/74 (9.5%) OR (95%CI) 3.6 (1.4-9.0) ↑			Women with REPL have an increased prevalence IR compared to matched controls	
Creus M, et al. Clinical chemistry and laboratory medicine : 2013;51: 693-699.	Case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	60 consecutive patients with >/= 3 unexplained RM and 30 healthy control women having at least one child but no previous miscarriage spain	Plasma Hcy levels, MTHFR gene mutation, red blood cell (RBC) folate and vitamin B12 serum levels RESULTS: studied. CONCLUSIONS: In the present study	No significant differences were observed neither in plasma Hcy levels, RBC folate and vitamin B12 serum levels nor in the prevalence of homozygous and heterozygous MTHFR gene mutation between the two groups			RM is not associated with hyperhomocysteinemia, and/or the MTHFR gene mutation.	

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D'Uva M, et al. Thrombosis journal 2007;5: 10.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	20 RPL 20 patients with unexplained female sterility 20 healthy women (selected)	Hcy Vit B12 Folate	median fasting total plasma homocysteine concentration was 19.2 ± 6.14 µM for RPL, while was 21.05 ± 8.78 µM for patients with unexplained sterility, vs 7.85 ± 3.31 µM of control group (p< 0.05). no significant differences were found in the levels of vitamin B 12 in the three groups, reduced folate concentrations were found in women with unexplained female sterility and RPL (p < 0.05 vs control group)				Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from Southern Italy: a preliminary report.
Govindaiah V, et al. Clin Biochem 2009;42: 380-386.	case-control study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	140 RPL (≥3PIs) 140 couples with normal reprod history	total plasma homocysteine, C677T MTHFR polymorphism and DNA damage The 95 percentiles of homocysteine levels in male and female controls were 19.6 µmol/L and 14.0 µmol/L- used as threshold for HHcy	Maternal [mean: 11.6+/-5.0 versus 8.6+/-4.2 micromol/L, OR 4.48] and paternal [mean: 19.6+/-9.5 versus 14.2+/-7.4 micromol/L, OR: 6.92] HHCycysteinemia, paternal age [OR: 1.16], paternal MTHFR 677T allele [OR: 2.30] and DNA damage were found to increase the risk for RPL. DNA damage showed positive correlation with plasma homocysteine and MTHFR 677T allele. Mean maternal homocysteine levels and mean paternal homocysteine levels were higher in cases than controls with 4.48 (95% CI: 2.30–8.70) and 6.92 (95% CI: 3.90–12.29) fold increased risk for RPL (p<0.0001). There was a correlation between maternal and paternal HCY levels with a correlation coefficient of 0.19.			Parental hyperhomocysteinemia, paternal age, paternal C677T MTHFR polymorphism and DNA damage are risk factors for RPL. DNA damage showed positive correlation with plasma homocysteine and MTHFR 677T allele	apart from MTHFR genotype, some genetic or non-genetic determinant also plays a role in increasing the homocysteine and might play an important role in the etiology of RPL The risk associated with paternal HHcycould be due to its effect on sperm quality by increasing DNA damage.
Gurbuz B, Yalti S, et al. Arch Gynecol	CS	☐ Selection bias controls are not discussed in methodology ☐Performance bias	58 unexplained RPL Control group s:	Day 3 serum levels of FSH, E2 and elevated FSH: LH ratios (>3.6)		FSH conc similar E2 and FSH:LH ratio elevated in		DOR should be considered in the workup of RPL.	role of DOR in unexplained RPL evidence for

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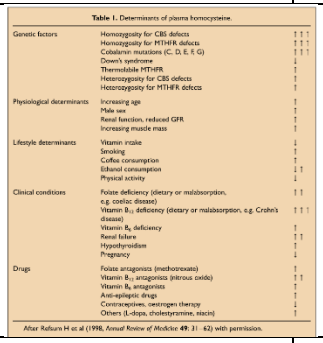
Obstet. 2004;270(1): 37-9.		☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** X **Unacceptable (-)**	22 explained RPL 27 controls (NOT DISCUSSED IN METHODS ??° Retrospective			unexplained RPL (p=0.006 and p=0.018) percentage of women with elevated FSH and/or E2 levels significantly higher in the unexplained RPL			elevated levels of hormones Control groups : relevant?? Clearly described??
Hague WM. Best practice & research Clinical obstetrics & gynaecology 2003;17: 459-469.	Review	NA		 Table 1. Determinants of plasma homocysteine. Genetic factors Homozygosity for CBS defects ↑↑↑ Homozygosity for MTHFR defects ↑↑↑ Cobalamin mutations (C, D, E, F, G) ↑↑↑ Down's syndrome ↓ Thermolabile MTHFR ↑ Heterozygosity for CBS defects ↑ Heterozygosity for MTHFR defects ↑ Physiological determinants Increasing age ↑ Male sex ↑ Renal function, reduced GFR ↑ Increasing muscle mass ↑ Lifestyle determinants Vitamin intake ↓ Smoking ↑ Coffee consumption ↑ Ethanol consumption ↓ ↑ Physical activity ↓ Clinical conditions Folate deficiency (dietary or malabsorption, e.g. coeliac disease) ↑↑ Vitamin B_{12} deficiency (dietary or malabsorption, e.g. Crohn's disease) ↑↑↑ Vitamin B_6 deficiency ↑ Renal failure ↑↑ Hypothyroidism ↑ Pregnancy ↓ Drugs Folate antagonists (methotrexate) ↑ Vitamin B_{12} antagonists (nitrous oxide) ↑↑ Vitamin B_6 antagonists ↑ Anti-epileptic drugs ↑ Contraceptives, oestrogen therapy ↓ Others (L-dopa, cholestyramine, niacin) ↑ After Refsum H et al (1998, *Annual Review of Medicine* **49**: 31–62) with permission.	Homocysteine and pregnancy. Narrative review Used in introduction only				
Hofmann GE, Khoury J, et al. Fertil Steril. 2000;74(6):1192-5.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** **X Unacceptable (-) controls infertile**	44 RPL 648: non RPL (infertile) Comparability : RPL were younger Retrospective	Clomiphene citrate challenge test (CCCT) FSH day 3 Day3E2 FSH day 10 Delivery rates (1y FU)		CCCT : Abnormal in 8/44 18% of RPL and 117/648 18% of controls DAY 3 FSH : lower in RPL (8.9 ± 7 vs. 11 ± 9 mIU/mL) DAY 3 E2and DAY 10 FSH: similar Delivery rates : similar for RPL and control; 36% and 37% resp in RPL and controls with normal CCCT	Incidence of DOR in RPL 18%	Ovarian reserve screening should be considered in the work-up of RPL before initiation of anticoagulant or immunotherapy.	Similar to infertile women, ovarian reserve testing can be used as a prognostic test.

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						poor in RPL or control with abnormal CCCT : 0/8 and 5/117 abnormal CCCT indien FSH> 25 13/36 36% vs. 0/8 0%			
Homburg R. Best Pract Res Clin Endocrinol Metab. 2006;20(2):281-92.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	good review				pcos increase miscarriage consistently risk worse if: obese, hyperinsulinaemic, increased PAI-1, high LH	good	
Ispasoiu CA, Chicea R, et al. Int J Endocrinol. 2013;2013:576926.	CCS	− Selection bias ?Assessment X Confounding +/−Statistics ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	N=65 idiopathic REPL (≥2 < 20 wks) uterus or chromosomal abnormalities, hypothyroid, hyperprolactinaemia, DM, PCOS, APS, genetic thrombophilia N=53 controls 1 live birth no PL	IR = HOMA-IR = fasting glucose x fasting insulin/ 405 Fasting insulin Fasting glucose	Higher Higher Lower	No additional statistics, no use of a cut off value		Fasting insulin and IR are higher in REPL than women without REPL and may be involved in the etiology of REPL.	**Limited statistics**

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Jordan J, Craig K, et al. Fertil Steril. 1994;62(1):54-62. (8005304)	CCS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Test population: 19 women (infertile/RPL) n=3 RPL 15 normal women (regular menses no additional comments)	tests performed in the same menstrual cycle: daily reproductive hormone levels, daily preovulatory follicle size, late luteal endometrial biopsies, and BBT charts. P levels (single and multiple) were used in an attempt to predict which patients had low integrated P levels.		Progesterone midluteal <80 ng*day/ml low sensitivity and/or specificity levels were found for the following tests: BBT charts, luteal phase length, and preovulatory follicle diameter.	1/3 (33,3%) vs. 2/15 (13%) NS	Best test for LPD is a midluteal phase single serum P level < 10 ng/mL or the sum of three serum P levels that is < 30 ng/mL. endometrial biopsy is a second line test	CRITERIA FOR LPD **Study conducted to evaluate a diagnostic method not to determine a prevalence/incidence** **Less information about controls**
Kaur R, Gupta K. Int J Appl Basic Med Res 2016;6: 79-83.	**SR**	**NA**	RPL	Endocrine dysfunction					Narrative review, only used in introduction
Kazerooni T, Ghaffarpasand F, et al. J Chin Med Assoc. 2013;76(5):282-8.	comparative study	☐ Selection bias no clear description control group ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Primary research question: association RPL and thrombophilia in patients with PCOS N=60 RPL≥ 3 < 20 wks (group 2) No PCOS, APS, no abnormalities uterine, karyotype N=60 healthy controls no RPL (group 4) Matched on age, BMI and parity	FI FG Insulin sensitivity check index (1/log(FI)+log(FG))		15.3 +-3.8 vs. 14.3 +-2.9 NS 76.3 +- 8.7 vs. 77.3 +- 5.8 NS 0.33 +- 0.008 vs. 0.33 +- 0.013 NS		Iran In women with RPL and PCOS LH, FSH and LH/FSH ratio are significantly elevated compared with RPL women without PCOS or healthy controls	
				Testosterone DHEAS		0.49 +-0.32 vs. 0.43 +- 0.3 NS 208.3 +- 36.8 vs. 216.8 +- 24.9 NS			
				LH FSH LH/FSH		9.42 +-1.2 significantly higher than 4.32 +-1.4 and 4.65 +- 0.9 6.31 +-1.5 higher than 4.98 +- 1.2 and 5.23 +- 1.4 1.48 +- 0.64 significantly higher than 1.37 +-0.83 and 0.89 +- 0.72			

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				homocysteine (Hcy) SERUM LEVELS	Patients in Group 1 had significantly higher levels of Hcy (p = 0.036) compared to group 3 Hcy levels Group 1: 12.4 ± 1.6 Group 2: 7.3 ± 1.1 (sign vs GR 1) Group 3: 9.65 ± 0.9 (sign GR 1-2) Group 4: 6.7±1.9 (sign vs GR 1-3)	Hyperinsulinemia, hyperandrogenemia, hypofibrinolysis, and hyperhomocysteinemia as well as APCR and factor V Leiden mutations are associated with RPL in patients with PCOS.	Correlation between thrombophilia and recurrent pregnancy loss in patients with polycystic ovary syndrome		
Ke RW. Obstet Gynecol Clin North Am. 2014;41(1):103-12. (24491986)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	REVIEW GOOD				PCOS associated with RM, WORSE WITH PAI-1,? Worse IR		
Lata K, Dutta P, , et al. Endocrine connections. 2013;2(2):118-24. PMID: 23802061	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	RM cases (100 pregnant and 25 non-pregnant) 2 or more consec Misc 21 and 35 years Controls: 100 pregnant women, no history of misc	Thyroid autoimmunity (TPOAb+ >34 U/ml) , subclinical hypothyroidism maternal and foetal complications (spontaneous abortion, hypertensive complications, gestational diabetes, intrahepatic cholestasis of pregnancy, preterm labour, IUGR, postdatism, preterm premature rupture of membranes and post partum	31% 18% in controls	subclinical hypothyroidism: 52% in TPOAb+ vs 16% in TPOAb- group (P=0.0002) TPOAb titre significantly higher in hypothyroid vs euthyroid RM (P=0.016) no difference in prevalence of miscarriage between hypothyroid and euthyroid individuals in TPOAb+. The odds ratio of having miscarriage was increased (5.62) when TPOAb+ with elevated TSH compared with normal values. no difference in the prevalence of miscarriage or obstetric outcomes between Rm and controls			Case-control maternal and foetal complications: influenced by the effect of levothyroxine (L-T4) therapy ??

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				haemorrhage / prematurity, APGAR score, birth weight and congenital malformation)		irrespective of TPO-status. !! TPOAb+ patients were treated with 25 mg L-T4 and titrated according to TSH at the time of recruitment. Patients with subclinical hypothyroidism were treated as deemed necessary.			
Lee GS, et al. Obstet Gynecol Sci 2016;59: 379-387.	cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)** **NO CONTROL GROUP**	178 RPL mean age 34.0+/-4.3 yrs mean number of Pls 2.69+/-1.11 (range, 2 to 11). Among of 178 women, 77 women were pregnant. After management of those women, LBR 84.4% and mean gestational weeks was 37.63+/-5.12.	chromosomal analysis, TSH, prolactin, blood glucose, PAI-1, natural killer cell proportion, ACA, aPLa, LA, anti-beta2GP-1 antibodies, ANA, protein C, protein S, antithrombin III, homocysteine, MTFHR gene, factor V Leiden mutation, and hysterosalphingography/hysteroscopic evaluation.	Anatomical cause (13.5%), chromosomal abnormalities (5.6%), and endocrine disorders (34.3%) were observed in RPL women Homocysteine >12 µmol/L : RPL: 4/178 (2.2%) Prim RPL : 3/145 (2.1%) Sec RPL: 1/33 (3.0%) 2PLs : 3/99 (3.0%) ≥ 3 Pls : 1/79 (1.3%)			Immunological factor including autoimmune and alloimmune disorders was most common etiology of RPL.	No controls
Li TC, Spuijbroek MD, et al. Bjog. 2000;107(12):1471-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X ☐ **Acceptable (+)** **Unacceptable (-)** **!! no controls**	No controls total cohort RPL N=144 N=106 women with REPL ≥3 (first trimester) No abnormalities: AC, LAC, karyotyping, HSG, coagulation	TSH > 5.0 mIU/l TSH<0.3 mIU/l	1/106 (1%) 0/106 (0%)			Delayed endometrium is associated with significant lower P levels	
				Day 3-5 PRL (>660 mIU/l)	3-122 (2.5%)				
				Endometrial biopsy Midluteal P<30 nmol/L		33/122 (27%) vs. 2/18 (11%) NS 8/24 (33,3%)			
				Testosterone > 3 nmol/L Androstenedione >10.2 nmol/L SHBG < 25 nmol/L	0/110 (0%) 3/90 (3.3%) 10/89 (11.2%) 13/89 (14.6%)				

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				Free androgen index >4.0					
				PCOS morphology LH >10 IU/L serumj Urinary hypersecretion LH LH/FSH ratio ≥3	8/102 (7.8%) 7/92 (8%) vs. 1/14 (7%) NS 0/38 (0%) vs. 0/8 (0%) 2/107 (1.9%)				
Li W, Ma N, et al. J Obstet Gynaecol. 2013;33(3):285-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-) no controls**	No controls N=177 women with REPL ≥3 (1rst trimester) No abnormalities: APA, karyotyping, HSG, coagulation	PRL (>660 mIU/l)	3/177 (1.7%)				
Liddell HS, Sowden K, et al. Aust N Z J Obstet Gynaecol. 1997;37(4):402-6.	CCS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Total cohort N=73 RPL ≥3 screened for PCOS morphology. N=17 PCOS, new pregnancy and no treatment in pregnancy N=31 no PCOS, new pregnancy and no treatment in pregnancy	PCOS morphology LBR and miscarriage rate LBR and miscarriage rate	26/73 (36%)	14.17 (82%) & 3/17 (18%) 25/31 (81%) & 6/31 (19%)		PCOS morphology in women with RPL does not predict a subsequent poor pregnancy outcome	**Relevant for prognostic value**

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Marai I, et al. Am j reprod immunol . 2004;51(3):235-40. PMID: 15209393	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	38 RM 20 infertility, but no misc 28 control parous women	Autoantibody Panel [antithyroglobulin (aTG), antithyroid peroxidase (aTPO), anticardiolipin (aCL), antiphosphatidyl-serine (aPS), antiprothrombin antibodies (aPT), anti-beta 2 glycoprotein 1 (ab2GP1), and anti-ENA].	Anti-TPO was the only antibody to be associated with RM (P = 0.01). 21% in RM vs 0% in infert 'aTG + aTPO + anti-ENA' panel: 31.6% in RM vs 0% in infert (P=0.001)				
Maryam K, Bouzari Z, et al. BMC Res Notes. 2012;5:133. (22405326)	CCS	? Selection bias – Assessment – Confounding + statistics ☐ No bias detected ---------- ☐ **High quality (++)** **x Acceptable (+)** ☐ **Unacceptable (-)**	N=50 cases ≥3PL <24 wks No DM, no PCOS N=50 controls 1 live birth 0-1 PL Matched age, BMI, no DM, no PCOS Iran	Insulin resistance = Fasting insulin≥20 mu/mL OR Fasting glucose to fasting insulin ratio <4.5	12/50 (24%) 4/50 (8%)	OR (95% CI) 3.6 (1.1-12.3)		In women with REPL IR is high. It is recommended to measure fasting glucose and fasting insulin in all REPL women	**Description study population is unclear**
Moini A, et al. Gynecol Endocrinol 2012;28: 590-593.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	184 women with history of RPL, of which 92 of them were diagnosed with PCOS and 92 patients were without known PCOS.	prevalence of thrombophilic disorders	70.7% of PCOS + RPL had thrombophilic disorders. The prevalence of protein C deficiency was significantly higher in PCOS+RPL compared to the non-PCOS+RPL group (21.7% vs. 10.9%, p = 0.04). Trend toward higher prevalence of protein S deficiency in PCOS group compared to controls (23.9% vs. 13%, p = 0.05). The prevalence of other thrombophilic disorders such as antithrombin III deficiency, homocysteine elevation, antiphospholipid antibody and Factor V Leiden was comparable between groups.			The prevalence of thrombophilic disorders was more common in PCOS women than the normal group	
Nardo LG, Rai R, et al. Fertil Steril. 2002;77(2):3	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias	N=344 ≥3RPL <12 wk no abnormalities: karyotype, APS, uterine	Day 8 testosterone high vs. normal LBR		192/344 (56%) Vs. 152/344 (44% abstract, 51.5% txt) Conclusion:NS		Pregnancy outcome in RPL not associated with T conc.	

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48-52.		☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**		Day 8 LH serum High > 10 IU/L Low < 4 IU/L PCOs LBR PCOS vs no PCOs LBR LH	32/344 (9.3%) 70/344 (20.4%) 174/344 (50.6%) 58.6% vs 50% NS NS			Not a significant relationship between pregnancy outcome and LH concentrations	**Prognosis /Prediction study** **no controls**
Nelen WL, Blom HJ, Steegers EA, den Heijer M, Eskes TK. Fertility and sterility 2000;74: 1196-1199.	meta-analysis	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	10 case-control studies After load = after methionine loading	Fasting Hcy (3 studies) (403 cases- 249 contr) Afterload Hcy (4 studies) (351 cases- 229 contr)	OR 2.7 (1.4-5.2) OR 4.2 (2.0 to 8.8) 3 studies found HHcy, fasting or afterload, to be a significant risk factor for REPL and 2 did not.	hyperhomocysteinemia = risk factor for REPL			
Ogasawara M, Kajiura S, et al. Fertil Steril. 1997;68(5):806-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** X **Unacceptable (-)**	197 RM patients excl. APS, uterine anomalies, endocrine disorders	prepregnancy P, Ez , and P/E2 ratio LPD = midluteal P<10 ng/ml	46 (23.4%) LPD without other endocrine abnormalities	38 (19.3%) suffered another abortion; 20.5% (31/151) of LPD-negative and 15.2% (7/46) of LPD-positive NS No difference in E2 or P/E2 ratio between those with another PL and those without PL.	midluteal serum P as a marker of a luteal phase defect	P, E2, and the P/E2 ratio may not predict future pregnancy loss in RM	**Predictive study** **No controls**
Okon MA, Laird SM, et al. Fertil Steril. 1998;69(4):682-90.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---	N=42 RPL ≥3 No APS no abnormalities karyotype, uterine N=18 fertile controls without RPL	Andostenedione Testosterone SHBG T/SHBG ratio Endometrial biopsy		↑ ↑ = =		T and androstenedione ↑ in women with RPL, which may have a	

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		☐ **High quality (++)** ☒**Acceptable (+) for PCOs morphology** ☒ **Unacceptable (-) due to absence controls for other variables (-)**		LH>10 IU/L PCOS morphology PCOS morphology and/or endocrinology	5/43 (11.6%) 7/43 (16.3%) vs. 0% NS 10/43 (23.3%)			detrimental effect on endometrial function (PP14↓ and endometrial biopsy)	
Ota K, , et al.. Eur J Immunol. 2015;45(11): 3188-99.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	recurrent PL	1,25-Dihydroxy-vitamin D3	CD69(+) activating receptor expression on NK cells was significantly decreased by incubation with 1,25(OH)2 D3 in a dose-dependent manner, while CD158a and CD158b inhibitory receptor expression was upregulated. The degranulation marker CD107a was significantly downregulated on NK cells following incubation with 1,25(OH)2 D3 . NK-cell conjugation with K562 target cells was not affected by 1,25(OH)2 D3 ; however, depolarization of perforin granules in conjugated NK cells was significantly increased. TLR4 expression on NK cells was significantly decreased and TNF-alpha and IFN-gamma production was significantly reduced by 1,25(OH)2 D3 through interference with NF-kappaB.			Our results suggest 1,25(OH)2 D3 has immune regulatory effects on NK cell cytotoxicity, cytokine secretion and degranulation process as well as TLR4 expression	
Ota K, Dambaeva S, et al. Hum Reprod. 2014;29(2):208-19.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☒ **Unacceptable (-) no controls**	N=133 RPL ≥3 < 20 wks USA	Low vitamin D (<30 ng/ml)	63/133 (47.4%)			Association between low vitamin D and APS & TPO	Study aim: relation between vit D deficiency and auto- and cellular immune abnormalities
Pils S, et al. PLoS One 2016;11: e0161606.	**CS**	Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **X Acceptable (+)**	78 explained RPL 66 idiopathic RPL	Anti-Mullerian hormone, basal follicle stimulating hormone, luteinizing hormone, estradiol, and age.	AMH and estradiol were significantly lower in women with idiopathic RPL (median 1.2 ng/ml, IQR 0.6-2.1, and median 36.5 pg/ml, IQR 25.8-47.3, respectively) than in women with explained RPL (median 2.0 ng/ml, IQR 1.1-2.7, and median 42.5 pg/ml, IQR 32.8-59.8, respectively; p<0.05). Optimized cut-off values for the prediction of idiopathic RPL were <39.5 pg/ml for estradiol (sensitivity: 63.3%, 95% CI: 50.9-75.1; specificity: 56.4%, 95% CI: 44.7-67.6) and <1.90				

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		Unacceptable (-)			ng/ml for AMH (sensitivity: 72.7%, 95% CI: 60.4-83.0; specificity: 52.6%, 95% CI: 40.9-64.0).				
Prakash A, Li TC, et al. Fertil Steril. 2006;85(6):1784-90.	other	X Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** **X Acceptable (+)** **Unacceptable (-)** **no clear study group (explained and unexplained mixed)**	34 RM ≥ 3 ab 1rst trimester 17 unexplained 6 APS 11 luteal phase defect 10 controls, no miscarriage + normal menstrua cycle) Similar age and length of follicular phase	Doppler assessment of blood flow to the follicle and the endometrium. (day 8-9) serum concentrations of AMH, inhibin B, FSH, LH, E2 and P (day 2-3) FSH, LH, E2 and P (day 8-9)	RM vs controls: No difference in doppler test: endo-metrial thickness, resistance indices, and systolic blood flow velocity for subendometrial and perifollicular vessels. Day 2-3: basal P level: significantly higher control No difference for AMH, inhibin B, FSH, LH, E2 (day 2-3) No difference for FSH, LH, E2 and P (day 8-9) correlation between ovarian and pituitary hormones was absent in RM (present in controls)			possibility of subtle derangements of the feedback mechanism responsible for regulation of follicle development in women with RM	RM vs healthy women; no differences in FSH, LH, E2
				Doppler assessment of blood flow to the follicle and the endometrium. (day 8-9) serum concentrations of AMH, inhibin B, FSH, LH, E2 and P (day 2-3) FSH, LH, E2 and P (day 8-9)	RM vs controls: No difference in doppler test: endo-metrial thickness, resistance indices, and systolic blood flow velocity for subendometrial and perifollicular vessels. Day 2-3: basal P level: significantly higher control No difference for AMH, inhibin B, FSH, LH, E2 (day 2-3) No difference for FSH, LH, E2 and P (day 8-9) correlation between ovarian and pituitary hormones was absent in RM (present in controls)				

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Puri M, et al Journal of perinatal medicine 2013;41: 549-554.	case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	107 women with 3 or more consecutive unexplained recurrent pregnancy losses and 343 women with 2 or more successful and uncomplicated pregnancies North Indian women	Plasma homocysteine, serum folate and vitamin B12 MTHFR C677T detection	MTHFR genotypic distribution among cases and controls showed no significant difference (P=0.409). However, MTHFR C677T polymorphism was found to be significantly associated with increased homocysteine in the case group (P=0.031). Hyperhomocysteinemia and vitamin B(1)(2) deficiency were found to be significant risk factors for recurrent pregnancy loss (RPL) (OR=7.02 and 16.39, respectively). Folate deficiency was more common in controls (63.47%) as compared to the case group (2.56%).			Low vitamin B12 increases homocysteine, specifically among T allele carrying case mothers, suggesting T allele is detrimental with B(1)(2) deficiency. The study emphasizes the importance of vitamin B(1)(2) in the prevention of RPL in North Indian women.	
Quere I, et al.. Fertility and sterility 2001;75: 823-825.	Non controlled study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	25 consecutive hyperhomocysteinemic patients, ages 20–37 years, who had no biological children, each patient having 3–5 episodes between the 8th and 16th week of amenorrhea	Treatment : 1-month high-dose folic acid, 15 mg daily, and vitamin B6, 750 mg daily	22 patients initiated a pregnancy during the 3-month period after the normalization of their Hcy 20 live births (4 preterm)				Treatment study
Rai R, Backos M, et al. Hum Reprod. 2000;15(3):6	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias	N=2199 RPL ≥3 Of them N=486 no abnormalities APS, uterine, karyotype	LBR Testosterone >3 nnmol/L		69.2% vs. 66% NS		Testosterone level is not predictive of pregnancy loss in RPL	**no controls** **Study on prognosis**

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12-5.		☐ No bias detected ---------- ☐ **High quality (++)** X ☐ **Acceptable (+)** **Unacceptable (-)**	Spontaneous pregnant and no treatment during pregnancy Overlap with the N=500 from Clifford et al. 1994	PCOS: ovaria > 9 ml, ≥10 cysts 2-8 mm LBR PCOS vs. no PCOS LBR LH>10 IU/L vs ≤10		895/2199 (40.7%) 142/233 (60.9%) vs. 148/253 (58.5%) NS 38/53 (72%) vs. 252/433 (58%) NS		PCOS morphology and high LH are not predictive of pregnancy loss in RPL	
Rao VR, Lakshmi A, et al. Indian J Med Sci. 2008;62(9):357-61.	Case control	– Selection bias – no major bias in assessment or confounding factors X No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	N=163 ≥ 2REPL ≤12 wk no cause for REPL Hypothyroid based on T3, T4, TSH Only normal levels presented no cut off values for hypothyroid	N=170 age matched controls ≥1 succesful pregnancy no miscarriages	Cases hypothyroid 7/163 (4.3%) Controls 1/170 (0.6%)	Not calculated		Hypothyroid significant related to REPL Diagnosis may Improve a next pregnancy outcome	
Regan et al. Lancet 1990;336: 1141-1144.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ High quality (++) X Acceptable (+) ☐ Unacceptable (-)	Total study population N=193; women with a spontaneous and regular cycle Micture of infertility, RPL and nulliparous Subpopulation to be studied: N=30 RPL ≥3 N=17 no previous PL and at least one successful pregnancy	Elevated LH serum (≥10 IU/L) LBR elevated LH vs. normal LH	9/30 (30%) vs. 1/17 (1.8%) P<0.05 2/6 (33%) vs. 15/16 (71%) (p<0.05)			Association between prepregnant elevated LH and pregnancy loss	Including prognostic study
Romero ST, et al. J Obstet Gynaecol Res. 2016;42: 763-768..	Case-control study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	117 women with unexplained RPL, defined as two or more pregnancy losses with no more than one live birth, 117 age-matched controls None had a diagnosis of pre-gestational or gestational diabetes	maternal serum fructosamine (a marker of glycemic control)	Fructosamine was higher in women with RPL (224.1 ± 28.79 µmol/mL) compared with controls (188.9 ± 19.3 µmol/mL, P < 0.001). This difference persisted when RPL patients and controls were stratified by BMI. The proportion of women with elevated fructosamine considered diagnostic of diabetes (>285 µmol/L) was similar in RPL patients and controls.				
Sagle et al. BMJ 1988;		☐ Selection bias ☐Performance bias	N=56 RPL ≥3	urinary pregnanediol – 3 alpha-glucuronide (metabolite	NS				

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297:1027		☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ High quality (++) X Acceptable (+) ☐ Unacceptable (-)	N=11 parous volunteers no RPL	progesterone) comparable in cases and controls					
Shah D, Nagarajan N. Indian J Endocrinol Metab. 2013;17(1):44-9.	Narrative review	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Studies have shown that surgical excision of corpus luteum (luteoctomy) before 7 weeks of gestation, uniformly precipitated an abrupt decrease in serum progesterone concentration followed by miscarriage.[5] The estimated onset of placental steroidogenesis occurs on the fifth gestational week. Transfer of luteal support to placenta occurs between seventh and ninth week and progesterone production from both sources continues to varying extent during the time period known as luteal-placental shift.[6] Progesterone secretion by the corpus luteum is required absolutely for the success of early human pregnancy. Progesterone not only supports the endometrial growth but also improves the blood flow and oxygen supply by increasing the nitric oxide production.[7,8] by the utero relaxing effect.[9] It keeps the myometrium quiescent They also potentially sustain the survival of the embryo by shifting the immune system towards production of T-helper (Th2) response.[10,1					REVIEW – non-systematic Used for information on progesterone secretion
Steegers-Theunissen RP, et al. Obstetrics and gynecology 2004;104: 336-343.	Case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	postpartum patients who had a history of vascular-related pregnancy complications. pregnancy-induced hypertension (n=37), pre-eclampsia (145), HELLP syndrome (105), recurrent early pregnancy loss (569), abruptio pla centae (135), intrauterine growth restriction (145), and intrauterine fetal death (105) The controls were postpartum patients who were comparable with the patient groups with regard to social class, geographic area, and age.		Hyperhomocysteinemia was associated with an approximately 2-fold to 3-fold increased risk for pregnancy-induced hypertension, abruptio placentae, and intra-uterine growth restriction. These associations lost their significance after adjustment for time interval and maternal age. Elevated fasting Hcy (>15µmol/l) and Hcy afterload (>51 µmol/l) were not associated with REPL (fasting Hcy: OR 1.2; 95% CI 0.6-2.6; Afterload Hcy: OR 1.2; 95% CI 0.5-2.7).		HHcy and vitamin deficiencies are largely determined by the interval between delivery and postpartum investigation and maternal age. These findings are inconsistent with earlier reports suggesting that hHHcy in most non-pregnant women is an important risk factor for vascular-related	Hyperhomocysteinemia, pregnancy complications, and the timing of investigation.

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								pregnancy complications.	
Stephenson MD. Fertil Steril. 1996;66(1):24-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X ☐ **Acceptable (+)** **Unacceptable (-)**	N=197 ≥3 REPL <20 wk consecutive and aneuploid abortions excluded	Serum TSH	Hypothyroid 6/197 (3.0%)	Not calculated	No info		No controls
Stephenson MD. Fertil Steril. 1996;66(1):24-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X ☐ **Acceptable (+)** **Unacceptable (-)**	N=197 ≥3 REPL <20 wk consecutive and aneuploid abortions excluded	Prevalence of endocrine factor: LPD = 2 late luteal phase endometrial biopsies with maturation delay of > 3 days	20% 39/197 34 LPD, 3.5% genetic 1/197 infectious 16% anatomical 20% autoimmune 84/197 unexplained		Frequency of etiologic factors		No controls available
Thangaratinam S, et al .: of evidence. BMJ 2011;342:d2616.	meta-analysis	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	30 articles with 31 studies (19 cohort and 12 case-control) - 12 126 women assessed the 5 studies with 12 566 women	thyroid autoantibodies Studies varied in the frequency and timing of the autoantibody testing, ranging from testing before pregnancy, in early pregnancy, and after delivery or miscarriage. The commonest threshold concentration of thyroid peroxidase for a diagnosis of positive thyroid autoantibodies was >100 U/ml.	association with miscarriage association in women with RPL association with preterm birth Effect of treatment	28 showed a positive association between thyroid autoantibodies and miscarriage. Meta-analysis of the cohort studies showed more than tripling in the odds of miscarriage with the presence of thyroid autoantibodies (odds ratio 3.90, 95% CI 2.48 to 6.12; P<0.001). For case-control studies the odds ratio for miscarriage was 1.80, 1.25 to 2.60; P=0.002) 13 studies (3 cohort, 10 case-control): The odds of miscarriage with thyroid autoantibodies was increased for women with recurrent miscarriages (4.22, 0.97 to 18.44; P=0.06) (heterogeneity I^2 =75%) doubling in the odds of preterm birth with the presence of thyroid autoantibodies (2.07, 1.17 to 3.68; P=0.01). 2 randomised studies: Both showed a fall in miscarriage			Association between thyroid autoantibodies and miscarriage and preterm birth

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					with levothyroxine on miscarriage	rates, and meta-analysis showed a significant 52% relative risk reduction in miscarriages with levothyroxine (relative risk 0.48, 0.25 to 0.92; P=0.03). One study reported on the effect of levothyroxine on the rate of preterm birth, and noted a 69% relative risk reduction (0.31, 0.11 to 0.90).			
Ticconi C, et al. Am j reprod immunol. 2011;66(6):452-9. PMID: 21623997	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	160 women with RM (2 or more consec Misc) 100 healthy women (at least 2 uncomplicated pregnancies at term and no history of miscarriage)	antithyroid autoantibodies (ATA) :thyreoglobulin (TG-Ab), thyroid peroxidase (TPO-Ab) and TSH receptor (TSHr-Ab)	Prevalence: RM vs controls : ATA: 28.75% vs 13% (p<0.05) TG-Ab : 22.5% vs 5% (p<0.05) TPO-Ab: 19.37% vs 8% (p<0.05) TSHr-Ab: 1.87% vs 2% (ns) No diff between 2Misc or >3 misc. 96.3% of RM and 93% of controls were euthyroid Positivity of other autoantibodies (mostly ANA, also dsDNA, AMA, celiac,…) ATA+ vs ATA- : 91.3% vs 53.1% (P<0.005) No diff 2 or more than 3 misc.		ATAs, particularly TG-Ab, are associated with RM and could be an expression of a more general maternal immune system abnormality leading to RM. ATA could have a role in RM irrespective of TSH	Case-control
Triggianese P, et al . Am J Reprod Immunol. 2015;73(1):56-65.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	primary infertility (n=31) and recurrent spontaneous abortion (n=69) fertile controls (n=30)	Prolactin and natural killer cells: basal PRL (bPRL), peak-time PRL (Pt-PRL), PRL absolute increase (aDPRL, [peak minus basal]), PRL relative increase (rDPRL, [(peak minus basal)/basal]), and decline-time PRL (Dt-PRL, +60 min PRL). A blunted PRL response was defined as a ≤ threefold PRL increase after TRH, and a brisk PRL response was defined as a ≥ 10-fold PRL increase after TRH administration.	**bPRL:** no significant difference between the RSA women and both the controls and the infertile women occurred **HPRL (defined as bPRL ≥15 ng/mL)** prevalence similar in RSA (15/69, 21.7%) vs infertile women (13/31, 41.9%) and controls (5/30, 16.7%) no significant differences between groups in the PRL response to TRH **NK cells** Higher percentage of NK cells were found in the RSA and in the infertile women compared with the controls P = 0.04 for both comparison). In multiple regression analyses, PRL was confirmed to be the only factor to			

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					have a significant effect on NK cell levels (coefficient of determination R2 0.74; P< 0.001) in the patients' group.				
Trout SW, Seifer DB. Fertil Steril. 2000;74(2):335-7.	CS	☐ Selection bias controls are known cause RPL this is not a correct control group ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** X **Unacceptable (-)**	57 evaluated for RPL ≥3 Ab 1rst trimester ⇨ 36 unexplained RPL ⇨ 21 known cause RPL = control Similar age, parity, and presence of infertility Retrospective	day 3 serum FSH day 3 E(2) levels		day 3 FSH and E(2) levels were elevated in unexplained RPL FSH >10 or E(2) >50 levels, or both elevated in 58% of U-RPL vs 19% of controls (odds ratio, 5.95 [95% CI, 1.7-21.3]; P<.004).		Role of DOR in unexplained RPL : Women with unexplained RPL have a greater incidence of elevated day 3 serum FSH and E(2) levels than do women with a known cause of RPL. Include in work-up	
Tulppala M, Bjorses UM, et al. Fertil Steril. 1991;56(1):41-4. (2065803)	CS	X Selection bias no real control group ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** X **Unacceptable (-)**	46 RM (>3) (27 primary and 19 secondary aborters) 3 x positive ACL 12 healthy control women 5 LB 7 no previous pregnancy	delay of greater than 2 days in endometrial maturation during two consecutive cycles Salivary P	17.4% results control group 0%?????	8 patients (17.4%, 5 primary and 3 secondary aborters) 38 normal ovulatory rise, but no diff in LPD or not, or healthy		endometrial maturation defect may be a factor in 17.4% of patients with habitual abortion, but this cannot be detected by salivary P assay.	Not use salivary P assay for diagnosis LPD **no clear study group (explained and unexplained mixed)**
Van den Boogaard E, Vissenberg R et al. Hum Reprod Update 2011;17(5):605-19	SR	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ High quality (++) X ☐ Acceptable (+) Unacceptable (-)	43 included studies; 38 eligible for meta-analysis. Scope review broader than only RPL.	Presence thyroid antibodies in euthyroid women associated with RPL N=447 vs. N=1880		OR 2,3 95%CI (1,5-3,5)			no controls, no clear study population

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
van Dijk MM, et al. Reprod Biomed Online 2016;33: 745-751.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	848 women with RPL 20 women with subclinical hypothyroidism (defined as thyroid-stimulating hormone >97.5th percentile mU/l with a normal thyroxine level) 10 with overt hypothyroidism 818 with normal thyroid function (control group)		subclinical hypothyroidism in only 2.4% of women with RPL no differences in live birth or miscarriage rate between women with subclinical hypothyroidism and euthyroid women LBR: 45% in women with subclinical hypothyroidism and 52% in euthyroid women (OR 0.69, 95% CI 0.28 to 1.71). The ongoing pregnancy rate : 65% versus 69% (OR 0.82, 95% CI 0.32 to 2.10) and the miscarriage rate was 35% versus 28% (OR 1.43, 95% CI 0.56 to 3.68). No differences were found when TSH 2.5 mU/l was used as cut-off level to define subclinical hypothyroidism.			In unexplained RPL, no differences were found in live birth, ongoing pregnancy and miscarriage rates between women with subclinical hypothyroidism and euthyroid women.	
Vissenberg R, , et al. Hum Reprod Update. 2015;21(3):378-87.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction.						Impact of thyroid disorders and AB on fertility and early pregnancy. No data on RPL, association,…
Wang LQ, et al. PLoS One 2016;11: e0165589.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	20 women undergoing primary miscarriage, 20 women with RM 20 women with normal pregnancy	Expressions of CYP27B1 mRNA and protein in villi and decidua The co-localization of CYP27B1 and certain cytokines including IL-10, IFN-gamma, TNF-alpha, and IL-2 expression .	Women with RM had a significantly lower expression of CYP27B1 mRNA and protein in villous and decidual tissues compared with the normal pregnant women (P = 0.000 in villus, P = 0.002 in decidua for mRNA; P = 0.036 in villus, P = 0.007 in decidua for protein.). Compared with the normal pregnancy, immunostaining for CYP27B1 was significantly decreased in villous trophoblasts and decidual glandular epithelial cells in RM women. No significant differences in the localization of CYP27B1, IL-10, IFN-gamma, TNF-alpha, and IL-2 expression were identified between the normal pregnant and RM women.			Women with RM have a lower level of CYP27B1 expression in chorionic villi and decidua compared with normal pregnant women, suggesting that reduced CYP27B1 expression may be associated with RM. The consistent localization of CYP27B1 and IL-10, IFN-gamma, TNF-alpha, and IL-2 expression in villous and decidual tissues suggests the importance of the local production of 1,25(OH)2D3 at the fetal-maternal interface to regulate cytokine responses.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Wang Y, Zhao H, et al. Gynecol Obstet Invest. 2011;72(4):245-51.	CCS	?/- Selection bias - Assesment X Confouding X Statistical issues ---------- ☐ High quality (++) X Acceptable (+) ☐ Unacceptable (-)	MEASURED IN PREGNANCY (China) N=97 women history REPL ≥2 Exclusie: abnormalities in hysteroscopy, thyroid function, karyotyping, APA, homocysteine, TORCH N=52 Women with no unhealthy pregnancies It is unclear if they all have previous pregnancies	OGTT HOMA-IR= fasting glucose x fasting insulin/ 22.5 Fasting glucose Fasting insulin Measured in 5th and 13th week of pregnancy	Higher glucose Higher insulin HOMA-IR = FG= FI=			Women with history REPL are at risk for IR during first trimester of a new pregnancy	
Watson H, Kiddy DS, et al. Hum Reprod. 1993;8(6):829-33.	CCS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X**Acceptable (+)** ☐ **Unacceptable (-)**	N=21 unexplained RPL ≥3<12 wk N=10 multiparous women No abnormalities: karyotype, APS, uterine	Midluteal Progesterone Testosterone PCO morphology LH, FSH (midluteal, midfollicular) Urinary LH elevated In RPL excessive LH secretion Oestrone 3 glucoride Urinary pregnanediol-3alpha-gluceronide	Ns 2.0 +- 0.54 vs.1.72 +- 0.22 (p<0.05) 17/21 (81%) vs. 1/10 (10%) NS 16/21 (76%) 249 +-135 vs. 126 +-62 In RPL elevated early to midluteal NS				
Yan X, et al. Arch Biochem Biophys 2016;606:128-133.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X**Acceptable (+)** ☐ **Unacceptable (-)**	40omen at 7-10 weeks gestation with RPL and 40 women of similar gestational age with a healthy pregnancy	vitamin D receptor (VDR) mRNA and protein in chorionic villi and decidua serum levels of VDR	Women with RPL had a significantly weaker expression of VDR mRNA in villi and decidual tissues compared with the control women (both $p < 0.0001$). Western blot analysis showed an approximately 46% decrease in VDR expression in villi and a 52% decrease in decidua in the RPL vs. the controls. Serum VDR levels were also significantly lower in the RPL group than in controls ($p = 0.003$). Significantly lower VDR expression in villous cytotrophoblasts and stromal cells, as well as in decidual glandular epithelial and stromal cells in RM compared to controls (all $p < 0.05$).			women with RPL have lower levels of VDR expression in chorionic villi, decidua and serum compared with normal pregnant women	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Zolghadri J, Tavana Z, et al. Fertil Steril. 2008;90(3):727-30.	CCS	?/- Selection bias - Assesment X Confouding - Statistical issues ---------- ☐ **High quality (++)** **Acceptable (+)** **X Unacceptable (-)**	N=164 women history REPL ≥3 Exclusie: abnormalities in hysteroscopy/HSG, thyroid function, karyotyping, APA, PRL, PT, PTT N=74 Women without REPL	OGTT	31/164 (18.9) 2 DM included 29/164 (17.6%) 4/74 (5.4%)	OR (95%CI) 1.34 (1.25-2.42) P=0.017 Recalculated 3.8 (1.3-11.3)		Study indicates a link between abnormal OGTT and history REPL	Iran Also intervention in study RQ11
Zammiti W, et al. Am J Reprod Immunol 2008;59: 139-145.	case-control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	350 RPL 200 healthy women	endothelial nitric oxide synthase (eNOS) functional polymorphisms: the 27-bp intron 4 repeat, the 894G/T of exon 7, and the promoter substitution -786T/C, homocysteine total plasma concentrations (tHcy)	None of the eNOS polymorphisms-related alleles, genotypes, and haplotypes were associated with RPL. The tHcy were similar between RPL and controls; no significant association between tHcy levels and eNOS genotypes could be evidenced tHcy (lmol ⁄ ml) ± S.D: RPL: 10.80 ±7.94 Contr: 8.72 ± 6.86			a lack of association between eNOS gene polymorphisms, the risk of RPL and tHcy levels	

Additional references included as background information

Bahn RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, Laurberg P, McDougall IR, Montori VM, Rivkees SA *et al.* Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Endocr Pract* 2011;**17:** 456-520.

Chan S, Boelaert K. Optimal management of hypothyroidism, hypothyroxinaemia and euthyroid TPO antibody positivity preconception and in pregnancy. *Clin Endocrinol (Oxf)* 2015;**82:** 313-326.

Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. *Eur Thyroid J* 2014;**3:** 76-94.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 2011;**21:** 1081-1125.

Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers GH, Eskes TK. Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. *J Nutr* 1998;**128:** 1336-1341.

9. WHAT IS THE VALUE OF ANATOMICAL INVESTIGATIONS IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Bohlmann MK, von Wolff M, et al. Reprod Biomed Online. 2010;21(2):230-6.	CS	☐ Selection bias (retrospective) ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Anatomical findings in HSC in women with history of 2 (87) vs 3 (119) miscarriages. Compare findings in US with HSC (retrospectively)	2D US vs HSC. HSC is done after US,		Se for US for synechia 0 %, for congenital uterine anomalies 52 %, for fibroids 68 %, polyp 60 %. Sp not specified.		No differences found. Women after exactly two early miscarriages can be advised that hysteroscopy will reveal uterine anomalies in more than 35% of patients, the majority of which are amenable to therapy	US vs HSC
Caliskan E, Ozkan S, et al. J Clin Ultrasound. 2010;38(3):123-7.	CS	☐ Selection bias (patients with abnormal HSG) ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	108 women by 2 gynecologists during the 1st 5 days after cessation of menstrual flow and then reexamined at the cycle days 20–24	2D US vs 3D US		For 3D US: Se 94.7%, Sp 75.0%, follicular phase, Se 100%, sp 93.7% luteal phase. 2DUS (Se 30.2% Sp 78.1% follicular phase, Se 42.1% Sp 81.2% luteal phase		Real-time 3DUS is an accurate method that can be used for the diagnosis of congenital mullerian defects	
Chan YY, Jayaprakasan K, et al. Hum Reprod Update. 2011;17(6):761-71.	SR	Appropriate question ? Rigorous search ? Yes Relevant studies included? Yes Quality of studies? Papers with no high quaoity not excluded Methodology ? --- ☐ **High quality (++)**	94 studies, 89 861 women	two-dimensional transvaginal ultrasound, hysteroscopy and HSG are suboptimal in this respect, as they all have a tendency to misclassify uterine abnormalities owing to their poorer accuracy when used as diagnostic tests in isolation. Historically, and still today, many authors considered the	5.5% in unselected population, 8.0% in infertile women, in those with a history of miscarriage and 24.5% in those with miscarriage and infertility	Not specified		Women with a history of miscarriage or miscarriage and infertility have higher prevalence of congenital uterine anomalies	

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		☐ **Acceptable (+)** ☐ **Unacceptable (-)**		combination of laparoscopy or laparotomy with hysteroscopy or HSG to be the gold standard for the diagnosis and differentiation of congenital uterine anomalies				compared with the unselected population	
Ferreira AM, Pires CR, et al. Int J Gynaecol Obstet. 2007;98(2):115-9.	Other	☐ Selection bias ☐Performance bias (interobserver bias) ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)** ☐ **NA**	43 women with recurrent pregnancy loss and 43 women with no history of abortion and at least 1 child born at term (control group).	transvaginal ultrasonography with uterine artery Doppler. PI and FVW				higher PI and a higher incidence of FVW of the A and B types—and thus a higher uterine artery impedance—were found among women with recurrent pregnancy loss.	Doppler, no intervention
Frates MC, Doubilet PM, et al. J Ultrasound Med. 1996;15(8):557-62.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	96 patients, prospectively, for RI during first trimester						Doppler RI has no predictive value for RM
Ghi T, Casadio P, et al. Fertil Steril. 2009;92(2):808-13.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)** ☐ **NA**	284 women with RM, 230 (81%) has normal 3D US, uterine anomaly was detected in 54 cases (19%).	3D US, and subsequent HSC for those without abnormal findings, HSC-LPS for those with UA diagnosed by 3D US		Not mentioned. 3D US was concordant with HSC diagnostic in 100 % of normal diagnostic, and detected 100 % of UA. Diagnostic (uterine anomaly type) was correct in all except 2 cases (3.7 %)		3D TV US appears to be extremely accurate for the diagnosis and classification of congenital uterine anomalies and may conveniently become the only mandatory step	3D TV US

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								in the assessment of the uterine cavity in patients with a history of recurrent miscarriage.	
Harger JH. Obstet Gynecol 2002;100: 1313-1327.	review	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	5 RCTs have offered significant information about elective cerclages performed for historical indications, and the expected neonatal survival rate with properly selected elective cerclages is around 87%. Transvaginal ultrasound studies have revealed new paradigms regarding normal cervical function in pregnancy and further understanding about the significance and predictive value of cervical changes at gestational ages between 20-37 weeks. Only two randomized clinical trials have been conducted regarding cerclage in women with decreasing cervical length or with cervical funneling. One of these two failed to demonstrate any resulting improvement in neonatal survival, and the other was too small to be conclusive.						Cerclage and cervical insufficiency: an evidence-based analysis.
Hooker AB, Lemmers M, et al. Hum Reprod Update. 2014;20(2):262-78.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Exclusion of women with RM						Included as background information of miscarriage
Jaslow CR, Kutteh WH. Fertil Steril. 2013;99(7):1916-22.e1.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	875 women with at least 2 miscarriages, primary and secondary	three-dimensional sonohysterography, confirmed by hysteroscopy/laparoscopy		Total frequency of patients with anomaliesa 19.3 (22.3 % in primary RM, 15 % in secondary RM) . Sono HSG less accurate to diagnose synequia (4 %)		These results support a recommendation for diagnostic imaging of the uterus after two losses in women with secondary RM as well as for those with primary RM.	In, good retrospective review

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Kassanos D, Salamalekis E, et al. Clin Exp Obstet Gynecol. 2001;28(4):266-8.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Women with a previous history of second trimester miscarriage due to cervical incompetence group I (n=27) elective cerclage was applied during the 14th week. Women in group II (n=28) were subjected to serial weekly evaluations of the cervix by transvaginal ultrasonograms. In 18 cases emergency cerclage was applied due to significant cervical changes	transvaginal sonography	In group I, labor started before the 33rd week in two cases (7.4%), between 33 and 37 weeks in nine (33.3%) and after the 37th week in 16 cases (59.2%). Out of the 18 patients in group II who had cervical cerclage after ultrasonographic evaluation, four (22.2%) delivered before the 33rd week, three (16.6%) between 33 and 37 weeks and 11 (61.1%) after the 37th week. No statistical difference was noted between the two groups referring to pregnancy outcome (p<0.1).			No evidence of benefit for US in second x miscarriage	
Ludwin A, Ludwin I, et al. J Obstet Gynaecol Res. 2011;37(3):178-86.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)** ☐ **NA**	83 women with history of RM or infertility, without distinction			SonoHSG Se 95.9%, Sp 88.9%, PPV 98.6%, NPV 72.7% for uterine malformations in general, (higher than those for HSG or HSC)		SonoHSG it is a cost-effective method to diagnose uterine abnormalities, in particular septate and bicornuate uterus	
Makris N, et al. Int J Gynaecol Obstet 2007;97: 6-9.	prospective study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	124 women with suspected intrauterine abnormality on 2-D ultrasonography or on hysterosalpingography	hysteroscopy, 3-DHS, and 3-D power Doppler (3-DPD) examination. (3-DHS could not be performed in 3 of the women because of cervical stenosis.)	Of the 121 women found to have an intracavitary abnormality, 20 had polyps, 11 had myomas, 2 had Mullerian duct anomalies, and 6 had synechiae on hysteroscopy.	There was agreement between hysteroscopy and 3-DHS in 19 of the polyp cases, 11 of the myoma cases, 2 of the Mullerian anomaly cases, and 4 of the synechiae cases. Examination with 3-DHS and 3-DPD reached a sensitivity of 91.9% and specificity of 98.8%, with a positive predictive value of 97.1% and a negative		Examination with 3-DHS and 3-DPD both allows for accurate assessment of intrauterine abnormalities.	Three-dimensional hysterosonography versus hysteroscopy for the detection of intracavitary uterine abnormalities.

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
						predictive value of 96.5%,			
Rimmer MP et al., J. Obstet. Gynaecol. Res. 2021;47: 689-697. 2021	Prospective cohort study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	344 Women with history of recurrent pregnancy loss or implantation failure over a 12-months follow-up period	the CD138+ cells/high powered field (hpf) was quantified using immunohistochemistry and image analysis of endometrial biopsies obtained during the secretory stage post ovulation.	AUC of 0.75 (95% CI 0.59–0.82, P = 0.01)	Women with a CD138+ score ≥ 16/hpf had a significantly higher risk of a miscarriage compared to those with a score 0–5 (RR 10.0, 95% CI 2.78, 36.02). Women with lower CD138+ scores showed levels of relative risk which were not statistically significant at a P-value <0.05 but were suggestive of increased risk with P-values <0.10	ROC analysis suggests an overall good performance for CD138+ as a diagnostic test with scores above a cutoff value of 4–6 cells/hpf suggesting higher risk for future RPL	Quantifying CD138+ cells by immunohistochemistry in women with a history of recurrent pregnancy loss is helpful to diagnose chronic endometritis and predict subsequent reproductive outcome.	
Robberecht C, Pexsters A, et al. Prenat Diagn. 2012;32(10): 933-42.	Other	**NA**	Products of conception from 51 couples with at least one previous miscarriage Not anatomical, but Chromosomal abnormalities in POC	embryoHSC to get samples, to be analyzed (POC) extracted DNA + array CGH + high resolution SNP arrays	Chromosomal aberrations were identified in 65.6% (21/32) of miscarriages and in 89% (8/9) of anembryonic cases. Interestingly, 4/11 chromosomally euploid embryos contained regions of loss of heterozygosity >5 Mb, suggesting the miscarriages might be due to an underlying lethal recessive disease			embryoHSC + array CGH is a usefull tool in RPL	
Saravelos SH, et al. Hum Reprod Update. 2008;14(5):415-29.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	625. Review about prevalence of uterine malformations in general population, infertile patients, and RM	2D US, HSC, HSG, MRI,	6.7 % in general population, 16.7 % in RM	Not mentioned	The relation between most congenital uterine anomalies and RM has been well documented in the literature; furthermore, it has been suggested that treatment of certain anomalies may result in an improved pregnancy outcome Therefore, any woman suffering from RM should be thoroughly investigated, to identify		Not a systematic review summarizing all evidence – good overview

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
							congenital uterine anomalies		
Saravelos SH, Yan J, et al. Hum Reprod. 2011;26(12): 3274-9.	CS	☐ Selection bias ☐Performance bias (lack of control group) ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	364 patients with RM	US and HSG	8.2 % of patients with RM had intrauterine fibroids, or distorting cavity	Not mentioned	Yes	Association between RM and intracavitary fibroids	Fibroids are associated with increased mid-trimester losses amongst women with RM. Resection of fibroids distorting the uterine cavity can eliminate the mid-trimester losses and double the live birth rate in subsequent pregnancies. Women with fibroids not distorting the uterine cavity can achieve high live birth rates without intervention
Tur-Kaspa I, Gal M, et al. Fertil Steril. 2006;86(6):1 731-5.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)**	1009	Saline SonoHSG in infertile patients	16.2% of infertile patients had intrauterine findings	Not mentioned	Yes	20 % of patients with Infertility have uterine malformations	Accuracy of saline sonoHSG

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
		☐ Unacceptable (-)							

Additional references included as background information

Grimbizis GF, Di Spiezio Sardo A, Saravelos SH, Gordts S, Exacoustos C, Van Schoubroeck D, Bermejo C, Amso NN, Nargund G, Timmerman D et al. The Thessaloniki ESHRE/ESGE consensus on diagnosis of female genital anomalies. Hum Reprod 2016;31: 2-7.

Hall-Craggs MA, Kirkham A, Creighton SM. Renal and urological abnormalities occurring with Mullerian anomalies. J Pediatr Urol 2013;9: 27-32.

Liddell HS, Lo C. Laparoscopic cervical cerclage: a series in women with a history of second trimester miscarriage. J Minim Invasive Gynecol 2008;15: 342-345.

Oppelt P, von Have M, Paulsen M, Strissel PL, Strick R, Brucker S, Wallwiener D, Beckmann MW. Female genital malformations and their associated abnormalities. Fertil Steril 2007;87: 335-342.

Ramanathan S, Kumar D, Khanna M, Al Heidous M, Sheikh A, Virmani V, Palaniappan Y. Multi-modality imaging review of congenital abnormalities of kidney and upper urinary tract. *World journal of radiology* 2016;8: 132-141.

Woelfer B, Salim R, Banerjee S, Elson J, Regan L, Jurkovic D. Reproductive outcomes in women with congenital uterine anomalies detected by three-dimensional ultrasound screening. Obstet Gynecol 2001;98: 1099-1103.

10. WHAT IS THE VALUE OF MALE SCREENING IN THE DIAGNOSIS OF RPL?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Bernardini LM, Costa M, et al. Reprod Biomed Online. 2004;9(3):312-20.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	20 couples suffering from three or more recurrent first trimester abortions. For a subset of this study population, additional experiments of multicolour fluorescence in-situ hybridization for chromosomes 4, 7, 12, 13, 15, 18, 21, and 22, were performed on the bases of the available data from abortive tissue karyotyping normal semen parameters (with or without RPL).	Experiments of double target in-situ hybridization were performed separately for chromosomes 1–17, 8–18 and sex chromosomes on sperm samples from	A markedly high rate of sperm disomy (14.5–15.5%) was scored in only two cases. For three other patients, the cumulative disomy rates for chromosomes 1, 17, 8, 18, X and Y also increased but at a lower level (7.8–9.5%). For the remaining 15 patients, the frequency of sperm aneuploidy was moderately increased or normal. Men with recurrent pregnancy loss (RPL) and poor semen quality had baseline sperm aneuploidy and diploidy rates higher than men with than men with normal semen parameters (with or without RPL). Using probes for chromosomes 1, 17, 8, 18, X and Y, significantly elevated frequencies of sperm aneuploidy (not diploidy) were found in 10% of men with a history of RPL. Their rate of sperm aneuploidy was 30–34%.				
Bhattacharya SM. Int Urol Nephrol. 2008;40(2):391-5.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	74 couples with the history of repeated early pregnancy Loss and 65 with proven fertility in past year	were analyzed as per WHO criteria, and DNA integrity was studied in each case by Acridine Orange staining test	No effects of semen analysis but significant differences were found in total motile sperms per ejaculate, percentage of motile sperm and, most importantly, in the DNA integrity				
Brahem S, Mehdi M, et al. Urology. 2011;78(4):792-6.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Semen samples from 31 patients with a history of recurrent pregnancy loss and 20 men with proven fertility	analyzed according to World Health Organization guidelines. Sperm DNA fragmentation was detected by the terminal deoxynucleotidyl transferase–mediated dUTP nickend labeling assay.	a significant difference was observed in sperm motility, but not in other parameters. The mean number of sperm cells with fragmented DNA was significantly increased in the RPL group (32.22 _ 6.14%) compared with control donors (10.20 _ 2.1%).				

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Bronet F, Martinez E, et al. Hum Reprod. 2012;27(7):1922-9. (22537817)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	prospective study evaluated DNA damage and the aneuploidy rate in fresh and processed (density gradient centrifugation) ejaculated sperm as well as the aneuploidy rate in biopsied embryos from fertility cycles. Fluorescence in situ hybridization	total of 154 embryos were evaluated from 38 patients undergoing PGD cycles; 35.2% of the embryos were chromosomally normal. Analysis of the same sperm samples showed an increased DNA fragmentation after sperm preparation in 76% of the patients. There was no correlation between DNA fragmentation and the aneuploidy rate in embryos or in fresh or processed sperm samples.		Sperm DNA fragmentation is not related to chromosomal anomalies in embryos from patients with recurrent miscarriage or implantation failure			
Carlini T, et al. Reprod. biomedicine online 2017;34: 58-65.	Case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	112 men from RPL couples and two control groups: 114 infertile men with one or more impaired semen parameters 114 fertile men with high-quality semen parameters.	Semen parameters were examined according to WHO criteria. Sperm DNA fragmentation (SDF) was evaluated using TdT-mediated dUDP nick-end labelling (TUNEL) assay.	With the exception of ejaculate volume, the seminal profile of patients with RPL was similar to that of fertile patients and better than the infertile ones. Sperm DNA integrity was impaired in the RPL group, with SDF values significantly higher than in fertile controls (18.8 +/- 7.0 versus 12.8 +/- 5.3, P < 0.001) and similar to those of infertile patients. SDF also showed a positive correlation with the age of patients with RPL and number of miscarriages			The results suggest a correlation between increased SDF and impaired reproductive capacity in terms of both fertilization and pregnancies carried to term.	
Carp H, Guetta E, et al. Fertil Steril. 2006;85(2):446-50.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Embryonic karyotype in recurrent miscarriage with parental karyotypic aberrations	One thousand one hundred eight patients with 3–16 miscarriages before 20 weeks gestation; 113 patients with and 995 without chromosomal aberrations. .	Karyotyping by standard G-banding techniques of both parents, and of 205 abortuses collected at curettage. Result(s): Two hundred three abortuses were successfully karyotyped. In 164 embryos of patients with no parental chromosomal aberrations, 23.2% (38/164) had chromosome aberrations. Of the 39 abortuses karyotyped in patients with chromosomal aberrations, 17 had normal karyotypes, 8 had balanced translocations, 2 had inversions identical to the parents, and 12 (30.8%) had abnormal karyotypes. This difference is not statistically significant (odd ratio 1.47, 95% confidence interval 0.63–3.39). Only 4 of the 39 karyotyped abortuses had an unbalanced translocation				Parental karyotyping was not particularly predictive of a subsequent miscarriage as a result of chromosomal aberrations as 43.5% of abortuses were euploidic, and the parental aberration was only passed on to the abortus in 10% of cases.

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Du Fossé NA et al., *Human reprod update* 2020;26: 650-669	Meta-analysis	Appropriate question? Yes Rigorous search? Yes Relevant studies included? Yes Quality of studies? Low to moderate Methodology? --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	9 included studies in the meta-analysis	association of advanced paternal age with spontaneous miscarriage during the first trimester of pregnancy	there is an increased risk for miscarriage for male age categories 30-34, 35-39 and 40-44 and this risk was higher for the ≥45 age category Similar pooled risk estimates for the first three age categories and a slightly higher pooled risk estimate for age category ≥45 years (1.74; 95% CI 1.26, 2.41			advanced paternal age is also associated with an increased risk of spontaneous miscarriage	
Du Fossé NA et al., Fert. Steril. 2022;117: 144-152	Meta-analysis	Appropriate question? Yes Rigorous search? Yes Relevant studies included? Yes Quality of studies? Low to moderate Methodology? --- **X High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	11 studies included. Six case-control studies, 4 prospective cohort studies and 1 retrospective study.	Six studies evaluated the association between preconceptional paternal smoking behavior and pregnancy loss, 2 studies focused on paternal alcohol consumption and pregnancy loss, and 3 studies addressed both exposures.	Risk estimate of pregnancy loss: 1–10 cigarettes per day: 1.01; 95% CI 0.97–1.06 11–19 cigarettes per day: 1.12; 95% CI 1.08–1.16 R20 cigarettes per day: 1.23; 95% CI 1.17–1.29.			No clear association was found between paternal alcohol consumption and pregnancy loss, based on 5 available studies.	Paternal smoking of>10 cigarettes per day in the preconception period was found to be associated with an increased risk of pregnancy loss, after adjustment for maternal smoking status
Gopalkrishnan K, Padwal V, et al. Arch Androl. 2000;45(2):111-7.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	51 fertile and 32 RM	sperm function tests, and ultrastructural studies of sperm	The functional tests were all normal except for a significant decrease in the capacity of nuclear chromatin to decondense in vitro.				

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Imam SN, Shamsi MB, et al. J Reprod Infertil. 2011;12(4):267-76.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Twenty infertile men with a history of iRPL and 20 fertile controls (having fathered a child a year earlier)	Conventional semen analysis was performed (concentration, motility, morphology; WHO criteria, 2010) within 1 hour of sample collection. Levels of reactive oxygen species (ROS) were assessed by luminol-dependant chemiluminescence. The total antioxidant capacity (TAC) was quantified by ELISA. The Sperm chromatin structure assay (SCSA) was performed by flow cytometry to determine DNA fragmentation Index (DFI)	No significant differences in age, seminal volume, liquefaction time, pH and sperm concentration were observed between the male partner of iRPL cases and the controls, but sperm morphology and motility were significantly ($p < 0.05$) lower in the male partner of cases with idiopathic recurrent spontaneous abortion (RSA). The mean ROS levels observed were 47427.00 relative light unit (RLU)/min/20 million sperm in the male partners as compared to 13644.57 RLU/ min/20 million sperm in the controls (normal <15000 RLU/min/20 million). The mean TAC levels in the controls (6.95 mM trolox) were significantly ($p < 0.05$) higher as compared to the male partners of women with IRPL (2.98 mM trolox). The average mean DFI of male partners were found to be 23.37±9.9 and the mean DFI of controls was 13.89±5.40. The mean DFI was significantly ($p < 0.05$) higher when compared to the controls. The range of DFI in male partners was 8.50–44.07. However, in the controls the range was 7.70–23.50.				
Kaare M, Painter JN, et al. Fertil Steril. 2008;90(6):2328-33.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	chromosome microdeletion study 40 male partners of women with RM	DNA from males was tested for Y chromosome microdeletions by analyzing 37 sequence tagged site					Y chromosome microdeletions were not found in spouses of patients.
Kamal A, Fahmy I, et al. Fertil Steril. 2010;94(6):2135-40.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	A detailed chart review of a cohort of 1,121 men with obstructive azoospermia who underwent intracytoplamic sperm injection (ICSI) was performed.		miscarriage (17.6% vs. 18.4%) rates did not differ between epididymal spermatozoa and testicular spermatozoa,				

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Khadem N, Poorhoseyni A, et al. Andrologia. 2014 ;46(2):126-30.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	30 couples with RSA and 30 fertile couples as control group completed the demographic data questionnaires	semen samples were analysed according to World Health Organization (WHO) standards (September 2009–March 2010) for evaluation of sperm DNA fragmentation, using sperm chromatin dispersion (SCD) technique.	The sperm from men with a history of RSA had a higher incidence of DNA fragmentation and poor motility than those of the control group, indicating a possible relationship between idiopathic RSA and DNA fragmentation.				
Li J et al;, Medicine 2021;100: e24828.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology? heterogeinity ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	24 case-control and cohort studies on Chinese couples including 1,690 male partners of women with RPL, and 1,337 male partners of fertile control women	Sperm density, viability, motility, morphology and DNA fragmentation	male partners of women with RPL had a significantly lower level of sperm density (SMD= – 0.53; 95%CI – 0.75 to – 0.30), sperm viability [standard mean deviation (SMD)= –1.03; 95%CI – 1.52 to – 0.54], sperm progressive motility rate (SMD= –0.76 95%CI –1.06 to –0.46), and normal sperm morphology rate (SMD= – 0.56, 95%CI – 0.99 to – 0.12), and had a significantly higher rate of sperm deformity rate (SMD=1.29; 95%CI 0.60-1.97), and sperm DNA fragmentation index (DFI) (SMD=1.60; 95%CI 1.04-2.17), when compared with the reference group. The 2 groups had no significant difference of semen volume (SMD= – 0.03; 95%CI – 0.14 to 0.08) and semen pH value (SMD= –0.23; 95%CI – 0.50 to 0.05)			an association of sperm density, sperm viability, sperm progressive motility rate, normal sperm morphology rate, sperm deformity rate, as well as sperm DFI with RPL.	
Miller D et al., Lancet 2019;393: 416-422.	RCT	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	2752 couples of whom 2752 were included in the primary analysis	1381 in the PICSI group and 1371 in the ICSI group	The term livebirth rate did not differ significantly between PICSI (27·4% [379/1381]) and ICSI (25·2% [346/1371]) groups (odds ratio 1·12, 95% CI 0·95–1·34; p=0·18). There were 56 serious adverse events in total, including 31 in the PICSI group and 25 in the ICSI group; most were congenital abnormalities, and none were attributed to treatment.			Compared with ICSI, PICSI does not significantly improve term livebirth rates.	

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Montagnoli et al 2021	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	53 studies	paternal exposure factors and lifestyles	Advanced age may impair male fertility and affect early pregnancy stages. Increased body mass index, smoking, alcohol and recreational drugs, all alter seminal fluid parameters. Hazardous alcohol use correlates with low birthweight in pregnancy and harmful behavioral lifestyles have been linked to congenital heart defects, metabolic and neurodevelopmental disorders in the offspring.			Measures targeting paternal health and lifestyle within the first 1,000 days' timeframe need to be implemented in couples undergoing reproductive decisions.	
Nicopoullos JD, Gilling-Smith C, et al. Fertil Steril. 2004;82(3):691-701.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Ten reports (734 cycles: 677 transfers) were identified as suitable to assess source of sperm; 9 reports (1,103 cycles: 998 transfers) to assess etiology; and 17 reports (1,476 cycles: 1,377 transfers) to assess the effect of cryopreservation		There was no difference in either IR or miscarriage rate between the two groups.				
Pasqualotto FF, Rossi-Ferragut LM, et al. J Urol. 2002;167(4):1753-6.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	166 consecutive patients (198 intracytoplasmic sperm injection cycles) with azoospermia were studied. Of these 198 cycles 68 were performed due to nonobstructive azoospermia using testicular spermatozoa and 130 were performed due to obstructive azoospermia using epididymal spermatozoa.		The pregnancy rate per cycle, pregnancy rate per patient and abortion rate were 30%, 39.8% and 28% for obstructive azoospermia, and 22%, 28.3% and 40% for nonobstructive azoospermia (p _0.05).				

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Pereza N, Crnjar K, et al. Fertil Steril. 2013;99(6):1663-7.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Male partners of 148 couples with at least three spontaneous pregnancy losses of unknown etiology, and 148 fertile men.	Azoospermia factor (AZF) regions were tested for Y chromosome microdeletions				None of the IRSA or control men had microdeletions in the AZFa, AZFb, or AZFc regions.	
Pu Y et al. et al Cogent Biology 2020;6: 1759393.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- x☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	326 male partners of women with recurrent pregnancy loss and 124 fertile men	The correla- tion between the specific type of sperm aneuploidy and recurrent miscarriage	Pooled data from three studies with sufficient data suggested that male partners of women with a history of RPL had significantly higher rates of total sperm aneuploidy compared with the partners of fertile control women (SMD: 1.07, 95% CI: 0.39–1.75, P < 0.01).			Qualitative analysis and quantitative analysis suggested an association between total sperm aneuploidy and RPL.	
Robinson L, Gallos ID, et al. Hum Reprod. 2012;27(10): 2908-17.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- x☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	16 cohort studies (2969 couples), 14 of which were prospective.	Eight studies used acridine orange-based assays, six the TUNEL assay and two the COMET assay. patients with high DNA damage compared with those with low DNA damage [risk ratio (RR) ¼ 2.16 (1.54, 3.03), P , 0.00001)]. A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (RR ¼ 3.94 (2.45, 6.32), P , 0.00001). limitations, reasons for caution: There is some variation in study	Meta-analysis showed a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage [risk ratio (RR) ¼ 2.16 (1.54, 3.03), P , 0.00001)]. A subgroup analysis showed that the miscarriage association is	with those with low DNA damage [risk ratio (RR) ¼ 2.16 (1.54, 3.03), P , 0.00001)]. A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (RR ¼ 3.94 (2.45, 6.32), P , 0.00001).			

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					strongest for the TUNEL assay (RR ¼ 3.94 (2.45, 6.32), P , 0.00001).				
Ruixue W, Hongli Z, et al. J Assist Reprod Genet. 2013;30(11): 1513-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	68 RPL couples and 63 randomly selected healthy controls.	Semen parameters were estimated by computer-assisted sperm analysis, and sperm nuclear status was detected with aniline blue (AB) staining.	There were no differences in sperm concentration, and motility between the groups (P >0.05). Significant odds ratio (OR) was found when occupational exposure and unhealthy habits were superimposed (OR: 11.965, P =0.005).				
Sbracia S, Cozza G, et al. Hum Reprod. 1996;11(1):117-20.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	120 previously selected couples with unexplained RSA were studied for sperm parameters retrospectively and prospectively	3 years of follow-up study: (i) 48 RSA couples who achieved a successful pregnancy; (ii) 39 RSA couples who experienced further abortions; and (iii) 33 RSA couples who experienced infertility during the follow-up period.	(RSA couples who achieved successful pregnancy and RSA couples who experienced miscarriages and no live birth during the follow-up) for sperm concentration (P < 0.01 and P < 0.01 respectively), sperm motility (P < 0.01 and P < 0.01 respectively)			Semen analysis is an important test in the clinical management of RSA couples.	
Talebi AR, Vahidi S, et al. Andrologia. 2012;44 Suppl 1:462-70.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	40 couples with a history of RSA and 40 couples with proven fertility were considered as case and control groups respectively.	sperm parameters and also sperm chromatin and DNA integrity assessed using cytochemical tests including aniline blue (AB), chromomycin A3 (CMA3), toluidine blue (TB), acridine orange (AOT) and nuclear chromatin stability assay.	In sperm chromatin evaluations, there were significant differences between the two groups in all of the tests.				

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Wettasinghe TK, Jayasekara RW, et al. Hum Reprod. 2010;25(12):3152-6.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	76 male partners of couples where the female partner had experienced three or more RPLs. One hundred and twenty random males from the general population were also analysed as a control group	DNA extracted from peripheral blood was tested for Y chromosome microdeletions in the azoospermic factor (AZF), AZFa, AZFb, AZFc regions using a multiplex PCR amplification system. Partial deletions within the AZFc region were also tested.			Y chromosome microdeletions do not appear to be important in the aetiology of RPL in this population in Sri Lanka.		
Zhang L, Wang L, et al. Int J Androl. 2012;35(5):752-7.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	111 men whose partners had a history of unexplained RSA (RSA group) and 30 healthy fertile men	reproductive outcome during the 12 months after they were enrolled in the study:	Sperm concentrations were significantly lower in the infertile subgroup (55.7 ± 24.1%) than in the controls (68.6 ± 27.8%). The rates of abnormal sperm chromatin integrity were significantly higher in the abortion (16.7 ± 7.7%) and infertile (16.3 ± 6.6%) subgroups, compared to the control group (13.0 ± 4.4%).			The sperm chromatin integrity was a significant predictor for future abortion	
Zhao J, Zhang Q, et al. Fertil Steril. 2014;102(4):998-1005 e8.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Infertility patient(s). pregnancy, 16 cohort studies (3,106 couples) miscarriage: 14 studies (2,756 couples, 965 pregnancies)	sperm DNA damage	pregnancy and miscarriage of IVF/ICSI. Meta-analysis showed that high-level sperm DNA fragmentation has a detrimental effect on outcome of IVF/ICSI, with decreased pregnancy rate and increased miscarriage rate. The stratified analysis by type of procedure (IVF vs. ICSI) indicated that high sperm DNA damage was related to lower pregnancy rates in IVF but not in ICSI cycles, whereas it was associated with higher miscarriage rates in both IVF and ICSI cycles.			The results indicate that assays detecting sperm DNA damage should be recommended to those suffering from recurrent failure to achieve pregnancy.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability Setting	Diagnostic test evaluated Reference standard test Include: Time interval and treatment	Prevalence	Accuracy (Se, Sp, PPV, NPV, LR+, LR-)	Reproducibility	Authors conclusion	Comments
Zidi-Jrah I, et al. Fertility and sterility 2016;105: 58-64.	Descriptive study		22 couples with history of RPL and 20 fertile men.	): Semen samples from case and control men were examined for differences in semen parameters, DNA fragmentation, chromatin condensation, and sperm aneuploidy.	Sperm progressive motility (30.2% vs. 51.5%) was significantly lower and abnormal morphology (74.8% vs. 54.2%) was significantly higher in the RPL group versus the control group, respectively. The percentage of fragmented DNA was significantly increased in the RPL group (17.1% vs. 10.2%) as well as the rate of spermatozoa with nuclear chromatin decondensation (23.6% vs. 11.8%). There was a significantly higher sperm aneuploidy rate among the RPL group as well			The increase in abnormal sperm parameters, sperm DNA fragmentation, nuclear chromatin decondensation, and sperm aneuploidy suggest possible causes of unexplained RPL.	
West R et al. Human Reprod. 2022	Secodary analysis of RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	1215 had measures of DNAq of which 1162 couples had embryo transfers	the assays of DNAq essentially following the published protocols for acridine orange (AO) staining, the alkaline comet assay, TUNEL assay, SCD assay and aniline blue (AB) assay.	All measures of HBS and DNAq discriminated normal from abnormal sperm samples ($p < 0.001$). SCD correlated negatively with the Comet ($r = -0.165$; $p < 0.001$) and TUNEL assays ($r = -0.200$; $p < 0.001$). HBS correlated negatively with AO ($r = -0.211$; $p < 0.001$), Comet ($r = -0.127$; $p < 0.001$) and TUNEL ($r = -0.214$; $p < 0.001$) and positively with SCD ($r = 0.255$; $p < 0.001$) and AB ($r = 0.127$; $p < 0.001$).	A parsimonious model for predicting live birth (and miscarriage) rates included treatment allocation (OR 2.167, 95% CI 1.084-4.464, p=0.031), female age (OR 0.301, 95% CI 0.133-0.761, p=0.013, per decade) and the AO assay (OR 0.79, 95% CI 0.60-1. 02.761, p=0.073, per 10 points rise). For couples failing to establish a clinical pregnancy, the model retained only the AB assay (OR 0.81, 95% CI 0.678-0.956-1.075, p=0.016, per 10 points rise).		PICSI adversely affected fertlisation rates and did not improve cumulative pregnancy rates.	

Overview studies assessing sperm paremeters in RPL couples and controls

	RPL	controls	pH	volume	Sperm motility	Sperm morphology	DNA fragmentation index	DNA integrity	Seminal viscosity	Sperm count
Gopalkrishnan, 2000	32	51	No diff	No diff	No diff	More head abnormality			Sign different	
Bhattacharya, 2008	74	65			Lower (total motile sperm and % of motile sperm)	No difference		Sign lower		
Brahem, 2011	31	20			Sign lower		Sign higher			
Imam, 2011	20	20			Sign lower	Sign lower	Sign higher			
Khadem, 2014	30	30		No diff	No difference in % motile	Sign lower % with normal morphol	Sign higher mean percentage DNA fragm (43.3% versus 16.7%, P = 0.024).		No diff	
Talebi, 2012	40	40			No difference in % progressively motile	No diff in % with normal morphol		Sign different		No diff
Sbracia, 1996	120	30		No diff	No diff	No diff in total no of alterations				
Zhang, 2012	111	30		No diff	No diff (forward motility)	No diff in % with normal morphol		No diff in % abnormal sperm chromatin integrity		No diff

Additional references included as background information

Aitken RJ, De Iuliis GN, McLachlan RI. Biological and clinical significance of DNA damage in the male germ line. *Int J Androl* 2009;**32:** 46-56.

Anifandis G, Bounartzi T, Messini CI, Dafopoulos K, Sotiriou S, Messinis IE. The impact of cigarette smoking and alcohol consumption on sperm parameters and sperm DNA fragmentation (SDF) measured by Halosperm((R)). *Arch Gynecol Obstet* 2014;**290:** 777-782.

Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. *Nat Rev Urol* 2010;**7:** 153-161.

Hsu PC, Chang HY, Guo YL, Liu YC, Shih TS. Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage. *Fertil Steril* 2009;**91:** 1096-1103.

Jensen TK, Gottschau M, Madsen JO, Andersson AM, Lassen TH, Skakkebaek NE, Swan SH, Priskorn L, Juul A, Jorgensen N. Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open* 2014;**4:** e005462.

Pacey AA, Povey AC, Clyma JA, McNamee R, Moore HD, Baillie H, Cherry NM, Participating Centres of Chaps UK. Modifiable and non-modifiable risk factors for poor sperm morphology. *Hum Reprod* 2014;**29:** 1629-1636.

Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol* 2013;**11:** 66.

Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2014**:** Cd007411.

Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online* 2014;**28:** 684-703.

11. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RM DUE TO GENETIC/CHROMOSOMAL CAUSES TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Basile N, Nogales Mdel C, et al. Fertil Steril. 2014;101(3): 699-704. (24424365)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias x☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	504 embryos undergone PGS 127 women, 40 RM	Time lapse embryoscope and day 3 biops Array cGH			t5 -t2 and CC3 can differentiate abnormal and normal embryos	Looking a morphokinetc analysis
Brezina PR, et al. Journal of assisted reproduction and genetics 2016;33: 823-832.	Systematic Review	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- X **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	various diagnostic platforms currently available to perform preimplantation genetic testing for aneuploidy and describe in a clear and balanced manner the various strengths and weaknesses of these technologies.	PGS is emerging as one of the most valuable tools to enhance pregnancy success with assisted reproductive technologies. While all of the current diagnostic platforms currently available have various advantages and disadvantages, some platforms, such as next-generation sequencing (NGS), are capable of evaluating far more data points than has been previously possible. The emerging complexity of different technologies, especially with the utilization of more sophisticated tools such as NGS, requires an understanding by clinicians in order to request the best test for their patients.				Information on the different techniques, not specific for RPL
De Krom G et al Human Reproduction, Vol.30, No.2 pp. 484–489, 2015	Other	NA	294 couples, RPL, carrying translocation	Genetic counselling and offered PGD				76.9% opted for PGD 8.8% not suitable for PGD
Dong Y, Li LL, et al. Genet Mol Res. 2014;13(2):2849-56. (24535899)	Other	☐ Selection bias ☐Performance bias x☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** x☐ **Acceptable (+)** ☐ **Unacceptable (-)**	control study 113 carrier couples 226 matched controls	No treatment	reproductive outcomes		Delivery rate the same in all groups Risk of misc same	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Franssen MT, Musters AM, et al. Hum Reprod Update. 2011;17(4):467-75. (21504961)	SR	Appropriate question ? Y Rigorous search ? Y Relevant studies included? Y Quality of studies? Methodology ? Good --- ☐ **High quality (++)** x **Acceptable (+)** ☐ **Unacceptable (-)**	Couples with structural Chromosomal abnormality and RM	NC vs PGD - no description of PGD methodology used			Insufficient data for PGD versus NC . no description of PGD methodology	SR – included studies up to April 2009
Ikuma S et al PLOS ONE June 17, 2015	other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	126 couples with RPL & translocation	Natural conception vs PGD PGD FISH on blastomeres				PGD birth rates same, but misc rates lowers
Maithripala 2018	Cohort study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	2321 couples who were referred to a highly specialized RPL clinic for ongoing clinical management + Couples who pursued PGD through local fertility centres during this study time (n = 13).	between January 2005 and December 2013		Thirty-six couples (1.6%) were found to be parental carriers of a structural chromosomal rearrangement. In this cohort, couples were twice as likely to pursue natural conception compared with IVF with PGD. No significant differences were observed in live birth rate between PGD and clinical management (66.6% vs. 53.3%, P = 0.717).	parental carriers of structural chromosomal rearrangement and history of RPL are more likely to pursue natural conception over IVF and PGD.	
Murugappan G, et al. Hum Reprod 2016;31: 1668-1674.	Retrospective cohort study	X Selection bias X Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** x☐ **Acceptable (+)**	300 RPL patients treated between 2009 and 2014. 2 academic fertility centers	IVF + PGS compared with expectant management (EM), 112 patients desired PGS 188 patients chose EM.	pregnancy rate and LB per attempt and CM rate per pregnancy. One attempt was defined as an IVF cycle followed by a	In the IVF group, 168 retrievals were performed and 38 cycles canceled their planned PGS. Cycles in which PGS was intended but cancelled had a significantly lower LB rate (15 versus 36%, P = 0.01) and higher CM rate (50 versus 14%, P < 0.01) compared with	Among all attempts at PGS or EM among RPL patients, clinical outcomes including	patients who elected for IVF/PGS may have had different clinical prognoses than patients who elected for expectant management

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
		☐ **Unacceptable (-)**			fresh embryo transfer or a frozen embryo transfer (PGS group) and 6 months trying to conceive (EM group).	cycles that completed PGS despite similar maternal ages. Of the 130 completed PGS cycles, 74% (n = 96) yielded at least one euploid embryo. Clinical pregnancy rate per euploid embryo transfer was 72% and LB rate per euploid embryo transfer was 57%. Among all attempts at PGS or EM, clinical outcomes were similar. Median time to pregnancy was 6.5 months in the PGS group and 3.0 months in the EM group.	pregnancy rate, live birth (LB) rate and clinical miscarriage (CM) rate were similar.	
Musters AM, Repping S, et al. Fertil Steril. 2011;95(6):2153-7, 7.e1-3. (21215967)	SR	Appropriate question ? Y Rigorous search ? Y Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** x **Acceptable (+)** ☐ **Unacceptable (-)**	Unexplained RM !!	limited FISH probes			LBR similar PGD vs NC unable to perform meta-analysis	SR – included studies up to Dec 2009
Sato T et al., Hum. Reprod. 2019;34: 2340-2348.	Prospective study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	A total of 171 patients were recruited for the study: an RPL group, including 41 and 38 patients treated respectively with and without PGT-A, and an RIF group, including 42 and 50 patients treated respectively with and without PGT-A. Patients in the RPL group had at least one case of aneuploidy ascertained through prior POC testing.	Women with RPL +PGT-A vs women with RPL without PGT-A From January 2017 to June 2018	LBR? miscarriage rate and the frequency of euploidy, trisomy and monosomy in the blastocysts	PGT-A was shown to reduce the biochemical pregnancy loss (12.5% vs 45.0%; aOR 0.14; 95%CI 1.28-10.95) and increase the live birth rate per embryo transfer in RPL women (52.4% vs 21.6%; aOR 3.89; 95%CI 1.16-13.1). However, there were no significant difference in the live birth rates per patient undergoing or not undergoing PGT-A (26.8% vs 21.1%).	A large portion of pregnancy losses in the RPL group might be due to aneuploidy, since PGT-A reduced the overall incidence of pregnancy loss in these patients.	
Shahine LK, et al. Fertility and sterility 2016;106: 1124-1128.	Prospective cohort	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---	239 patients with RPL, defined as two or more clinical miscarriages, were screened for inclusion. 102 cycles in patients with unexplained RPL resulted in at	IVF with blastocyst biopsy and aneuploidy screening of all 23 chromosome pairs. Outcomes were compared by ovarian reserve test results, with diminished ovarian	Rate of aneuploidy in blastocysts and incidence of IVF cycles with no transfer owing to no euploid blasts.	Patients with DOR had a higher percentage of aneuploid blastocysts (57% vs 49%) and a higher incidence of no euploid embryos to transfer (25% vs 13%). The higher rate of aneuploidy in blastocysts was most significant in	RPL patients with DOR have a higher percentage of aneuploid blastocysts and risk of no	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
		☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	least one euploid embryo transferred.	reserve (DOR) defined as a cycle day 3 FSH >10 IU/mL and/or antimullerian hormone <1 ng/mL.		patients aged <38 years (67% vs 53%). Implantation rates after transfer of euploid blastocysts were similar (61% compared with 59%), and miscarriage rates were low (14% and 10%).	euploid embryo to transfer compared with RPL patients with normal ovarian reserve.	

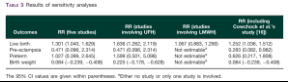
Additional references included as background information
None

12. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RPL DUE TO THROMBOPHILIA + ANTIPHOSPHOLIPID SYNDROME TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
de Jong PG, et al. The Cochrane database of systematic reviews 2014;7: CD004734.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- X **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Recurrent miscarriage 1228 women (≥2 RPL up to 24 weeks) 9 RCTs with or without inherited thrombophilia: where possible subgroup with inherited thrombophilia	Anticoagulant (Aspirin , and/or heparin - UFH,LMWH-) treatment was started at a maximum of 12 weeks' gestation and continued beyond 32 weeks' gestation or until end of pregnancy	LBR	LMWH versus aspirin (3 RCTS): RR 1.16 (0.93-1.45, n=325 , I^2=67%) LMWH vs no treatment (3RCTs): RR 1.23 (0.84-1.81, n=453, I^2=80%) LMWH+aspirin vs no treatment (2 RCTs): RR 1.01 (0.87-1.16, n=322) *Subgroup; women with inherited thrombophilia; potential benefit for LMWH - aspirin, but underpowered (RR 1.25, 95% CI 0.74 to 2.12).* LMWH and aspirin versus aspirin: (2RCTs): RR 1.11, 0.94-1.30, n=327) LMWH with aspirin versus LMWH: (1RCT) RR 0.91,0.72-1.15, n=126) LMWH with or without aspirin versus no treatment : (5 RCTs) : RR 1.07; 0.99-1.15- n=793) Aspirin vs placebo : (2RCTs) RR 0.94, 0.80-1.11, n=256) *Subgroup; inherited thrombophilia; RR* 1.08 (0.0.63-1.85- 1RCT) Obstretric complications not sign affected by treatment LMWH+aspirin increased risk for bleeding 40% local skin reactions		
Hamulyak EN et al., AJOG 1996;174:	Cochrane review	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies?	Eleven studies (1672 women); nine randomised controlled trials and two quasi-RCTs.	The dose and type of heparin and aspirin varied among studies. One study compared aspirin alone with placebo; no studies compared		A benefit of heparin (UFH or LMWH) and aspirin, as compared to aspirin alone, with regard to live birth was reported (RR 1.27; 95%CI	The ombination of heparin (UFH or LMWH) plus aspirin during	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
1584-1589		Methodology ? --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**		heparin alone with placebo and there were no trials that had a no treatment comparator arm during pregnancy; five studies explored the efficacy of heparin (either UFH or LMWH) combined with aspirin compared with aspirin alone; one trial compared LMWH with aspirin; two trials compared the combination of LMWH plus aspirin with the combination of UFH plus aspirin; two studies evaluated the combination of different doses of heparin combined with aspirin. All trials used aspirin at a low dose. Aspirin versus placebo		1.09-1.49, 5 studies, n= 1295). Heparin plus aspirin may reduce the risk of pregnancy loss (RR 0.48; 95%CI 0.32 to 0.71, 5 studies, n=1295).	the course of pregnancy may increase live birth rate in women with persistent aPL when ompared with aspirin treatment alone. The observed beneficial effect of heparin was driven by one large study in which LMWH plus aspirin was compared with aspirin alone.	
Empson M,et al The Cochrane database of systematic reviews 2005: Cd002859.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	RPL + aPL/LAC Pregnant women with at least one fetal loss and evidence of antiphospholipid antibodies. (aCL or LA) 13 RCTs N=849 (same studies as Wisloff 2004 + Vaquero 2001)	aspirin, unfractionated heparin, low molecular weight heparin, prednisone, intravenous immunoglobulin and plasmapheresis.	Pregnancy loss Preterm delivery, …	<u>Heparin</u> uFH/Asp vs Asp : RR 0.46 – CI 0.29-0.71 (2RCTs-n=140) LMWH vs asp RR 0.78 – CI 0.39-1.57 (1RCT-n=98) LMWH vs IVIG; RR 0.37 – CI 0.12-1.16 (1RCT-n=40) UFH vs LMWH : no studies high dose UFH/asp vs low dose UFH/Asp : RR 0.83 – CI 0.29-2.38 (1RCT, n=50) <u>Aspirin</u> vs placebo : RR 1.05 – CI0.66-1.68 (3RCTs-n=71) <u>Prednisone</u> Pred+ASP vs placebo or asp: RR 0.85 – CI 0.53-1.36 (2RCTs- n=122) Pred+ASP vs Hep/Asp: RR 1.17 – CI 0.47-2.93 (1RCT, n=45) Adverse outcomes with prednisone: preterm		<u>Prednisone :</u> Based on Laskin 1997 + Silver 1993 AND Cowchock 1992 <u>IVIG</u> : Based on Branch 2000, Triolo 2003 and Vaquero 2001

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						delivery, neonatal intensive care unit admission, rate of pre-eclampsia , hypertension, gestational diabetes, lower birth weight <u>IVIG</u> No reduction in pregnancy loss in any of the 3 RCTs; One study had no pregnancy loss in either the treatment group or the control group (Branch 2000).		
Glueck CJ, et al. Blood coagulation & fibrinolysis 2015;26: 736-742.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	1014 patients with thrombotic events 123 (12%) recurrent miscarriage. Tested for Genes; - MTHFR C677T-A1298C, - factor V Leiden G506A, - prothrombin G20210A serologic - factor VIII - factor XI homocysteine	126 of 1014 (12.4%) patients, had high homocysteine L-methyl folate (5 mg), vitamin B6 (100 mg), and vitamin B12 (2 mg/day),		Median pretreatment homocysteine level (15.6mmol/l) fell to 10.0 on treatment (P<0.0001), and in 56 of the 74 patients (76%), the homocysteine level fell to normal		No discussion of RM group
Kutteh WH. Am J obstet gynecol 1996;174: 1584-1589.								Included in systematic reviews. Only details on when and how to treat are added to the guideline, as additional information
Laskin CA, et al. J Rheumatol 2009: 36: 279-287.								Included in systematic reviews. Only details on when and how to treat are added to the guideline, as additional information

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Mak A, et al Rheumatology. 2010;49(2):281-8.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	RPL + aPL 5/6 RCTs N= 334	Heparin + aspirin vs aspirin only	live birth rate sec: pre-eclampsia, birth weight, prema-turity, premature rupture of membranes (PROM) and fetal death.	hep/asp vs asp only Higher LBR (5RCTs): 74.3% vs 55.8%; RR 1.301: CI 1.40-1.629; NNT 5.6) less pre-eclampsia (RR 0.471; CI 0.096, 2.314) no diff in preterm labour, birth weight 	The combination of heparin and aspirin is superior to aspirin alone in achieving more live births in patients with positive aPL antibodies and RPL.	
Middeldorp S. Hematology Am Soc Hematol Educ Program 2014; 393-399.				Associations between the types of thrombophilia and types of complications, Currently available clinical trial evidence regarding the use of aspirin and heparin to prevent these pregnancy complications. In women with antiphospholipid syndrome, guidelines recommend prescribing aspirin and heparin to women with recurrent miscarriage. The same regimen is suggested for late pregnancy complications by some, but not all, experts. Aspirin or low-molecular-weight heparin to improve pregnancy outcome in women with unexplained recurrent miscarriage has no benefit and should not be prescribed. Whether anticoagulant therapy prevents recurrent miscarriage in women with inherited thrombophilia or in women with severe pregnancy complications remains controversial because of inconsistent results from trials. Aspirin modestly decreases the risk of severe preeclampsia in women at high risk.			Used in the justification as it provides additional information to interpret the results of the systematic reviews
Perricone R, et al. Rheumatology. 2008;47(5):646-51.	Cs	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	24 SLE + recurrent spontaneous abortion	High dose IVIG versus prednisolone and NSAIDs (control)	Pregnancy outcome LBR / Miscarriages C-section / Preterm delivery clinical response [lupus activity index-pregnancy (LAI-P)] symptoms ANA, anti-dsDNA, anti Ro/SS-A or La/SS-B, aCL, LAC, C4, C3	IVIG vs control 100% vs 75% 0 vs 3 (week 7,11 and 23) 91.7% vs 66.7% 25% vs 55.6% Sign decrease (0.595) at the end vs beginning of pregnancy for IVIG group (p<0.0001), not in control group.		SLE patients

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Skeith L, et al. Blood 2016.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	women with inherited thrombophilia and prior late (>/=10 weeks) or recurrent early (<10 weeks) pregnancy loss. 8 RCTS 483 WOMEN	LMWH versus no LMWH (if aspirin in both arms it was ignored)	LBR	LMWH compared to no LMWH (RR 0.81, 95% CI, 0.55 to 1.19, p=0.28), no significant difference	no benefit of LMWH in preventing recurrent pregnancy loss in women with inherited thrombophilia.	
Zhang T, et al. for Medicin. 2015;94(45): e1732.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Recurrent Miscarriage:	Antithrombotic Treatment	LBR Bayesian Network Meta-Analysis and Systematic Review.			RELEVANT ??
			<u>Patients With or Without Thrombophilia</u> 2391 patients - 362 aspirin, - 801 LMWH - 388 LMWH + aspirin 840 placebo or intensive surveillance group		treatments vs placebo : no significant effect of improving LBR LMWH vs aspirin:OR2.02, 95% CI 1.13–3.95);LMWH had the highest SUCRA (85.10%) and showed the greatest probability (61.48%) of being ranked first to improve LBR - aspirin had the lowest SUCRA (7.00%) and showed the greatest probability of being least beneficial (82.04%). Consistent in pairwise analysis Other comparisons not sign.		
			<u>Patients with APS</u> 543 patients - 232 aspirin, - 80 LMWH - 103 LMWH + aspirin - 108 UFH+aspirin - 20 placebo		treatments vs placebo : no significant effect of improving LBR UFH and aspirin had the highest SUCRA (75.50%) and showed the greatest probability (75.15%) of being at the top 2 positions in the effect of reducing PL, followed by LMWH (SUCRA,71.00%; being in the top 2 places with probability of 65.87%). Whereas aspirin had the lowest SUCRA (23.00%) pair-wise meta-analysis (PW) and sensitivity analysis (SA): UFH plus aspirin vs aspirin: (PW: OR 2.47, 95% CrI 1.36–4.52; SA:OR2.54, 95% CI 1.54–4.31) LMWH alone vs aspirin (PW: OR 2.42, 95% CI 1.04–5.66; SA: OR 2.42, 95% CI 1.09–5.62) significantly improved live births		
Ziakas PD et al. Obstet Gynecol.	SR	Appropriate question ? Rigorous search ? Relevant studies included?	RPL + APS 5 RCTs	Heparin + aspirin vs aspirin only	First trim losses ()	<u>LMWH or UFH+ ASP vs ASP:</u> OR 0.39, 95% CI 0.24–0.65 number needed to treat 6, I^2=10%).	UFH and aspirin confers a significant	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
2010;115(6): 1256-62.		Quality of studies? Methodology ? --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	N=398		late-pregnancy losses	Combo=better UFH: OR 0.26, CI 0.14-0.48 , NNT 4; 3RCTs, n=212 LMWH: OR 0.70, CI 0.34-1.45; 2 RCTs, n=186 LMWH or UFH+ ASP vs ASP: (OR 1.07, 95% CI 0.36–3.16 – n=291) UFH: OR 0.52, CI 0.11-2.46; 3RCTs, n=141 LMWH: OR 2.28, CI 0.43-12.13; 2 RCTs, n=150 UFH versus LMWH: comparablel effectiveness (Noble – Stephenson)	benefit in live births. The efficacy of LMWH plus aspirin remains unproven	

Additional references included as background information

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO, American College of Chest P. VTE, thrombophilia, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012;**141:** e691S-736S.

Bates SM, Rajasekhar A, Middeldorp S, McLintock C, Rodger MA, James AH, Vazquez SR, Greer IA, Riva JJ, Bhatt M et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: venous thromboembolism in the context of pregnancy. Blood advances 2018;2: 3317-3359.

Skeith L, Bates SM, Bates V, Rodger MA. The challenges and lessons learned in conducting clinical trials in pregnant women with antiphospholipid syndrome. Thrombosis research 2020;194: 54-56.

13. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RPL WITH SUSPICION OF IMMUNOLOGICAL BACKGROUND TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Laskin CA, et al. N Engl J Med 1997;337: 148-153.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	773 nonpregnant RPL women were screened for ANA, anti-DNA, antilymphocyte, and anticardiolipin antibodies and lupus anticoagulant. 385 women had at least one autoantibody, 202 who later became pregnant women were stratified according to age (18-34 years or 35-39 years) and the week of gestation at which the previous fetal losses had occurred (< or = 12 or > 12 weeks).	202 pregnant (RPL with at least 1 Ab) were randomly assigned in equal numbers to receive either prednisone (0.5 to 0.8 mg per kilogram of body weight per day) and aspirin (100 mg per day) or placebo for the duration of the pregnancy.	successful pregnancy Live infants were born to 66 women in the treatment group (65 %) and 57 women in the placebo group (56 %, P=0.19). More infants were born prematurely in the treatment group than in the placebo group (62% vs. 12%, P<0.001). The major side effects of therapy in the mothers were hypertension (treatment group, 13 %; placebo group, 5 %; P=0.05) and diabetes mellitus (15 % and 5 %, P=0.02).		Treating women who have autoantibodies and recurrent fetal loss with prednisone and aspirin is not effective in promoting live birth, and it increases the risk of prematurity.	
Moraru M, Carbone J, et al. Am J Reprod Immunol. 2012;68(1):75-84. (22509929)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	157 women with previous recurrent miscarriage and/or recurrent implantation failure after in vitro fertilization Consecutively 64 selected with CD56(+) cell expansion, no apparent underlying disease and who maintained their desire to conceive	Intravenous immunoglobulin therapy => 40 patients received IVIG during pregnancy	clinical pregnancy rate for the women under IVIG therapy was 92.5% and the live birth rate was 82.5%. Significantly lower pregnancy and live birth rates (25% and 12.5%, respectively) were observed for the patients with recurrent pregnancy loss and NK/NKT-like cells expansion without IVIG. After three cycles of IVIG, NK cell percentages decreased significantly and these values persisted throughout gestation.		IVIG for women with recurrent reproductive failure and NK or NKT-like cell expansion was a safe and beneficial therapeutic strategy that associated with high clinical pregnancy and live birth rates.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Nielsen HS, Christiansen OB. Hum Reprod. 2005;20(6):1720-8.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	women with recurrent miscarriage negative for the lupus anticoagulant.	No therapy	Prognostic impact of anticardiolipin antibodies			Q5 : prognostic value of ACL Ab
Stricker RB, Winger EE. Am J Reprod Immunol. 2005;54(6):390-6. (16305665)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	99 women were prospectively evaluated for immunologic abortion, which was defined as three or more miscarriages and the presence of specific immunologic abnormalities. The average age of the women was 37 years (range: 28-49), and the average number of miscarriages was 3.8 (range: 3-12).	Prior to the next conception, patients were treated with IVIG at a dose of 0.2 g/kg. Once conception was achieved, IVIG treatment was continued on a monthly basis through 26-30 weeks of pregnancy. 72 women received initial IVIG treatment, and 50 subsequently became pregnant.	42/50 women (84%) had a successful term pregnancy. Of the 27 women who refused IVIG therapy, 20 became pregnant and 18 (90%) miscarried. The difference in pregnancy success rate between the IVIG-treated and untreated groups was significant (P = 0.001). Four women had mild allergic reactions during IVIG infusion, and these reactions resolved when the IVIG brand was changed. Fetal abnormalities were not observed.		low-dose IVIG therapy is safe and effective for older women with immunologic abortion.	
Tang AW, Alfirevic Z, et al. Hum Reprod. 2013;28(7):1743-52. (23585559)	Pilot RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- X **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	160 eligible women were screened. The endometrium was sampled 5-9 days after the LH surge, stained using immunohistochemistry for CD56 and the sub-epithelial region analysed with image analysis. Women with a high uNK cell density (>5%) (n=72) were invited to contact the clinic at 4-6 weeks gestation for randomization.	prednisolone (20 mg for 6 weeks, 10 mg for 1 week, 5 mg for 1 week) or identical placebo tablets. 40 women were randomized	Prednisolone was associated with side effects of insomnia and flushing. Live birth rate : 12/20 (60%) with prednisolone and 8/20 (40%) with placebo (RR 1.5, 95% CI 0.79-2.86, absolute difference 20% CI-10%, +50%), (not significant) There were no pregnancy complications or serious adverse fetal outcomes.		It was feasible to recruit women with idiopathic RM into a 'screen and treat' trial despite their desire for active medication.	
Thangaratinam S, et al .: of evidence. BMJ 2011;342:d2	meta-analysis	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias	30 articles with 31 studies (19 cohort and 12 case-control) - 12 126 women	thyroid autoantibodies Studies varied in the frequency and timing of the autoantibody testing, ranging from testing	association with miscarriage	28 showed a positive association between thyroid autoantibodies and miscarriage.	Association between thyroid autoantibodies and miscarriage	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
616.		☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	assessed the 5 studies with 12 566 women	before pregnancy, in early pregnancy, and after delivery or miscarriage. The commonest threshold concentration of thyroid peroxidase for a diagnosis of positive thyroid autoantibodies was >100 U/ml.		Meta-analysis of the cohort studies showed more than tripling in the odds of miscarriage with the presence of thyroid autoantibodies (odds ratio 3.90, 95% CI 2.48 to 6.12; P<0.001). For case-control studies the odds ratio for miscarriage was 1.80, 1.25 to 2.60; P=0.002)	and preterm birth	
					association in women with RPL	13 studies (3 cohort, 10 case-control): The odds of miscarriage with thyroid autoantibodies was increased for women with recurrent miscarriages (4.22, 0.97 to 18.44; P=0.06) (heterogeneity I^2 =75%)		
					association with preterm birth	doubling in the odds of preterm birth with the presence of thyroid autoantibodies (2.07, 1.17 to 3.68; P=0.01).		
					Effect of treatment with levothyroxine on miscarriage	2 randomised studies: Both showed a fall in miscarriage rates, and meta-analysis showed a significant 52% relative risk reduction in miscarriages with levothyroxine (relative risk 0.48, 0.25 to 0.92; P=0.03). One study reported on the effect of levothyroxine on the rate of preterm birth, and noted a 69% relative risk reduction (0.31, 0.11 to 0.90).		
Winger EE, Reed JL. Am J Reprod Immunol. 2008;60(1):8-16. (18422811)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)**	75 pregnancies in patients with a history of RSA Patient populations in the three treatment groups were similar in terms of age, past	Divided into 3 groups: group I: 21 patients treated with AC (anticoagulants), group II: 37 patients treated with AC and IVIG, and group III: 17 patients treated with AC, IVIG and the TNF inhibitor	The live birth rate was 19% (4/21) in group I, 54% (20/37) in group II, and 71% (12/17) in group III. There was significant improvement in pregnancy outcome in group II versus group I (P = 0.0127) and in group III versus group I (P = 0.0026). The live birth rate in group III compared to group II was not significantly different (P = 0.3723). Side effects of AC, IVIG and TNF inhibitor treatment were		In women with RSA, addition of either IVIG or a TNF inhibitor + IVIG to the AC regimen appears to improve live	

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		☐ **Acceptable (+)** ☐ **Unacceptable (-)**	miscarriages, inherited thrombophilia and autoimmunity.	Etanercept (Enbrel) or Adalimumab (Humira). IVIG was administered at least once during the cycle of conception and/or at least once after a positive pregnancy test. Adalimumab or Etanercept was administered according to standard protocols.	minimal in these patients, and no birth defects were identified in their offspring.		birth rates compared to the treatment with AC alone.	

Additional references included as background information
None

14. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RPL DUE TO METABOLIC ABNORMALITIES OR HORMONAL ABNORMALITIES TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Aghajafari F, et al. BMJ 2013;346: f1169.	meta-analysis	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	3357 studies were identified and reviewed for eligibility. 31 eligible studies were included in the final analysis.	association between serum 25-OHD levels during pregnancy and the outcomes of interest (pre-eclampsia, gestational diabetes, bacterial vaginosis, caesarean section, small for gestational age infants, birth weight, birth length, and head circumference).	Insufficient serum levels of 25-OHD were associated with gestational diabetes (pooled OR 1.49, 95% CI 1.18 to 1.89), pre-eclampsia (1.79, 1.25 to 2.58), and small for gestational age infants (1.85, 1.52 to 2.26). Pregnant women with low serum 25-OHD levels had an increased risk of bacterial vaginosis and low birthweight infants but not delivery by caesarean section.		Vit D insufficiency is associated with an increased risk of gestational diabetes, pre-eclampsia, and small for gestational age infants.	
Al-Biate MA. Taiwan J Obstet Gynecol. 2015;54(3):266-9.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	106 nondiabetic pregnant women with PCOS who became pregnant while using metformin	metformin throughout pregnancy (metformin group - 56) vs discontinuation of metformin once pregnant (control group – n=50).	The rate of early pregnancy loss in the metformin group was 8.9% (5/56) compared with 36% (18/50) in the control group ($p < 0.001$). metformin group: 25 cases with a positive history of EPL in previous pregnancies and 31 had a negative history. For patients with previous EPL, the rate of pregnancy loss was 45% (35 miscarriages/15 live births /50 pregnancies). (no metformin treatment): reduction in rate of PL from 45% to 8.9% In the control group, 20 of the 50 women had a history of previous PL: rate of pregnancy loss was 36% (9 miscarriages/16 live births/25pregnancies) No sign reduction in rate of PL Metformin was well tolerated in all patients. No cessation or reduction in the treatment dose. No side effects or serious complications were observed.		Metformin therapy in pregnant women with PCOS was associated with a significant reduction in the rate of early pregnancy loss.	

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Andrade C. J Clin Psychiatry. 2016;77(4):e411-4.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Review of the safety of metformin administered during pregnancy, with focus on psychological disorders for which metformin is also prescribed.		The available data suggest that metformin exposure during the first trimester is not associated with major congenital malformations; that metformin reduces the risk of early pregnancy loss, preeclampsia, preterm delivery, and GDM in women with PCOD; that metformin is associated with at least comparable benefits relative to insulin treatment in women with mild GDM; and that neurodevelopmental outcomes at age 1.5–2.5 years are comparable after gestational exposure to metformin and insulin.			Not specific for RPL
Bernardi LA, Cohen RN, et al. Fertil Steril. 2013;100(5):1326-31. (23954357)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	286 women with a history ofR2 pregnancy losses <10 weeks.	From 2004–2007, no treatment for women with SCH ([TSH] >2.5 mIU/L with a normal free thyroxine or free thyroxine index); from 2008 onward, levothyroxine treatment prepregnancy to maintain TSH≤2.5 mIU/L.		prevalence of SCH was 55 (19%) The cumulative LBR was 27 (69%) of 39 for women with SCH versus 104 (74%) of 141 for euthyroid women. The per-pregnancy LBR was 34 (49%) of 69 for SCH versus 129 (58%) of 221 for euthyroid women. When the LBR was compared between treated and untreated SCH, the cumulative LBR was 17 (71%) of 24 versus 10 (67%) of 15, respectively. The per-pregnancy LBR for SCH treated versus untreated women was 22 (48%) of 46 versus 12 (52%) of 23, respectively.		
Chen H, et al. The Cochrane database of systematic reviews 2016;7:Cd008883.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	assess the effectiveness and safety of different types of dopamine agonists in preventing future miscarriage given to women with idiopathic hyperprolactinemia and RPL	One study (recruiting 48 women with idiopathic hyperprolactinemia) met our inclusion criteria; 46 women (42 pregnancies - 4/46 women did not conceive during the study period) were included in the analysis. The study compared the use of a dopamine agonist (bromocriptine, 2.5 mg to 5.0 mg/day until the end of the ninth week of gestation) versus a no-treatment control. The study was judged as being at a high risk of bias. It was not possible to carry out meta-analysis due to insufficient data.The study reported both of this review's primary outcomes of miscarriage and live birth. Results from this single study suggest that, compared to no treatment, oral bromocriptine was effective in preventing future miscarriage (risk ratio (RR) 0.28, 95% confidence interval (CI) 0.09 to 0.87, 46 participants (low-quality evidence)) in women with idiopathic hyperprolactinemia. There was no clear difference with regard to the other primary outcome of live births (RR 1.50, 95% CI 0.93 to 2.42, 46 participants (very low-quality evidence)).There was no difference with regard to this review's secondary outcome of			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
				conception (RR 0.92, 95% CI 0.77 to 1.09, 46 participants (very low-quality evidence)) between the group of women who received dopamine (21 out of 24 women conceived) and women in the no-treatment group (21 out of 22 women conceived).				
Clifford K, Rai R, et al. Bmj. 1996;312(7045):1508-11. (8646142)	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	106 ovulatory women with a history of recurrent miscarriage, polycystic ovaries, and hypersecretion of luteinising hormone.	pituitary suppression with a luteinising hormone releasing hormone analogue followed by low dose ovulation induction and luteal phase progesterone (group 1) or were allowed to ovulate spontaneously and then given luteal phase progesterone alone or luteal phase placebo alone (group 2).	Conception and live birth rates over six cycles.	conception rates in the pituitary suppression and luteal phase support groups were 80% (40/50 women) and 82% (46/56) respectively (NS). Live birth rates were 65% (26/40) and 76% (35/46) respectively (NS). In the luteal phase support group there was no difference in the outcome of pregnancy between women given progesterone and those given placebo pessaries.	Prepregnancy suppression of high luteinising hormone concentrations in ovulatory women with recurrent miscarriage and hypersecretion of luteinising hormone does not improve the outcome of pregnancy.	
Coomarasamy A, , et al. N Engl J Med. 2015;373(22):2141-8.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	PROMISE trial 836 women with unexplained recurrent miscarriages 18 to 39 years of age actively trying to conceive naturally RM = 3 or more consecutive or nonconsecutive losses of pregnancy in the first trimester Exclusion criteria: - unable to conceive naturally within 1 year after recruitment; - APS or other thrombophilic conditions;	Twice-daily vaginal suppositories containing either 400 mg of micronized progesterone or matched placebo from a time soon after a positive urinary pregnancy test (and no later than 6 weeks of gestation) through 12 weeks of gestation.	Live birth after 24 weeks of gestation newborn survival	rate of live births was 65.8% in the progesterone group vs 63.3% in placebo group (RR 1.04; 95% CI 0.94 to 1.15; rate difference, 2.5 percentage points; 95% CI, −4.0 to 9.0). There were no significant between-group differences in the rate of adverse events. no significant between-group differences in the rates of clinical pregnancy (at 6 to 8 weeks), ongoing pregnancy (at 12 weeks), ectopic pregnancy, miscarriage, stillbirth, and neonatal outcomes, as well as in the median gestational age at miscarriage	Progesterone therapy in the first trimester of pregnancy did not result in a significantly higher rate of live births among women with unexplained RM	Unexplained RM

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			- uterine cavity abnormalities - abnormal parental karyotype, - other identifiable cause of RM such as diabetes, thyroid disease, or SLE - currently receiving heparin therapy; Contraindications to progesterone					
De-Regil LM, et al. The Cochrane database of systematic reviews 2016: CD008873.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	15 trials assessing a total of 2833 women, 9 compared the effects of vitamin D alone versus no supplementation or a placebo 6 trials compared the effects of vitamin D and calcium with no supplementation. Risk of bias in the majority of trials was unclear and many studies were at high risk of bias for blinding and attrition rates.	To examine whether oral supplements with vitamin D alone or in combination with calcium or other vitamins and minerals given to women during pregnancy can safely improve maternal and neonatal outcomes.	Vitamin D alone versus no supplementation or a placebo Data from seven trials involving 868 women consistently show that women who received vitamin D supplements alone, particularly on a daily basis, had higher 25-hydroxyvitamin D than those receiving no intervention or placebo, but this response was highly heterogeneous. Also, data from two trials involving 219 women suggest that women who received vitamin D supplements may have a lower risk of pre-eclampsia than those receiving no intervention or placebo (8.9% versus 15.5%; risk ratio (RR) 0.52; 95% CI 0.25 to 1.05, low quality). Data from two trials involving 219 women suggest a similar risk of gestational diabetes among those taking vitamin D supplements or no intervention/placebo (RR 0.43; 95% CI 0.05, 3.45, very low quality). There were no clear differences in adverse effects, with only one reported case of nephritic syndrome in the control group in one study (RR 0.17; 95% CI 0.01 to 4.06; one trial, 135 women, low quality). Given the scarcity of data for this outcome, no firm conclusions can be drawn. No other adverse effects were reported in any of the other studies. With respect to infant outcomes, data from three trials involving 477 women suggest that vitamin D supplementation during pregnancy reduces the risk preterm birth compared to no intervention or placebo (8.9% versus 15.5%; RR 0.36; 95% CI 0.14 to 0.93,		Supplementing pregnant women with vitamin D in a single or continued dose increases serum 25-hydroxyvitamin D at term and may reduce the risk of pre-eclampsia, low birthweight and preterm birth. Data on adverse effects were lacking in all studies.	

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					moderate quality).			
Dhillon-Smith RK et al., NIHR Journals Library 2019	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	1420 women were eligible and 952 were randomised to receive levothyroxine (n = 476) or placebo (n = 476)	levothyroxine at a dose of 50 µg daily or placebo. (n = 476) or placebo (n = 476) 49 hospitals across the UK between 2011 and 2016	LBR; gestation at delivery; birthweight; appearance, pulse, grimace, activity and respiration (Apgar) scores; congenital abnormalities; and neonatal survival at 28 days of life.	The live birth rate was 37% in the levothyroxine group and 38% in the placebo group, (RR 0.97; 95%CI 0.83-1.14, P= 0.74).	Levothyroxine therapy in a dose of 50 µg per day does not improve live birth rate in euthyroid women with thyroid peroxidase antibodies	
Hirahara F, Andoh N, et al. Fertil Steril. 1998;70(2):246-52. (9696215)	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	24 RM patients with hyperprolactinemia and 24 RM patients with occult hyperprolactinemia. no other etiologic abnormalities, including ovarian or endocrinologic disturbances such as luteal phase dysfunction, polycystic ovaries, hypersecretion of LH, galactorrhea, or thyroid hormone disorders. normal weight	Bromocriptine (2.5–5.0 mg/d, depending on individual response) From before conception until the end of the 9th week of gestation (n=24) No treatment (n=22) 2 drop-outs	Successful pregnancy (live birth)	The percentage of successful pregnancies was higher in the bromocriptine-treated group than in the group that was not treated with bromocriptine (85.7% versus 52.4%, P < .05). Serum prolactin levels during early pregnancy (5–10 weeks of gestation) were significantly higher in patients who miscarried (31.8–55.3 ng/mL) than in patients whose pregnancies were successful (4.6–15.5 ng/mL, P < .01 or P < .05).	Appropriate circulating levels of prolactin may play an important role in maintaining early pregnancy, especially in cases of hyperprolactinemic RPL.	

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Jakubowicz DJ, Iuorno MJ, et al. J Clin Endocrinol Metab. 2002;87(2):524-9. (11836280)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	96 women with PCOS that became pregnant	Metformin during pregnancy (n=65) versus no treatment (n=31)	Early pregnancy loss rate	8.8% (6 of 68 pregnancies), vs 41.9% (13 of 31 pregnancies) in controls (P < 0.001). Subset with a prior history of miscarriage: ,11.1% (4 of 36 pregnancies) versus 58.3% (7 of 12 pregnancies) (P = 0.002).	Metformin administration during pregnancy reduces first-trimester pregnancy loss in women with the polycystic ovary syndrome.	Not RM patients
Johnson P, Pearce JM. Bmj. 1990;300(6718):154-6. (2105793)	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	42 women with polycystic ovarian disease and primary recurrent spontaneous abortions	Ovulation was induced by clomiphene or pituitary suppression with buserelin followed by pure follicle stimulating hormone.		Spontaneous abortions occurred in 11 of 20 women given clomiphene compared with two of 20 who had pituitary suppression.	Pituitary suppression before induction of ovulation significantly reduces the risk of spontaneous abortion in women with polycystic ovarian disease and primary recurrent spontaneous abortions.	pituitary suppression before induction of ovulation
Khan I et al., Expert Rev Clin Pharmacol 2017;10: 97-109.	RCT	☐ Selection bias X Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	160 pregnant women were enrolled and equally dis-tributed into groups A (LMWH) and B (placebo)	Group A received a daily dose of 40 mg enoxaparin (LMWH) subcutaneously and group B women received a placebo in the form of multivitamin tablets during April 2013 to January 2014	LBR	The groups were similar in terms of mean age, gestational age and body mass index. Our results showed no statistically significant difference in live birth rates between the two groups, with 78.8% and 73.8% for group A and B, respectively (p=0.0574). A RR of 1.07 (95% CI 0.9 - 1.3) was calculated for group A.	Subcutaneous enoxaparin in a once daily dose of 40 mg did not improve the chance of live births in nonthrombophilic women with unexplained RPL when compared with	

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							the placebo.	
Khattab S, Mohsen IA, et al. Gynecol Endocrinol. 2006;22(12):680-4. (17162710)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	prospective cohort study 200 non-diabetic PCOS patients under ART 120 pregnant control group: 80 who discontinued metformin use at the time of conception or during pregnancy comparable groups	metformin before pregnancy, continued taking metformin at a dose of 1000-2000 mg daily throughout pregnancy	Rates of early pregnancy loss	11.6% in metformin group vs 36.3% in the controls ($p < 0.0001$; OR 0.23, 95% CI 0.11-0.42).	Administration of metformin throughout pregnancy to women with PCOS was associated with a marked and significant reduction in the rate of early pregnancy loss.	NOT RPL
Lata K, Dutta P, et al. Endocr Connect. 2013;2(2):118-24. (23802061)	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	100 pregnant women with recurrent miscarriage 31 thyroid autoimmunity (thyroid peroxidase antibody (TPOAb(+)) >34 U/ml) Rm= 2 or more consecutive miscarriages Control: 100 pregnant women without a history of miscarriage 27.0+/-3.1 years.	levothyroxine (l-T4) therapy. All patients with TPOAbC were treated with 25 mg L-T4 and titrated according to TSH at the time of recruitment into the study. The patients who had subclinical hypothyroidism were treated as deemed necessary.	obstetric outcome spontaneous abortion, hypertensive complications, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy, preterm labour, IUGR, postdatism, preterm premature rupture of membranes and post partum haemorrhage. Neonatal outcomes :prematurity	The incidence of subclinical hypothyroidism was higher in TPOAb(+) group than in TPOAb(-) group (52 vs 16%; P=0.0002). no difference in the prevalence of miscarriage or obstetric outcomes between recurrent miscarriage and healthy pregnant women group irrespective of TPO status.		Conclusion unclear; no comparison treated vs not treated

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					(delivery between 20 and 37 weeks), APGAR score, birth weight and congenital malformation.			
Lepoutre T, et al. Gynecologic and obstetric investigation 2012;74: 265-273.	Case control	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	537 consecutive iodine-supplemented women with a singleton pregnancy [441 TPOAb- controls and 96 TPOAb+ women (47 nontreated and 49 treated)] if TSH exceeded 1 mU/l in TPOAb+ women, 50 microg of levothyroxine (L-T4) was prescribed.	thyroid and obstetric parameters.	The miscarriage rate was significantly higher in the nontreated TPOAb+ group compared with the treated group (16 vs. 0%; p = 0.02). Compared to the control group, TSH in TPOAb+ patients was higher at the first prenatal visit prior to L-T4 treatment (p < 0.01), while free thyroxine was higher than in the control group after the 20th week (p < 0.05).		Our study supports the potential benefit of universal screening and L-T4 treatment for autoimmune thyroid disease during pregnancy.	
Li TC, Ding SH, et al. Fertil Steril. 2001;75(2):434-7. (11172853)	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	21 subjects with otherwise unexplained recurrent miscarriage who had retarded endometrial development in the mid-luteal phase.	Controlled ovarian stimulation using human menopausal gonadotropins and repeat endometrial biopsy in the treatment cycle in 13 subjects.	Histological dating of endometrial biopsy in treatment cycles and miscarriage rate in treatment and nontreatment cycles.	11 (85%) of the 13 biopsies in the treatment cycle were found to be normal. The miscarriage rate in the treatment group, 2 of 13, was significantly lower than that in the nontreatment group (7/12) (chi2 5.0, P<.05).	preliminary experience suggests that controlled ovarian stimulation by human menopausal gonadotropins in the follicular phase is an effective treatment for luteal phase defect associated with recurrent pregnancy loss.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Morley LC, Simpson N, et al. Cochrane Database Syst Rev. 2013;1:Cd008611.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Women with a history of three or more consecutive unexplained miscarriages prior to 24 weeks of gestation, who had a confirmed pregnancy. The target population of this review were women with truly unexplained miscarriage after routine investigations. 5 RCTS/ 596 women (El-Zibdeh 2005;Harrison 1985; Harrison 1992; Quenby 1994; Svigos 1982).	Human chorionic gonadotrophin versus control	Primary outcomes 1. First trimester pregnancy loss (less than 12 completed weeks of gestation) 2. Second trimester pregnancy loss 3. Stillbirth Secondary outcomes 1. Threatened miscarriage 2. Low birthweight (less than 2500 g) 3. Prematurity (gestation less than 37 completed weeks) 4. Neonatal death (less than 28 days of delivery) 5. Adverse effects: maternal and fetal 6. Cost	1st trimester miscarriage: statistically significant benefit in using hCG (risk ratio (RR) 0.51, 95% CI 0.32 to 0.81; 5 studies, 302 women, I2 = 39%) With the random-effects model applied to all 5 studies, the risk ratio was 0.55 (95% CI 0.28 to 1.09) Adverse effects hCG in pregnancy was safe for both mother and baby. None of the studies reported any adverse effects from the use of hCG. congential defects The RR calculated from the results of El-Zibdeh 2005 and Svigos 1982 was 1.05 (CI 0.16 to 7.12), suggesting no increased risk of congential defects when using hCG.		Review also included in UNEXPLAINED RM !
Negro R, et al. Hum Reprod. 2005 Jun;20(6):1529-33.			484 euthyroid women Undergoing ART 412 TPOAb negative 72 TPO-Ab+ group A (n = 36) underwent LT4 treatment, group B (n = 36) placebo	levothyroxine (LT4) versus placebo All controlled ovarian stimulation	pregnancy rate, miscarriage rate and delivery rate.	No differences in pregnancy rate were observed between the three groups. Miscarriage rate was higher in TPOAb (+) in comparison to TPOAb (-) [relative risk: 2.01 (95% CI = 1.13-3.56), P = 0.028].	The pregnancy rate is not affected either by presence of TPOAb or treatment with LT4. However, TPOAb (+) women show a poorer delivery rate compared to TPOAb (-). LT4 treatment in TPOAb (+) does not affect the delivery	not RM patients

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
							rate.	
Negro R, et al. J Clin Endocrinol Metab. 2006 Jul; 91(7):2587-91. 16621910	prospective, randomized trial	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	984 pregnant women first trimester TSH of 0.3-4.2 mU/l, (not subclinically hypothyroid) TPOAb+ 869 TPOAb negative (C) 115 TPO-Ab+ group A (n = 57) underwent LT4 treatment, group B (n = 58) placebo TPOAb(+) had higher TSH compared with TPOAb(-)	levothyroxine	rate of obstetrical complications	Groups A and C showed a similar miscarriage rate (3.5 and 2.4%, respectively), which was lower than group B (13.8%) [P < 0.05; relative risk (RR), 1.72; 95% confidence interval (CI), 1.13-2.25; and P < 0.01; RR = 4.95; 95% CI = 2.59-9.48, respectively]. Group B displayed a 22.4% rate of premature deliveries, which was higher than group A (7%) (P < 0.05; RR = 1.66; 95% CI = 1.18-2.34) and group C (8.2%) (P < 0.01; RR = 12.18; 95% CI = 7.93-18.7).		not RM patients
Negro Ret al. J Clin Endocrinol Metab 2010;95: 1699-1707.	Comparative Study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	4562 women were randomly assigned to the universal screening or case-finding group. Women in both groups were stratified as high risk or low risk based on risk factors for thyroid disease. All women in the universal screening group, and high-risk women in the case-finding group, were immediately tested for free T(4), TSH, and thyroid peroxidase antibody. Low-risk women in the case-finding group had their	Intervention included levothyroxine in women with a TSH above 2.5 mIU/liter in TPO antibody-positive women and antithyroid medication in women with a undetectable TSH and elevated free T(4).	Total number of adverse obstetrical and neonatal outcomes	No significant differences were seen in adverse outcomes between the case-finding and universal screening groups. Adverse outcomes were less likely to occur among low-risk women in the screening group than those in the case-finding group.	Universal screening compared with case finding did not result in a decrease in adverse outcomes. Treatment of hypothyroidism or hyperthyroidism identified by screening a low-risk group was associated with a	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
			sera tested postpartum.				lower rate of adverse outcomes.	
Ota K, et al. Human reproduction 2014;29: 208-219.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Women with three or more consecutive spontaneous abortions prior to 20 weeks of gestation.	Serum vitamin D level, cellular activity and autoimmune parameters in vivo and in vitro were measured.	Sixty-three out of 133 women (47.4%) had low vitamin D (<30 ng/ml). APA: significantly higher in low vitamin D group (VDlow) (39.7%) than in the normal vitamin D group (VDnl) (22.9%) (P< 0.05) (adjusted odds ratio 2.22; 95% CI 1.0-4.7) ANA: VDlow versus VDnl; 23.8% versus 10.0%, (OR 2.81, 95% CI 1.1-7.4), anti-ssDNA (19.0% versus 5.7%, OR 3.76, 95% CI 1.1-12.4) TPOAB: (33.3% versus 15.7%, OR 2.68, 95% CI 1.2-6.1) Peripheral blood CD19(+) B and CD56(+) NK cell levels and NK cytotoxicity at effector to target cell (E:T) ratio of 25:1 were significantly higher in VDlow when compared with those of VDnl (P < 0.05 each). No differences in Th1/Th2 ratios between VDlow and VDnl.		Assessment of vitamin D level is recommended in women with RPL. Vitamin D supplementation should be explored further as a possible therapeutic option for RPL.	
Stephenson MD, et al. Fertility and sterility 2017;107: 684-690.e682.	Observational cohort study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Women seen between 2004 and 2012 with a history of two or more unexplained pregnancy losses <10 weeks in size; endometrial biopsy (EB) performed 9-11 days after LH surge; and one or more subsequent pregnancy(ies). Women were excluded if concomitant findings, such as endometritis, maturation delay, or glandular-stromal dyssynchrony 116 women met the inclusion criteria	Vaginal micronized P was prescribed at a dose of 100-200 mg every 12 hours starting 3 days after LH surge (luteal start) if glandular epithelial nuclear cyclin E (nCyclinE) expression was elevated (>20%) in endometrial glands or empirically despite normal nCyclinE (</=20%). Women with normal nCyclinE (</=20%) who did not receive P were used as controls.	ongoing pregnancy >10 weeks in size	51% (n = 59) had elevated nCyclinE and 49% (n = 57) had normal nCyclinE. Pregnancy success in the 59 women with elevated nCyclinE significantly improved after intervention: 6% (16/255) in prior pregnancies versus 69% (57/83) in subsequent pregnancies. Pregnancy success in subsequent pregnancies was higher in women prescribed vaginal micronized P compared with controls: 68% (86/126) versus 51% (19/37); odds ratio = 2.1 (95% confidence interval, 1.0-4.4).	In this study, we found that the use of luteal start vaginal micronized P was associated with improved pregnancy success in a strictly defined cohort of women with RPL.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Takeda E et al., J. Obstet. Gynaecol. Res. 2020;46: 567-574.	Obs study	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	386 patients with RPL between November 2014 and January 2019.	Examine attitudes toward PGT-A in patinets with RPL		Overall, 25.1% of patients desired PGT-A and 35.2% answered that they knew about it. Regarding the reasons for wanting PGT-A, 42.3% thought that it would insure a live birth and with complete case analysis, showed that the patients' wish for PGT-A as a means of giving live birth was affected by their IVF-EThistory (adjusted odds ratio 2.7, 95% CI 1.2–7.2) and whether they had any knowledge of PGT-A (2.4, 1.1–5.3). Those with a higher total family income (3.5, 1.2–10.1) and a previous IVF-ET (4.6, 2.0–10.3) tended to want PGT-A as a means of avoiding miscarriage.	The majority had no opinion or a poor knowledge of PGT-A. More patients who self-assessed as knowing about PGT-A or who had undergone IVF-ET had the above type of misunderstanding.	
Thangaratinam S, et al .: of evidence. BMJ 2011;342:d2616.	meta-analysis	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	30 articles with 31 studies (19 cohort and 12 case-control) - 12 126 women assessed the 5 studies with 12 566 women	thyroid autoantibodies	Effect of treatment with levothyroxine on miscarriage	Results treatment only 2 randomised studies: Both showed a fall in miscarriage rates, and meta-analysis showed a significant 52% relative risk reduction in miscarriages with levothyroxine (relative risk 0.48, 0.25 to 0.92; P=0.03). (NEGRO 2005 + 2006) One study reported on the effect of levothyroxine on the rate of preterm birth, and noted a 69% relative risk reduction (0.31, 0.11 to 0.90).	Association between thyroid autoantibodies and miscarriage and preterm birth	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Van Dijk MM et al., The Lancet Diabetes Endocrinol. 2022;10: 322-329.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	187 women randomly assigned to receive levothyroxine (n=94 women) or placebo (n=93 women)	Levothyroxine (daily dose ranged from 0.5 to 1.0 µg/kg bodyweight) or placebo until the end of pregnancy. Between January 2013 and September 2019,	The primary outcome was live birth, defined as the birth of a living child beyond 24 weeks of gestation. Secondary outcomes included ongoing pregnancy, pregnancy loss, preterm delivery, adverse events and time to conception leading to live birth.	Live birth occurred in 47 women (50%) in the levothyroxine group and in 45 women (48%) in the placebo group (risk ratio, 1.03; 95% confidence interval [CI], 0.77 to 1.38; absolute risk difference 1.6%; 95% CI, -12.7% to 15.9%).	Levothyroxine treatment did not result in higher live birth rates in euthyroid women with recurrent pregnancy loss positive for TPO-Ab compared to placebo.	
Vissenberg R, et al. Human reproduction update 2012;18: 360-373.	systematic review	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	22 articles were included for the systematic review and 11 were appropriate for meta-analyses.		Eight studies reported on hyperthyroidism. Propylthiouracil (PTU) and methimazole reduce the risk for preterm delivery [risk ratio (RR): 0.23, confidence interval (CI): 0.1-0.52], pre-eclampsia (RR: 0.23, CI: 0.06-0.89) and low birthweight (RR: 0.38, CI: 0.22-0.66). The nine studies that reported on clinical hypothyroidism showed that levothyroxine is effective in reducing the risk for miscarriage (RR: 0.19, CI: 0.08-0.39) and preterm delivery (RR: 0.41, CI: 0.24-0.68). For treatment of subclinical hypothyroidism, current evidence is insufficient. The five studies available on thyroid autoimmunity showed a not significant reduction in miscarriage (RR: 0.58, CI: 0.32-1.06), but significant reduction in preterm birth by treatment with levothyoxine (RR: 0.31, CI: 0.11-0.90).		For hyperthyroidism, methimazole and PTU are effective in preventing pregnancy complications. For clinical hypothyroidism, treatment with levothyroxine is recommended. For subclinical hypothyroidism and thyroid autoimmunity, evidence is insufficient to recommend treatment with levothyroxine.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Wagner CL, et al. . Reviews in endocrine & metabolic disorders 2017.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**		The main finding of these more recent studies was that a 4000 IU/d dose of vitamin D3 safely elevates circulating 25(OH) D to a concentration that, regardless of race, fully normalizes vitamin D metabolism and calcium homeostasis in the pregnant women. Further, in these trials, which cumulatively involved more than 2000 pregnant women, were without a single adverse event observed attributable to vitamin D supplementation (Table 2). Of major interest, data from Hollis et al. studies [25, 92, 93] when analyzed on an intent-to-treat basis, clearly demonstrated increased vitamin supplementation decreased complications of pregnancy and C-section births [24, 80]. Further, RCT data have clearly demonstrated that higher doses of vitamin D during pregnancy improve birth outcome data [80, 82].				
Wang Y, et al. Gynecologic and obstetric investigation 2011;72: 245-251.		☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Pregnant women with a history of recurrent miscarriage were included in the patient group (n = 97), while those with no history of abnormal pregnancy were included in the control group (n = 52).	an oral glucose tolerance test and insulin-releasing test between the 5th and 13th weeks of pregnancy.	(1) Levels of fasting plasma glucose, fasting plasma insulin, homeostasis model assessment of insulin resistance index, and homeostasis model assessment beta function were not statistically significantly different ($p < 0.05$) between the two groups. (2) The area under the curve of glucose and area under the curve of insulin were higher in the patient group than in the control group. The composite insulin sensitivity index of the patient group was lower than that of the control group. The differences in these three parameters between the groups were statistically significant ($p < 0.05$).		Women with a history of recurrent miscarriage are at an increased risk for insulin resistance during the first trimester of a new pregnancy	
Zolghadri J, Tavana Z, et al. Fertil Steril. 2008;90(3):727-30. (18001723)	CCS	?/- Selection bias - Assesment X Confouding - Statistical issues ---------- ☐ **High quality (++)** X **Acceptable (+)** **Unacceptable (-)**	N=164 women history REPL ≥3 Exclusie: abnormalities in hysteroscopy/HSG, thyroid function, karyotyping, APA, PRL, PT, PTT N=74 Women without REPL	OGTT placebo or metformin	31/164 (18.9) 2 DM included 29/164 (17.6%) 4/74 (5.4%)	OR (95%CI) 1.34 (1.25-2.42) P=0.017 Recalculated 3.8 (1.3-11.3) All patients with abnormal GTT divided in 4 groups (PCOS or not, placebo or metformin) and abortion rate compared. The abortion rate was significantly decreased after metformin therapy in the patients without PCOS compared to the placebo group (15% vs. 55%; OR 2.4, 95% CI 0.35–		Study indicates a link between abnormal OGTT and history REPL

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						4.4, P=0.02) and although the abortion rate decreased after metformin therapy in the patients with PCOS, the P value was not statistically significant (25% vs.66%; P=0.42).		

Additional references included as background information

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 2017;27: 315-389.

Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. *Eur Thyroid J* 2014;**3:** 76-94.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 2011;**21:** 1081-1125.

McAree T, Jacobs B, Manickavasagar T, Sivalokanathan S, Brennan L, Bassett P, Rainbow S, Blair M. Vitamin D deficiency in pregnancy - still a public health issue. *Maternal & child nutrition* 2013;9: 23-30.

Maraka S, Mwangi R, McCoy RG, Yao X, Sangaralingham LR, Singh Ospina NM, O'Keeffe DT, De Ycaza AE, Rodriguez-Gutierrez R, Coddington CC, 3rd *et al.* Thyroid hormone treatment among pregnant women with subclinical hypothyroidism: US national assessment. *BMJ (Clinical research ed)* 2017;356: i6865.

15. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RM DUE TO UTERINE ABNORMALITIES TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
AAGL_ J Minim Invasive Gynecol. 2012;19(2):152-71.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- X **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Not mentioned. Literature search for Cochrane database SR by Dec,2010				Recommendations about diagnostic and treatment of fibroids in general. Submucosal fibroids mentioned	Recommendations about diagnostic
Alborzi, et al. Archives of gynecology and obstetrics 2015;291:1167-1171.	Observational study	X Selection bias ☐ Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	26 women with double uterine cavities (22 bicornuate and 4 didelphic uteri) with history of recurrent pregnancy loss undergoing laparoscopic Metroplasty	14 followed up for 1 y, 9 had full term pregnancy, and 3 had miscarriage			Laparoscopic metroplasty by developing single uterine cavity with a suitable volume and minimal adhesion formation can be a substitute for laparotomy technique.	Low number of cases
Bailey et al Women's health (London, England) 2015;11:161-167.	NS review	X Selection bias ☐ Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Review about surgical options for women having UA and RPL	Efficiency of surgical techniques is not evaluated			Anatomic abnormalities, both acquired and congenital, account for about 20% of the explainable causes of RPL. Minimally invasive surgery is suitable for correction of the majority of these	Conclusion not proved

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							abnormalities. In general, pregnancy rates are significantly improved after surgical correction	
Carrera M et al;, J. Minim. Invasive Gynecol. 2021	MA	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Eleven studies were included in the quantitative synthesis: one randomized controlled trial and 10 observational studies involving reproductive outcomes from 1589 patients with either complete or partial uterine septum.	women undergoing hysteroscopic resection of the uterine septum and those with expectant management		The pooled OR for miscarriage was 0.45, (95% CI, 0.22-0.90). -Complete septum subgroup: Misscarriage: OR 0.16; 95% CI, 0.03-0.78 -Partial septum subgroup: Miscarriage: OR = 0.36; 95% CI, 0.19-0.71 PBR: OR = 0.30, 95% CI, 0.11-0.79 Risk of fetal malpresentation: OR = 0.32, 95% CI, 0.16-0.65. -studies not differentiating between complete or partial septum: OR 0.58; 95% CI, 0.20-1.67	The results of the present meta-analysis support that hysteroscopic metroplasty is effective in reducing the risk of miscarriage in patients with complete or partial uterine septum, although these data should be confirmed with a well-designed randomized controlled trial.	
Choe JK, Baggish MS. Fertil Steril. 1992;57(1):81-4. (1730335)	Other	X Selection bias ☐ Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	19 patients with uterine septum and RM	Neodynium-Yag laser HSC	13 were pregnant, 10 full term	87 % full term delivery rate after surgery, vs 11 % preoperative		In, although it is old, and low number of patients

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Colacurci N, De Franciscis P, et al. J Minim Invasive Gynecol. 2007;14(5):622-7.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	One hundred-sixty patients with septate uterus and a history of recurrent abortion (58)vor primary infertility (102)	HSC: versapoint vs resectoscopy with monopolar.			Both techniques had similar outcomes. 70 % of patients with RM got pregnant, 18 % had a miscarriage	
Drakeley AJ, et al. Cochrane Database of Systematic Reviews 2003, Issue 1. Art. No.: CD003253.	SR	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias X No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	2175 women,			The use of a cervical stitch should not be offered to women at low or medium risk of mid trimester loss, regardless of cervical length by ultrasound. The role of cervical cerclage for women who have short cervix on ultrasound remains uncertain as the numbers of randomised women are too few to draw firm conclusions.		
Ghahiry AA, Refaei Aliabadi E, et al. Int J Fertil Steril. 2014;8(2):129-34.	CS	X Selection bias X Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	65 patients with primary and secondary infertility, recurrent abortion and structural uterine defects reported in sonography or hysterosalpangography (HSG) Only 8 patients with RM	HSC metroplasty		6 patients with RM had a full term pregnancy after HSC metroplasty (75 %)	We show improvement in conceptional outcome and in patient's chief complaints after hysteroscopy surgery of these anomalies.	Small number of cases, although percentage is similar to other papers
Giacomucci E, Bellavia E, et al. Gynecol Obstet Invest. 2011;71(3):183-8.	CS	X Selection bias X Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	352 patients having RM and UM, got HSC metroplasty, 170 patients having RM and uterine septum, T-shaped uterus, or arcuate uterus	Obstetric outcomes of pregnanct women after HSC metroplasty	Miscarriage rate reduced from 88% to 14 % (results from 16 retrospective studies)	Before surgery, the overall term delivery rate was 5.5%. After surgery, the overall term delivery rate was 59% (66.7% for T-shaped uterus, 62.8% for septum/partial septum and 55.6% for arcuate uterus)	a randomized controlled trial on the effectiveness of the uterine cavity morphology is needed in patients with recurrent miscarriage	Evidence in favour of HSC metroplasty for UM. Good obstetric outcomes

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Homer HA, Li TC, et al. Fertil Steril. 2000;73(1):1-14.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Non systematic review about septate uterus, including RPL. 658 patients from 16 papers, having RPL and a HSC metroplasty	Global reduction for miscarriages from 88 to 5.9 % after metroplasty		Abdominal metroplasty is obsolete.	A metaanalysis of published retrospective data comparing pregnancy outcome before and after hysteroscopic septoplasty indicated a marked improvement after surgery,	overall miscarriage rate from 88%– 5.9% after HSC metroplasty. Therefore, it appears that in women with RPL, the presence of a uterine septum is an indication for metroplasty
Hooker AB, Lemmers M, et al. Hum Reprod Update. 2014;20(2):262-78.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	Patients with RPL not included	HSC to find out prevalence of IUA in women having miscarriages (not RPL)		Recurrent miscarriages and D&C procedures were identified as risk factors for adhesion formation.	Treatment strategies are proposed to minimize the number of D&C in an attempt to reduce IUAs.	Outcome after removal IUA in women having RPL not specified
Jaslow CR. Obstet Gynecol Clin North Am. 2014;41(1):57-86.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	Bibliography review for UA and RPL, some treatment options reviewed				See summary	Use as background information

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Krishnan M et al., Arch. Gynecol. Obstet.2021; 303: 1131-1142.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	Seven studies involving 407 women with hysteroscopic septum resection and 252 with conservative management were included in the meta-analysis.	women with uterine septum and a history of subfertility and/or poor reproductive outcomes treated by hysteroscopic septum resection against control	The primary endpoint was live birth rate, whereas clinical pregnancy, miscarriage, preterm birth and malpresentation rates were secondary outcomes	Hysteroscopic septum resection was associated with a lower rate of miscarriage (OR 0.25, 95% CI 0.07-0.88) compared with untreated women. No significant effect was seen on live birth, clinical pregnancy rate or preterm delivery. However, there were fewer malpresentations during labour in the treated group (OR 0.22, 95% CI 0.06-0.73).	no significant effect of hysteroscopic resection on live birth.	
Kowalik CR, Goddijn M, et al. Cochrane Database Syst Rev. 2011(6):Cd008576.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	RM + septate uterus	Hysteroscopic metroplasty	Hysteroscopic metroplasty in women with recurrent miscarriage and a septate uterus is being performed in many countries to improve reproductive outcomes in women.This treatment has been assessed in non-controlled studies, which suggested a positive effect on pregnancy outcomes. However, these studies are biased due to the fact that the participants with recurrent miscarriage treated by hysteroscopic metroplasty served as their own controls. Until now, the effectiveness and possible complications of hysteroscopic metroplasty have never been considered in a randomised controlled trial.Taking this into account there is insufficient evidence to support this treatment in these women			No RCTS found
Makino T, Umeuchi M, et al. Int J Fertil. 1992;37(3):167-70.	Other	X Selection bias XPerformance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	1,200 married women with a history of repeated reproductive wastage.	hysterosalpingography intervention : metroplasty .	Out of 1,200 hysterosalpingographies, 188 revealed congenital uterine anomaly (15.7%). The incidence of repeated spontaneous abortion in cases with low-grade anomalies is as high as the incidence among cases with more severe anomalies (based on X/M ratio). A significant improvement in maintaining pregnancy was observed after metroplasty; more than 84% of postoperative pregnancies were successfully maintained, whereas none of the 233 presurgical pregnancies had lasted full term. As a control group, 47 other women with anomalies were randomly chosen, and their subsequent pregnancies were monitored, without metroplasty. Of their pregnancies, 94.4% terminated spontaneously before 12 weeks of gestation			incidence of congenital uterine anomalies among infertile patients

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Mollo A, Nazzaro G, et al. J Minim Invasive Gynecol. 2011;18(1):112-7.	Other	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒**Acceptable (+)** ☐ **Unacceptable (-)**	66 patients with RPL, 59 underwent inpatient resectoscopic surgery after 3D ultrasound diagnosis of septate uterus. Laparoscopy was performed in the remaining 7 patients	Metroplasty (HSC), wither under 3D US control, or laparoscopy			Efficiency not evaluated for RPL, just for anatomical correction	
Pang LH, Li MJ, et al. Int J Gynaecol Obstet. 2011;115(3):260-3.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	138 patients diagnosed with subseptate uterus Women were divided in 2 groups: group A comprised women with a history of recurrent spontaneous abortion (RSA), and was subdivided into control (A1) and surgery (A2) groups; group B comprised women with no history of poor reproductive outcomes, and was subdivided into control (B1) and surgery (B2) groups Not randomized, patient choose surgery or expectant management.	Surgery (septum resection) or No treatment	women were enrolled in group A. Among 32 patients who underwent expectant management (group A1), there were 18 (56.3%) pregnancies, of which 9 (50.0%) ended in spontaneous abortion, 5 (27.8%) ended in preterm delivery, and 4 (22.2%) ended in term delivery. Among the 46 participants who underwent hysteroscopic septum resection (group A2), there were 37 (80.4%) pregnancies after the operation, of which 8 (21.6%) ended in spontaneous abortion, 2 (5.4%) ended in preterm delivery, and 27 (73.0%) ended in term delivery. The rate of pregnancy was significantly higher in group A2 than in group A1 ($P < 0.05$). The reproductive outcomes also differed between the 2 groups ($P< 0.05$) There was no difference in pregnancy rate, incidence of RSA, or preterm or term delivery between group B1 and group B2.		Hysteroscopic septoplasty significantly improved pregnancy outcomes in women with a history of RSA	
Papp Z, Mezei G, et al. J Reprod Med. 2006;51(7):544-52. (16913545)	CS retrospective	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	157 consecutive women who underwent surgery during a 25-year period. One hundred fifty-seven patients with a subseptate, septate or bicornuate uterus and history of recurrent abortions (124 cases) or infertility (33 cases) were included in this study.	Operative technique was similar to the procedure first described by Bret and Guillet and by Tompkins.		The fetal survival rate increased from 0.0% before surgery to 81.9% postoperatively in the recurrent abortion group and to 92.8% in the infertility group. Among women having undergone surgery, 63.8% gave birth to at least 1 healthy child, the proportion of previous habitually miscarrying and infertile women was 70.2% and 32.0%, respectively. No uterine rupture or	Conventional transabdominal metroplasty seems to be a safe procedure in women with symmetric uterine anomalies and RM or infertility. No perioperative	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						any other complication was observed.	or subsequent peripartum complications were observed.	
Porcu G, Cravello L, et al. Eur J Obstet Gynecol Reprod Biol. 2000;88(1):81-4. (10659922)	CS	X Selection bias XPerformance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X **Acceptable (+)** ☐ **Unacceptable (-)**	63 patients consulting for septate uterus and repeated pregnancy loss or abnormal fetal presentation	HSC resection of uterine septum		the rate of first-trim abortions goes from 90 to10–20% after treatment	hysteroscopic section of uterine septa significantly improves the prognosis of the pregnancies in patients with a history of severe obstetrical accidents	Heterogeneous population
Pritts et al. Fertility and sterility 2009;91: 1215-1223.	SR	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias X No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	women with and without fibroids,	myomectomy	Clinical pregnancy rate, spontaneous abortion rate, ongoing pregnancy/live birth rate, implantation rate, and preterm delivery rate in.	Women with subserosal fibroids had no differences in their fertility outcomes compared with infertile controls with no myomas, and myomectomy did not change these outcomes compared with women with fibroids in situ. Women with intramural fibroids appear to have decreased fertility and increased pregnancy loss compared with women without such tumors, but study quality is poor. Myomectomy does not significantly increase the clinical pregnancy and live birth rates, but the data are scarce. Fibroids with a submucosal component led to decreased clinical pregnancy and implantation rates compared with infertile control subjects. Removal	Fertility outcomes are decreased in women with submucosal fibroids, and removal seems to confer benefit. Subserosal fibroids do not affect fertility outcomes, and removal does not confer benefit.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						of submucous myomas appears likely to improve fertility.		
Rikken JFW et al., Hum. Reprod 2020;35: 1578-1588	Retrospective study	☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ High quality (++) X Acceptable (+) ☐ Unacceptable (-)	257 women with a septate uterus in 21 centres	Allocation to septum resection (n= 151 women) or expectant management (n=106 women) January 2000 until August 2018	The primary outcome was live birth (LBR). Secondary outcomes included pregnancy loss, preterm birth (PBR) and foetal malpresentation.	Septum resection vs expectant management: Hazard ratios HR: LBR: 53.0% vs 71.7%; HR 0.71 95% CI 0.49–1.02 Pregnancy loss rate: 46.8% vs 34.4%; OR 1.58; 95%CI 0.81–3.09 PBR: 29.2% vs 16.7%; OR 1.26; 95% CI 0.52–3.04 Foetal malpresentation: 19.1% vs 34.6%; OR 0.56; 95% CI 0.24–1.33.		
Rikken JFW et al., Hum. Reprod 2021;36: 1260-1267	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- X High quality (++) ☐ Acceptable (+) ☐ Unacceptable (-)	79 women with a septate uterus	randomly assigned to septum resection (n=39) or expectant management (n=40),	PLR, LBR, CPR, OPR, PBR	no evidence for benefit from septum resection in term of pregnancy loss (RR 2.3; 95%CI 0.86-5.9), clinical pregnancy (RR 1.2; 95%CI 0.77-1.2), ongoing pregnancy (RR 0.95, 95%CI 0.52-1.8), live birth (RR 0.88, 95%CI 0.47-1.7) or preterm birth (RR 1.3; 95%CI 0.37-4.4) rates	septum resection does not lead to improved reproductive outcomes compared to expectant management for women with a septate uterus	
Roy KK, Singla S, et al. Arch Gynecol Obstet. 2010;282(5): 553-60.	CS	X Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	186 patients (50.5 % of them with RPL) having submucosal fibroids	hysteroscopic myomectomy by monopolar electrode loop. Second look HSC	Miscarriage rate dropped from 69.1% to 23.3% (RPL subgroup)	Removal of submucous myoma has significant increase in fecundity in infertile patients with no other underlying cause	Hysteroscopic myomectomy is relatively safe and cost effective surgical procedure with good reproductive outcome	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Saravelos SH, Yan J, et al. Hum Reprod. 2011;26(12): 3274-9.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ High quality (++) ☐ Acceptable (+) ☐ Unacceptable (-)	966 women reviewed retrospectively, and then 25 women having distorting-cavity fibroids, vs 54 women having non distorting-cavity fibroids prospective The main limitation of this study is the lack of a control group for the women who underwent myomectomy.	TV 2D US and hysterosalpingography, HSC fibroid resection		prevalence of fibroids 8.2%, submucosal 2.8 %	Fibroids are associated with increased mid-trimester losses amongst women with RM. Resection of fibroids distorting the uterine cavity can eliminate the mid-trimester losses and double the live birth rate in subsequent pregnancies. Women with fibroids not distorting the uterine cavity can achieve high live birth rates without intervention	Also in Q 8
Sugiura-Ogasawara M, et al Journal of obstetrics and gynaecology 2015;35: 155-158.	Prospective trial	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias XNo bias detected ---------- **XHigh quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	170 patients with congenital uterine anomalies suffering two or more miscarriages	Surgery (metroplasty), vs no surgery	In favor of metroplasty (live birth rate 81.3% in treated group vs 61.5% without surgery	Surgery showed no benefit in patients with a bicornuate uterus for having a baby, but tended to decrease the preterm birth rate and the low birth weight	The possibility that surgery has benefits for having a baby in patients with a septate uterus suffering recurrent miscarriage could not be excluded	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Sugiura-Ogasawara M, Ozaki Y, et al. Curr Opin Obstet Gynecol. 2013;25(4):293-8.	Other	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	??	HSC metroplasty	Live birth rate ranges from 33 to 65 % and miscarriage rate decreases from 87-77 % to 44-17 % in different studies included		There are currently no good studies that support surgery as increasing the live birth rate in cases of Mullerian anomalies	Evidence in favor of intervention (HSC metroplasty)
Valle RF, Ekpo GE. J Minim Invasive Gynecol 2013;20: 22-42.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** ☒**Acceptable (+)** ☐ **Unacceptable (-)**	Patients with RPL not included 29 studies included.	HSC metroplasty	Pregnancy rate, live birth rate	The results achieved with hysteroscopic metroplasty surpass those of previous invasive abdominal metroplasty procedures, with a rate of viable pregnancies . 80% in patients with a history of repeated abortion Although no prospective randomized studies have been performed with an adequate number of patients to demonstrate the efficacy of treatment vs no treatment, the overall success reported indicates its efficacy and reaffirms the place of minimally invasive treatment such as hysteroscopic metroplasty as the criterion standard and method of choice for treatment of this septate uterus.		Meta-analysis : all studies included in Valle can be excluded (colored red below)
Valli E, Vaquero E, et al. J Am Assoc Gynecol Laparosc. 2004;11(2):240-4.	CS	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	48 consecutive women with septate uterus and RSA	LPS-HSC resection of the septum		Reproductive outcome in terms of term pregnancy was significantly improved after hysteroscopic metroplasty compared with controls (76% vs. 20%)	HSC septum resection seems to be an effective, simple, and safe procedure, associated with low morbidity, that can improve live birth rate in patients affected with poor previous reproductive outcome.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Venturoli S, Colombo FM, et al. Arch Gynecol Obstet. 2002;266(3): 157-9.	Other	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	141 patients having HSC metroplasty (Group I (69 patients) presented with infertility and Group II (72 patients) with recurrent abortion.			Miscarriage rate reduced from 100 % to 25 %. But fertility decreases (only 52 % of women are able to get pregnant)	Hysteroscopic resection is a feasible, safe and effective procedure for achieving normal uterine architecture Hysteroscopic metroplasty seems to be particularly indicated in patients with RM	Retrospective, lack of control group
Zolghadri J, Younesi M, et al. Journal of obstetrics and gynaecology research 2014; 40(2):[375-80 pp.].	RCT	☐ Selection bias xPerformance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	33 singleton pregnancies with 2nd X RPL	Single McDonalds vs Double cerclage		Both same effectiveness, but double gets longer gestation (37 vs 34 weeks). Small groups. Perinatal outcome not mentioned.	The double cervical cerclage method seems to provide better cervical support, as compared with the classic McDonald cerclage method, in those suffering from recurrent pregnancy loss, due to cervical incompetence.	Lack of control group, they compare 2 cerclage techniques. Low number of patients

Additional references included as background information

Alborzi S, Asadi N, Zolghadri J, Alborzi S, Alborzi M. Laparoscopic metroplasty in bicornuate and didelphic uteri. Fertil Steril 2009;92: 352-355.

Di Spiezio Sardo A, Mazzon I, Bramante S, Bettocchi S, Bifulco G, Guida M, Nappi C. Hysteroscopic myomectomy: a comprehensive review of surgical techniques. Hum Reprod Update 2008;14: 101-119.

Drakeley AJ, Quenby S, Farquharson RG. Mid-trimester loss--appraisal of a screening protocol. Hum Reprod 1998;13: 1975-1980.

Grimbizis GF, Camus M, Tarlatzis BC, Bontis JN, Devroey P. Clinical implications of uterine malformations and hysteroscopic treatment results. Hum Reprod Update 2001;7: 161-174.

Hall M, Vousden N, Carter J, Hezelgrave N, Shennan AH. Prevention of mid-trimester loss following full dilatation caesarean section: a potential role for transabdominal cervical cerclage. J Obstet Gynaecol 2015;35: 98-99.

Kodaman PH, Arici A. Intra-uterine adhesions and fertility outcome: how to optimize success? Curr Opin Obstet Gynecol 2007;19: 207-214.

Lieng M, Istre O, Qvigstad E. Treatment of endometrial polyps: a systematic review. Acta Obstet Gynecol Scand 2010;89: 992-1002.

Lieng M, Istre O, Sandvik L, Qvigstad E. Prevalence, 1-year regression rate, and clinical significance of asymptomatic endometrial polyps: cross-sectional study. J Minim Invasive Gynecol 2009;16: 465-471.

Salim S, Won H, Nesbitt-Hawes E, Campbell N, Abbott J. Diagnosis and management of endometrial polyps: a critical review of the literature. J Minim Invasive Gynecol 2011;18: 569-581.

Santamaria X, Cabanillas S, Cervello I, Arbona C, Raga F, Ferro J, Palmero J, Remohi J, Pellicer A, Simon C. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study. Hum Reprod 2016;31: 1087-1096.

Smit JG, Kasius JC, Eijkemans MJ, Koks CA, van Golde R, Nap AW, Scheffer GJ, Manger PA, Hoek A, Schoot BC et al. Hysteroscopy before in-vitro fertilisation (inSIGHT): a multicentre, randomised controlled trial. Lancet 2016;387: 2622-2629.

Story L, Shennan A. Cervical cerclage: an established intervention with neglected potential? Eur J Obstet Gynecol Reprod Biol 2014;176: 17-19.

Tulandi T, Alghanaim N, Hakeem G, Tan X. Pre and post-conceptional abdominal cerclage by laparoscopy or laparotomy. J Minim Invasive Gynecol 2014;21: 987-993.

16. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH RPL DUE TO MALE FACTOR TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Chavarro JE, et al. Fertil Steril 2010;93: 2222-2231.	Cross sectional study	**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	483 male partners of subfertile couples.	Standard semen analysis, sperm DNA fragmentation, and serum levels of reproductive hormones.	As expected, body mass index (BMI) was positively related to estradiol levels and inversely related to total testosterone and sex hormone-binding glogulin (SHBG) levels. There was also a strong inverse relation between BMI and inhibin B levels and a lower testosterone: LH ratio among men with a BMI ≥ 35 kg/m² BMI was unrelated to sperm concentration, motility, or morphology. Ejaculate volume decreased steadily with increasing BMI levels. Further, men with BMI ≥ 35 kg/m(2) had a lower total sperm count than normal weight men (adjusted difference in the median [95% CI] = -86 x 106 sperm [-134, -37]). Sperm with high DNA damage were significantly more numerous in obese men than in normal-weight men			
Cho CL, et al. Asian J Androl 2016;18: 186-193.		**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ? OK** --- ☐ **High quality (++)** **x Acceptable (+)** ☐ **Unacceptable (-)**			A significantly higher chances for pregnancy after varicocelectomy than either no treatment or medication in patients with clinical varicoceles and at least one abnormal semen parameter (OR:2.87; 95% CI: 1.33–6.20; P < 0.001) was reported. No beneficial effect of varicocele repair on fertility potential could be demonstrated in men with subclinical varicocele. Varicocelectomy in men with varicocele and normal semen parameters did not show a clear benefit over observation. Studies examining sperm DNA damage and pre- and post-varicocele treatment indicate that patients with varicoceles have significantly higher sperm DNA damage than controls, with a mean difference of 9.84% (95% CI: 9.19–10.49; P < 0.00001). It has been also shown that varicocelectomy decrease sperm DNA fragmentation with a mean difference of −3.37% (95% CI: −4.09—2.65; P < 0.00001) compared to no treatment.		

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
De Ligny W et al., Cochrane Database of Systematic Reviews 2022.	Cochrane SR	**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	90 studies with a total population of 10,303 subfertile men, aged between 18 and 65 years,	any type, dose or combination of oral antioxidant supplement with placebo, no treatment, or treatment with another antioxidant,	In six studies reporting miscarriage, no significant difference was found in miscarriage rate between couples randomized to antioxidant therapy compared to placebo (OR 1.46; 95%CI 0.75-2.83). Live birth rate was higher in couples randomized to treatment (OR 1.43; 95%CI 1.07-1.91, 12 RCTs, n=1283, I2= 44%, very low-quality evidence). When studies at high risk of bias were removed from the analysis, there was no evidence of increased live birth (Peto OR 1.22, 95%CI 0.85-1.75, 8 RCTs, n= 827, I2= 32%)			
Donnelly ET, et al. Hum Reprod 2000;15: 1552-1561.		**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** --- **☐ High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	Semen samples (n = 25) were prepared by discontinuous Percoll density centrifugation (95.0:47.5).	DNA integrity was determined using a modified alkaline single cell gel electrophoresis (Comet) assay. DNA fragmentation, possibly indicative of apoptosis, was detected by TUNEL Mitochondrial transmembrane potential was determined using the mitochondrial probe 5,5',6,6'-tetrachloro-1,1', 3,3'-tetraethyl benzimidazolyl carbocyanine iodide (JC-1).	The DNA integrity of prepared spermatozoa was significantly greater than that of semen ($P < 0.005$). Further, the percentage of spermatozoa with fragmented DNA and the degree of fragmentation within these cells in prepared spermatozoa is significantly less than in semen ($P < 0.005$). There is a significant correlation between DNA damage quantified using the Comet assay and DNA fragmentation determined using TUNEL ($R = 0.562$, $P < 0.01$). The percentage of spermatozoa with dysfunctional, possibly apoptotic, mitochondria was significantly lower in prepared spermatozoa than in neat semen samples ($P < 0.001$). There was a negative correlation between the percentage of spermatozoa with dysfunctional mitochondria and the percentage of progressively motile spermatozoa ($R = -0.67$, $P < 0.01$).			
Lepine S et al., The Cochrane database of systematic reviews 2019;7: Cd010461	Cochrane SR	**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** --- **X High quality (++)** **☐ Acceptable (+)** **☐ Unacceptable (-)**	eight RCTs (4147 women)	patients (not RPL) undergoing PICSI compared with ICSI with sperm selected using standard methods	two RCTs reported live birth and there may be little or no difference between PICSI and ICSI (RR 1.09; 95%CI 0.97-1.23, 2 RCTs, n=2903, I2=0%, low-quality evidence). In contrast, three RCTs showed a significant decrease in pregnancy loss rates among couples in the PICSI group (RR 0.61; 95%CI 0.45-0.83, 3 RCTs, 3005 women, I2=0%, although low-quality evidence). This impact of HA-ICSI sperm selection was also observed when the pregnancy loss was calculated per clinical pregnancy (RR 0.62; 95%CI 0.46-0.82, 3 RCTs, n=1065, I2=0%, low-quality evidence).	The current evidence suggests that advanced sperm selection strategies in assisted reproductive technologist (ART) may not result in an inc ease in the likelihood of live birth.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Miller D et al., Lancet 2019;393: 416-422.	RCT	☐ Selection bias ☒Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	2752 couples of whom 2752 were included in the primary analysis	1381 in the PICSI group and 1371 in the ICSI group	The term livebirth rate did not differ significantly between PICSI (27·4% [379/1381]) and ICSI (25·2% [346/1371]) groups (odds ratio 1·12, 95% CI 0·95–1·34; p=0·18). There were 56 serious adverse events in total, including 31 in the PICSI group and 25 in the ICSI group; most were congenital abnormalities, and none were attributed to treatment.		Compared with ICSI, PICSI does not significantly improve term livebirth rates.	
Pasqualotto FF, et al. J Androl 2012;33: 239-243.		**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	169 men undergoing varicocele repair before ICSI when compared with 79 couples forgoing repair.		no significant difference in spontaneous implantation, pregnancy, or miscarriage rates significant improvement in fertilization rates between the two groups (73.2% vs 64.9%, P = 0.0377)			
Sakkas D, et al. Hum Reprod 2000;15: 1112-1116.		**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Semen samples were collected, washed and one part of the semen spread on a slide, the remainder was prepared using the swim-up, PureSperm((R)) or Percoll((R)) techniques. Spermatozoa from different fractions were fixed on slides and assessed.		Sperm samples (n) from different men were stained using the chromomycin A(3) (CMA(3)) fluorochrome, which indirectly demonstrates a decreased presence of protamine (n = 31 for swim-up; n = 45 for PureSperm((R)); n = 39 for Percoll((R))). Spermatozoa prepared using PureSperm((R)) (n = 35) and Percoll((R)) (n = 37) were also examined for the presence of endogenous DNA nicks. Good quality spermatozoa should not possess DNA nicks and not stain (i.e. fluoresce) with CMA(3). When prepared using the swim-up technique the spermatozoa recovered showed no significant improvement with the CMA(3) staining. When spermatozoa were prepared using the PureSperm((R)) and Percoll((R)) techniques, a significant (P < 0.001) decrease in both CMA(3) positivity and DNA strand breakage was observed. These results indicate that both the PureSperm((R)) and Percoll((R)) techniques can enrich the sperm population by separating out those with nicked DNA and with poorly condensed chromatin.			

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Wang YJ, et al. Reprod Biomed Online 2012;25: 307-314.	meta-analysis	**Appropriate question ?** **Rigorous search ?** **Relevant studies included?** **Quality of studies?** **Methodology ?** --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	12 were selected that measured similar types of reactive sperm DNA damage. Seven studies determined the damage of sperm DNA in varicocele-associated patients and six studies evaluated the efficacy of varicocelectomy. One study was a duplicate because both outcomes were included.		The overall estimate showed that patients with varicoceles have significantly higher sperm DNA damage than controls, with a mean difference of 9.84% (95% CI 9.19 to 10.49; P<0.00001). A varicocelectomy can improve sperm DNA integrity, with a mean difference of -3.37% (95% CI -4.09 to -2.65; P<0.00001). In conclusion, there is increased sperm DNA damage in patients with varicoceles and varicocelectomy may be a possible treatment; however, more studies with appropriate controls are needed to confirm this finding.			
West R et al., Hum Reprod 2022.	Secodary analysis of RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	1215 had measures of DNAq of which 1162 couples had embryo transfers	the assays of DNAq essentially following the published protocols for acridine orange (AO) staining, the alkaline comet assay, TUNEL assay, SCD assay and aniline blue (AB) assay.	All measures of HBS and DNAq discriminated normal from abnormal sperm samples (p < 0.001). SCD correlated negatively with the Comet (r = -0.165; p < 0.001) and TUNEL assays (r = -0.200; p < 0.001). HBS correlated negatively with AO (r = -0.211; p < 0.001), Comet (r = -0.127; p < 0.001) and TUNEL (r = -0.214; p < 0.001) and positively with SCD (r = 0.255; p < 0.001) and AB (r = 0.127; p < 0.001).	A parsimonious model for predicting live birth (and miscarriage) rates included treatment allocation (OR 2.167, 95% CI 1.084-4.464, p=0.031), female age (OR 0.301, 95% CI 0.133-0.761, p=0.013, per decade) and the AO assay (OR 0.79, 95% CI 0.60-1. 02.761, p=0.073, per 10 points rise). For couples failing to establish a clinical pregnancy, the model retained only the AB assay (OR 0.81, 95% CI 0.678-0.956-1.075, p=0.016, per 10 points rise).	PICSI adversely affected fertlisation rates and did not improve cumulative pregnancy rates.	

Additional references included as background information

Aitken RJ, De Iuliis GN, McLachlan RI. Biological and clinical significance of DNA damage in the male germ line. *Int J Androl* 2009;**32:** 46-56.

Aitken RJ, Jones KT, Robertson SA. Reactive oxygen species and sperm function--in sickness and in health. J Androl 2012;**33**: 1096-1106.

Aitken RJ, Drevet JR. The Importance of Oxidative Stress in Determining the Functionality of Mammalian Spermatozoa: A Two-Edged Sword. Antioxidants (Basel, Switzerland) 2020;9.

Arabi M. Nicotinic infertility: assessing DNA and plasma membrane integrity of human spermatozoa. Andrologia 2004;36: 305-310.

Doshi SB, Khullar K, Sharma RK, Agarwal A. Role of reactive nitrogen species in male infertility. Reprod Biol Endocrinol 2012;10: 109.

Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. *Nat Rev Urol* 2010;**7:** 153-161.

Hsu PC, Chang HY, Guo YL, Liu YC, Shih TS. Effect of smoking on blood lead levels in workers and role of reactive oxygen species in lead-induced sperm chromatin DNA damage. *Fertil Steril* 2009;**91:** 1096-1103.

Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril 2011;95: 116-123.

Nagler HM, Luntz RK, Martinis FG. Varicocele. In Lipshultz LI and Howards S (eds) Infertility in the male Mosby-Year Book. 1997. Inc., , St Louis, USA, pp. 336-359.

Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol* 2013;**11:** 66.

Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2014**:** Cd007411.

Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online* 2014;**28:** 684-703.

17. WHICH THERAPEUTIC INTERVENTIONS SHOULD BE OFFERED TO PATIENTS WITH UNEXPLAINED RPL TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Barad DH, et al. Fertil Steril 2014;101: 710-715.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	141 consecutive, unselected, consenting women with no history of renal disease, sickle cell disease, or malignancy who were undergoing IVF The mean age for the whole study group was 39.59 +/- 5.56 years (G-CSF: 39.79 +/- 5.13 years; placebo: 39.38 +/- 6.03 years).	endometrial perfusion with granulocyte colony-stimulating factor in IVF cycles 73 patients to receive G-CSF (Filgrastim, Amgen, 300 mug/1.0 mL) and 68 to receive placebo (saline).	endometrial thickness clinical pregnancy rates embryo implantation rates	Endometrial thickness statistically significantly increased over the 5-day observation period for the whole group by approx. 1.36 mm. The increase in the G-CSF group was not statistically significantly different from the control group. clinical pregnancy and implantation rates : no effect of G-CSF treatment. No adverse events for either treatment group.	In normal IVF patients, G-CSF does not affect endometrial thickness, implantation rates, or clinical pregnancy rates.	Included in review Cavalcante 2015 Not RPL specific
Cavalcante MB, et al . Iran J Reprod Med 2015;13: 195-202.	Review	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)** ☐ **Unacceptable (-)**	The search of electronic databases resulted in 215 citations (PubMed/ Medline: 139 and Scopus: 76), of which 38 were present in both databases. Of the remaining 177 publications, seven studies were included in the present review.	Granulocyte colony-stimulating factor in patients who have difficulty conceiving and maintaining pregnancy		Describes 2 studies on RPL (Scarpellini 2009 and Santjohanser 2013) but no meta-)analysis was performed due to difference in studies		(both included studies are discussed in the evidence table and guideline)
Christiansen OB, et al. Acta Obstet Gynecol Scand. 1994;73(3):261-8.	RCT	☒ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☒ **Acceptable (+)**	Patients with unexpl RM, 3 or more misc.	43 patients got donor LIT before and in pregnancy 23 patients got autologous lymphocytes (placebo)		23% increased LBR in all patients with LIT 38% increased LBR after LIT in primary RM (p = 0.02)	LIT increased LBR in primary RM	Data included in review Wong 2014 – mentioned for details on side effects

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
		☐ **Unacceptable (-)**						
Clark DA. Am J Reprod Immunol. 1994;32(4):290-3.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☒ **Unacceptable (-)**						Mice experiment, intralipid seems to reduce resorption rate in mice matings Used as background information
Coomarasamy A, , et al. N Engl J Med. 2015;373(22) :2141-8.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	PROMISE trial 836 women with unexplained recurrent miscarriages 18 to 39 years of age actively trying to conceive naturally RM = 3 or more consecutive or nonconsecutive losses of pregnancy in the first trimester Exclusion criteria: - unable to conceive naturally within 1 year after recruitment; - APS or other thrombophilic conditions; - uterine cavity abnormalities - abnormal parental karyotype, - other identifiable cause of RM such as diabetes, thyroid disease, or SLE	Twice-daily vaginal suppositories containing either 400 mg of micronized progesterone or matched placebo from a time soon after a positive urinary pregnancy test (and no later than 6 weeks of gestation) through 12 weeks of gestation.	Live birth after 24 weeks of gestation newborn survival	rate of live births was 65.8% in the progesterone group vs 63.3% in placebo group (RR 1.04; 95% CI 0.94 to 1.15; rate difference, 2.5 percentage points; 95% CI, −4.0 to 9.0). There were no significant between-group differences in the rate of adverse events. no significant between-group differences in the rates of clinical pregnancy (at 6 to 8 weeks), ongoing pregnancy (at 12 weeks), ectopic pregnancy, miscarriage, stillbirth, and neonatal outcomes, as well as in the median gestational age at miscarriage	Progesterone therapy in the first trimester of pregnancy did not result in a significantly higher rate of live births among women with unexplained RM	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
			- currently receiving heparin therapy; Contraindications to progesterone					
Coomarasamy A, , et al. N Engl J Med. 2019;380: 1815-1824.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	4153 women, randomly assigned to receive progesterone (2079 women) or placebo (2074 women). The percentage of women with available data for the primary outcome was 97% (4038 of 4153 women).	vaginal suppositories containing either 400 mg of progesterone or matching placebo twice daily, from the time at which they presented with bleeding through 16 weeks of gestation	Primary outcome: the birth of a live-born baby after at least 34 weeks of gestation.	The incidence of live births after at least 34 weeks of gestation was LBR: 75% vs 72 (relative rate, 1.03; 95% CI 1.00-1.07; P = 0.08). The sensitivity analysis, in which missing primary outcome data were imputed, resulted in a similar finding (relative rate, 1.03; 95% CI, 1.00 to 1.07; P = 0.08). The incidence of adverse events did not differ significantly between the groups.	Among women with bleeding in early pregnancy, progesterone therapy administered during the first trimester did not result in a significantly higher incidence of live births than placebo	
Coomarasamy A, , et al., AJOG 2020;223: 167-176.	MA	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	The PROMISE trial studied 836 women from 45 hospitals in the United Kingdom and the Netherlands The PRISM trial studied 4153 women from 48 hospitals in the United Kingdom	PROMISE: 400 mg of micronized progesterone taken vaginally twice daily from no later than 6 weeks until 12 weeks of gestation vs placebo PRISM: 400 mg of micronized progesterone taken vaginally or rectally twice daily from randomization until 16 weeks of gestation vs placebo	PROMISE: LBR ≥24 weeks PRISM: LBR ≥34 weeks	A key finding, first observed in the PROMISE trial, and then replicated in the PRISM trial, was that treatment with vaginal micronized progesterone 400 mg twice daily was associated with increasing live birth rates according to the number of previous miscarriages. For the <u>subgroup of women with a history of 1 or more miscarriage(s) and current pregnancy bleeding,</u> • LBR: 75% (689/914) with progesterone vs 70% (619/886) with placebo (rate difference 5%; RR 1.09,	women with a history of miscarriage who present with bleeding in early pregnancy may benefit from the use of vaginal micronized progesterone 400 mg twice daily.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						95% CI 1.03-1.15; P=.003). The benefit was greater for the subgroup of women with 3 or more previous miscarriages and current pregnancy bleeding: • LBR: 72% (98/137) with progesterone vs 57% (85/148) with placebo (rate difference 15%; RR 1.28, 95% CI, 1.08-1.51; P=.004). No short-term safety concerns were identified from the PROMISE and PRISM trials.		
de Jong PG, et al. Cochrane Database Syst Rev. 2014;7:Cd004734.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☒ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	RM patients with 2 or more misc. idiopathic or heritable thrombophilia 5 trials included	410 got heparin +/- LDA 383 got no treatment		All trials hep +/- LDA vs no: RR 1.07 (0.99-1,15) Good trials hep + LDA vs no: RR 1.01 (0.87-1.16) Trials comparing hep vs LDA: no difference		
Eapen A et al., Hum Reprod. 2019;34: 424-432.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- ☒ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	150 women with a history of unexplained recurrent pregnancy loss: 76 women (median age, 32[IQR, 29-34] years; mean BMI, 26.3[SD, 4.2]) and 74 women (median age, 31[IQR, 26-33] years; mean BMI, 25.8[SD, 4.2]) were randomized to placebo.	Daily subcutaneous injections of recombinant human granulocyte - colony stimulating factor 130 µg or identical appearing placebo from as early as three to five weeks of gestation for a maximum of 9 weeks. between 23 June 2014 and 05 June 2016.	The primary outcome was clinical pregnancy at 20 weeks of gestation. Secondary outcomes included miscarriages, livebirth, adverse events, stillbirth, neonatal birth weight, changes in clinical laboratory variables following study drug exposure, major congenital	The clinical pregnancy rate at 20 weeks, as well as the live birth rate, was 59.2% (45/76) in the rhG-CSF group, and 64.9% (48/74) in the placebo group, giving a relative risk of 0.9 (95% CI: 0.7-1.2; P = 0.48). There was no evidence of a significant difference between the groups for any of the secondary outcomes. Adverse events (AEs) occurred in 52 (68.4%) participants in rhG-CSF group and 43 (58.1%) participants in the placebo group. Neonatal congenital anomalies were observed in 1/46 (2.1%) of babies	No significant increase in clinical pregnancy or live births with the use of rhG-CSF in the first trimester of pregnancy.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
					anomalies, preterm births and incidence of anti-drug antibody formation.	in the rhG-CSF group versus 1/49 (2.0%) in the placebo group (RR of 0.9; 95% CI: 0.1-13.4; P = 0.93).		
Egerup P, ET al PloS one 2015;10: e0141588.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Recurrent Miscarriage 11 RCTs + 4 observ studies for harms	Intravenous Immunoglobulins	proportion of women not giving live birth women, Serious adverse events infants experiencing SAEs	No significant difference in the number of 'no live birth' was found when IVIg was compared with placebo or treatment as usual (107/265 (40%) versus 113/266 (42%); RR: 0.92, 95% CI 0.75–1.12, p = 0.42). (n=1008)		
Gomaa MF, Archives of gynecology and obstetrics 2014;290: 757-762.	RCT	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☒ ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Unexplained recurrent miscarriage no significant differences between groups	Oral prednisolone (5mg/day) + Heparin + Low dose Aspirin Control : Placebo + Heparin + Low dose Aspirin	Ongoing pregnancy rate Miscarriage rate	Pred: 70.3% Placebo: 9.2% RR 7.63 (3.7-15.7) NNT 1.63 29.7% vs 90.8%		10 lost to follow-up
Haas DM and Ramsey PS. Cochrane Database Syst Rev 2013;10: Cd003511.	**SR**	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☒ ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	14 RCTs (2158 women)	progestogen versus placebo or no treatment	The meta-analysis of all women, regardless of gravidity and number of previous miscarriages, showed no statistically significant difference in the risk of miscarriage between progestogen and placebo or no treatment groups (Peto odds ratio (Peto OR) 0.99; 95% confidence interval (CI) 0.78 to 1.24) and no statistically significant difference in the incidence of adverse effect in either mother or baby. A subgroup analysis of placebo controlled trials did not find a difference in the rate of miscarriage with the use of progestogen (10 trials, 1028 women; Peto OR 1.15; 95% CI 0.88 to 1.50).In a subgroup analysis of four trials involving women who had recurrent miscarriages (three or more consecutive miscarriages; four trials, 225 women), progestogen treatment showed a statistically significant decrease in miscarriage rate compared to placebo or no treatment (Peto OR 0.39; 95% CI 0.21 to 0.72). However, these four trials were of poorer methodological quality. No statistically significant differences were found between the route of administration of			Progestogen for preventing miscarriage

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
					progestogen (oral, intramuscular, vaginal) versus placebo or no treatment. No significant differences in the rates of preterm birth, neonatal death, or fetal genital anomalies/virilization were found between progestogen therapy versus placebo/control.			
Haas DM et al., Cochrane Database Syst Rev 2019;2019	**SR**	Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	12 RCTs (1,856 women)	progestogens with placebo or no treatment	The meta-analysis of all women suggests that there may be a reduction in the number of miscarriages for women given progestogen supplementation compared to placebo/controls (RR 0.73, 95% CI 0.54 to 1.00, 10 trials, 1684 women, moderate-quality evidence). A subgroup analysis comparing placebo-controlled versus non-placebo-controlled trials, trials of women with three or more prior miscarriages compared to women with two or more miscarriages and different routes of administration showed no clear differences between subgroups for miscarriage. None of the trials reported on any secondary maternal outcomes, including severity of morning sickness, thromboembolic events, depression, admission to a special care unit, or subsequent fertility. There was probably a slight benefit for women receiving progestogen seen in the outcome of live birth rate (RR 1.07, 95% CI 1.00 to 1.13, 6 trials, 1411 women, moderate-quality evidence). We are uncertain about the effect on the rate of preterm birth because the evidence is very low-quality (RR 1.13, 95% CI 0.53 to 2.41, 4 trials, 256 women, very low-quality evidence). No clear differences were seen for women receiving progestogen for the other secondary outcomes including neonatal death, fetal genital abnormalities or stillbirth. There may be little or no difference in the rate of low birthweight and trials did not report on the secondary child outcomes of teratogenic effects or admission to a special care unit.			For women with unexplained recurrent miscarriages, supplementation with progestogen therapy may reduce the rate of miscarriage in subsequent pregnancies.
Hekmatdoost A, et al. PLoS One 2015;10: e0143569.	RCT	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	220 Women with 3 or more idiopathic recurrent abortion, aged 20 to 45 years	randomly assigned to receive either folic acid or 5-MTHF daily 1 mg 5-methylentetrahydrofolate or 1 mg folic acid from at least 8 weeks before conception to the 20th week of the pregnancy.	ongoing pregnancy rate at 20th week of pregnancy, serum folate and homocysteine at the baseline, after 8 weeks, and at the gestational age of 4, 8, 12, and 20 weeks, MTHFR gene C677T and A1298C polymorphisms.	There was no significant difference in abortion rate between two groups. Serum folate increased significantly in both groups over time; these changes were significantly higher in the group receiving 5-MTHF than the group receiving folic acid (value = 2.39, p<00.1) and the result was the same by considering the time (value = 1.24, p<0.01). Plasma tHcys decreased significantly in both groups over time; however these changes were	The results do not support any beneficial effect of 5-MTHF vs. folate supplementation in women with recurrent abortion with any MTHFR C677T and/or A1298C polymorphism.	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						not significantly different between the groups (value = 0.01, p = 0.47).		
Hutton B, Sharma R, et al. Bjog. 2007;114(2): 134-42.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Patients with unexplained RM included in RCTs	172 Ivlg 173 placebo	Yes	All pts: RR LBR after Ivlg: 1,.28 (0.78-2.10) Sec RM: RR LBR after Ivlg 2.71 (1.09-6.73)	Ivlg may improve preg, outcome in secondary RM	No unjustified exclusions of RCTS or patients. Most recent two RCTs not included.
Kumar A, Begum N, et al. Fertil Steril 2014;102: 1357-1363.e1353.	Other	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Patients with RM with 3 or more misc.	175 pts got progesterone LBR 93.1% 173 pts got placebo. LBR 83.2% Treatment started when +FHA by ultrasound		LBR sign higher in progesterone treated pts	Progesterone reduces miscarriage rate in RM	Inclusion late in pregnancy
Laskin CA, et al. N Engl J Med 1997;337: 148-153.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	773 nonpregnant RPL women were screened for ANA, anti-DNA, antilymphocyte, and anticardiolipin antibodies and lupus anticoagulant. 385 women had at least one autoantibody, 202 who later became pregnant women were stratified according to age (18-34 years or 35-39 years) and the week of gestation at which the previous fetal losses had occurred (< or = 12 or > 12 weeks).	202 pregnant (RPL with at least 1 Ab) were randomly assigned in equal numbers to receive either prednisone (0.5 to 0.8 mg per kilogram of body weight per day) and aspirin (100 mg per day) or placebo for the duration of the pregnancy.	successful pregnancy Live infants were born to 66 women in the treatment group (65 %) and 57 women in the placebo group (56 %, P=0.19). More infants were born prematurely in the treatment group than in the placebo group (62% vs. 12%, P<0.001). The major side effects of therapy in the mothers were hypertension (treatment group, 13 %; placebo group, 5 %; P=0.05) and diabetes mellitus (15 % and 5 %, P=0.02).		Treating women who have autoantibodies and recurrent fetal loss with prednisone and aspirin is not effective in promoting live birth, and it increases the risk of prematurity.	
Lashley EE, et al. Am J Reprod	SR	☐ Selection bias ☐Performance bias	the effect of antipaternal antibodies on pregnancy complications		risk ratio for HLA class I and class II antibodies on	The seventeen studies that were selected for meta-analysis showed high level of statistical and clinical	No consistent conclusions can be drawn from	the effect of antipaternal antibodies on

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Immunol 2013;70: 87-103.		☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**			pregnancy complications. risk for first- and third-trimester complications	heterogeneity. In the meta-analysis, we found no significant effect of HLA class I or class II antibodies on pregnancy outcome.	the meta-analysis. Discrepancies in the meta-analysis are the result of different screening techniques, varying time points of screening, and use of incorrect control groups.	pregnancy complications is unclear
Meng L, et al. Arch Gynecol Obstet 2015;294: 29-39.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X Acceptable (+)** **☐ Unacceptable (-)**	76 patients in the intralipid group and 78 in the IVIG group	intralipid or IVIG	rate of successful pregnancy comparisons of peripheral NK cell activities were accessed by flow cytometry the effects of intralipid on trophoblasts were investigated using a Matrigel assay with the JEG-3 cell line	There were no statistically significant differences in successful pregnancy rates between the two groups (92.1 vs 88.2 %, P = 0.415). The reduced NK cell concentrations revealed the cytotoxic effects of the treatments in both groups. The invasive ability of JEG-3 cells was inhibited during co-culture with patient PBMCs. However, the inhibitory effect could be alleviated if the patient PBMCs were stimulated with intralipid.	Intralipid can be used as an alternative treatment to IVIG for URSA, and its potential mechanism of action may occur by regulating NK cell function and promoting trophoblast invasion.	
Pasquier E, et al. Blood 2015;125: 2200-2205.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **☐ High quality (++)** **X ☐ Acceptable (+)** **☐ Unacceptable (-)**	258 pregnant women with a history of unexplained recurrent miscarriage (>/=2 consecutive miscarriages before 15 weeks' gestation) and a negative thrombophilia workup. (mean age 32 years, >/=3 miscarriages: 72%; mean gestational age 39 days of amenorrhea)	one daily subcutaneous injection of enoxaparin (low-molecular-weight heparin - 40 mg) or placebo until 35 weeks' gestation.	LBR	66.6% of 138 who received enoxaparin had a live birth vs 72.9% of 118 who received placebo. The absolute difference was -6% (95% CI, -17.1 to 5.1), excluding a 10% increase in the rate of live-birth on enoxaparin (P = .34).	enoxaparin (40 mg once daily) did not improve the chance of a live birth in nonthrombophilic women with unexplained recurrent	LMWH for unexplained recurrent miscarriage

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
							miscarriage	
Roussev RG, Acacio B, et al. Am J Reprod Immunol. 2008;60(3):258-63.	CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	50 patients with abnormal NK activity results (NKa)	intralipid 20% i.v. (9 mg/mL total blood volume - corresponds to 2 mL of intralipid 20% diluted in 250 mL saline; or 18 mg/mL - corresponds to 4 mL of intralipid 20% diluted in 250 mL saline) infusions	NK activity results (flow cytometry using K562 cells as targets)	39 (78%) showed NKa suppression within the normal range the first week after infusion, 11 (22%), showed suppression, but still above the normal threshold. They received second infusion 2-3 weeks later. In 10, the Nka activity was normalized the following week. Four patients had three intralipid infusions in 2-week periods in between and after the third infusion, and all showed NKa normal activity. In 47 patients the suppressive effect of the Intralipid after the normalization of NKa lasted between 6 and 9 weeks, in two patients this benefit lasted 5 weeks, and in one patient the effect was 4 weeks.	Intralipid is effective in suppressing in vivo abnormal NK-cell functional activity. The results suggest that Intralipid can be used successfully as a therapeutic option to modulate abnormal NK activity in women with reproductive failure.	
Saccone G et al. Fertility and sterility 2017;107:430-438. e433.	**SR**	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)** ☐ **Unacceptable (-)**	Women with RPL : 802 patients receiving progesterone and 784 receiving placebo	Progesterone versus placebo	women randomized to the intervention group had a lower risk of recurrent miscarriage (RR 0.72; 95% CI 0.53-0.97) and higher live birth rate (RR 1.07; 95% CI 1.02-1.15) compared with those who did not. Discrepancies in the conclusion of this meta-analysis with the largest included trial were explained by the differences in progesterone supplement, and the inclusion of 7 trials published before 1990 when the quality standards for RCTs were lower			recent meta-analysis combined 10 trials, including the trials of Kumar and Coomarasamy
Santjohanser et al Arch Immunol Ther Exp (Warsz) 2013;61:159-164	Retrosp CS	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- ☐ **High quality (++)** **X Acceptable (+)**	127 patients with history of RPL undergoing IVF 199 IVF cycles	G-CSF (n=49): 11 patients received 34x106 IU once per week and 38 patients received 13×106 IU twice per week starting on the day of embryo transfer until the 12th week of gestation	Pregnancy rate Live birth rate	G-CSF: PR of 47% LBR of 32% Other medications group: PR 27% (p=0.016) LBR of 14% (p=0.006)		Included in review Cavalcante 2015 Not RPL specific

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
		☐ **Unacceptable (-)**		Controls: Not treated (n=33) or treated with other Medications (n=45): enoxaparin 40 mg subcutaneously once per day, acetylsalicylic acid (100 mg/day), folic acid (5 mg/day) or prednisone/ dexamethasone (2.5-5.0 mg/0.5 mg/day) starting in the middle of the previous cycle until the evidence of an embryonic heart beat and doxycycline (100 mg/day for 5 days) beginning at ET. All patients received folic acid (0.5 mg) and progesterone vaginally (600 mg/day in the luteal phase until the 12th week of pregnancy)		no medications group: PR 24% (p=0.016) LBR of 13% (p=0.016).		
Scarpellini F, Sbracia M. Hum Reprod. 2009;24(11): 2703-8.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected ---------- X☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Patients with RM, 4 or more miscarriages who have previously miscarried after Ivlg	35 pts got G-CSF (1 µg (100,000 IU)/kg/day of Filgrastim subcutaneously from the sixth day after ovulation until onset of menstruation or the end of the 9th week of pregnancy. 33 got saline All miscarried pregnancies Had normal male or female karyotype	LBR	All women became pregnant spontaneously within 3 months G-CSF: LBR 29/35 (82.8%) saline: LBR 16/33 (48.5%) OR 5.1; 95%CI 1.5-18.4 NNT 2.9 (95%CI: 2.1- 10.3) During pregnancy, the patients treated with rG-CSF also had higher levels of β-hCG compared with those in placebo group Treated group ; 1 case of skin rash and 2 cases of leukocytosis (WBC count >25,000 mL) In the placebo group: 1 gestational hypertension		Included in review Cavalcante 2015
Schleussner E, et al. Ann	RCT	☐ Selection bias X☐Performance bias	449 women with at least 2 consecutive early miscarriages	Low-molecular-weight heparin:	ongoing pregnancy at 24 weeks'	At 24 weeks' gestation, 191 of 220 pregnancies (86.8%) and 188 of 214	Daily LMWH injections do	Placebo injections were not used, and

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Intern Med 2015;162: 601-609.		☐ Attrition bias X Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable (-)** FUNDING SOURCE: Pfizer Pharma.	or 1 late miscarriage included during 5 to 8 weeks' gestation after viable pregnancy was confirmed by US	control group received multivitamin pills, and the intervention group received vitamins and 5000 IU of dalteparin-sodium for up to 24 weeks' gestation.	gestation. live-birth rate late pregnancy complications. RESULTS:	pregnancies (87.9%) were intact in the intervention and control groups, respectively (absolute difference, -1.1 percentage points [95% CI, -7.4 to 5.3 percentage points]). LBRs were 86.0% (185 of 215 women) and 86.7% (183 of 211 women) in the intervention and control groups, resp (absolute difference, -0.7 percentage point [CI, -7.3 to 5.9 percentage points]). There were 3 intrauterine fetal deaths (1 woman had used LMWH); 9 cases of preeclampsia or the hemolysis, elevated liver enzyme level, and low platelet count (HELLP) syndrome (3 women had used LMWH); and 11 cases of intrauterine growth restriction or placental insufficiency (5 women had used LMWH).	not increase ongoing pregnancy or live-birth rates in women with unexplained RPL.	neither trial staff nor patients were blinded.
Shaaban OM, et al. Clinical and applied thrombosis/ hemostasis 2016:	RCT	☐ Selection bias XPerformance bias X Attrition bias X Detection bias ☐ No bias detected ---------- ☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Unexplained Recurrent Miscarriage With Negative Antiphospholipid Antibodies. 150 intervention 150 control There was no significant difference between both groups as regards age, parity, or number of previous miscarriages	Low-Molecular-Weight Heparin 150 patients receiving LMWH (Tinzaparin sodium 4500 IU) subcutaneous daily injection with 500 microg folic acid once daily orally started once positive pregnancy test till the 20th week of gestation. The control group included 150 patients receiving the same dose of folic acid alone.	rate of continuation of a viable pregnancy after 20 weeks of gestation	.There was a significant increase in women who continued their pregnancy beyond 20 weeks in the study group compared to the control group (73.3% vs 48%, respectively; P = .002). The take-home baby rate was also significantly higher in the LMWH group compared to the control group (P = .001).	Early start of LMWH decreases the incidence of miscarriage in the first 20 weeks of pregnancy in women with unexplained RM negative for APAs.	
Selhub J, Rosenberg		Na	These safety concerns are contrary to the 2015 WHO statement that "high folic acid intake has not reliably been shown to be associated with negative health effects". In the folic acid post-fortification era, we have shown that in elderly participants in NHANES 1999-2002, high plasma folate					Data on negative effects of high dose

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IH. Biochimie 2016;126: 71-78.			level is associated with exacerbation of both clinical (anemia and cognitive impairment) and biochemical (high MMA and high Hcy plasma levels) signs of vitamin B12 deficiency. Potential detrimental effects of high folic acid intake may not be limited to the elderly nor to those with B12 deficiency. A study from India linked maternal high RBC folate to increased insulin resistance in offspring. Our study suggested that excessive folic acid intake is associated with lower natural killer cells activity in elderly women. In a recent study we found that the risk for unilateral retinoblastoma in offspring is 4 fold higher in women that are homozygotes for the 19 bp deletion in the DHFR gene and took folic acid supplement during pregnancy. In the elderly this polymorphism is associated with lower memory and executive scores, both being significantly worse in those with high plasma folate. These and other data strongly imply that excessive intake of folic acid is not always safe in certain populations of different age and ethnical/genetic background.					folic acid
Tang AW. Hum Reprod 2013;28: 1743-1752.	RCT	X ☐ **Acceptable (+)**	2 * 20 patients with idiopathic recurrent miscarriage + high uterine natural killer cell density	prednisolone (20 mg for 6 weeks, 10 mg for 1 week, 5 mg for 1 week) or placebo when pregnant	live birth rate	12/20 (60%) with prednisolone and 8/20 (40%) with placebo (RR 1.5, 95% CI 0.79–2.86) Compliance with medication was reported to be 100%. Prednisolone side effects: insomnia and flushing		Feasibility trial
Wang S-W et al. Reprod BioMedicine Online 2016; 33: 720-36.	SR	X ☐ **Acceptable (+)**	metaanalysis. excluded two smaller trials from the Egerup analysis but included two Chineese trials only published in Chinese journals.	Ivlg treatment		the effect was strongest in secondary RPL, and in the total group of RPL the livebirth rate after Ivlg was borderline significantly increased compared with placebo, RR = 1.25, 95% CI 1.00-1.56). Interesting they found that in studies where the treatment started before conception, the treatment increased the livebirth rate highly significantly compared with placebo: RR 1.67, 95% CI 1.30-2.24), p< 0.0001.		maybe advocate for studies tetsing preconceptional Ivlg treatment.
Wong LF, Porter TF, et al. Cochrane Database Syst Rev. 2014;10:Cd0	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ----------	Unexpl. RM, 3 misc, max one previous birth	Ivlg, lymphocyte immunization (LIT) or trophoblast injection			No effect of any of the treatments	Exclusion of whole RCTs or subsets of patients without giving reason. Includes patients with 2 miscar. at

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
00112.		☐ **High quality (++)** X ☐ **Acceptable (+)** ☐ **Unacceptable (-)**						odds with. stated inclusion criteria
Yajnik CS, et al. Diabetologia 2008;51: 29-38.		NA	700 consecutive eligible pregnant women	measured maternal nutritional intake and circulating concentrations of folate, vitamin B12, tHcy and methylmalonic acid (MMA) at 18 and 28 weeks of gestation. These werecorrelated with offspring anthropometry, body composition (DEXA scan) and insulin resistance [HOMA-R] at 6 years.	Two-thirds of mothers had low vitamin B12 (<150 pmol/l), 90% had high MMA (>0.26 micromol/l) and 30% had raised tHcy concentrations (>10 micromol/l); only one had a low erythrocyte folate concentration. Although short and thin (BMI), the 6-year-old children were relatively adipose compared with the UK standards (skinfold thicknesses). Higher maternal erythrocyte folate concentrations at 28 weeks predicted higher offspring adiposity and higher HOMA-R (both p < 0.01). Low maternal vitamin B12 (18 weeks; p = 0.03) predicted higher HOMA-R in the children. The offspring of mothers with a combination of high folate and low vitamin B12 concentrations were the most insulin resistant.		Low maternal vitamin B12 and high folate status may contribute to the epidemic of adiposity and type 2 diabetes	Data on negative effects of high dose folic acid Study in India
Yamada H et al., EClinicalMedicine 2022;50: 101527.	RCT	☐ Selection bias ☐Performance bias ☐ Attrition bias ☐ Detection bias ☐ No bias detected --- **X High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	50 women received IVIG and 49 women received placebo in the ITT population	women with primary RPL of unexplained aetiology received 400 mg/kg of IVIG daily or placebo for five consecutive days starting at 4-6 weeks of gestation From June 3, 2014 to Jan 29, 2020	The primary outcome was ongoing pregnancy rate at 22 weeks of gestation (OPR), and the live birth rate (LBR) was the secondary outcome	IVIG group vs placebo group in the ITT population: • OPR: (31/50 [62·0%] vs. 17/49 [34·7%]; odds ratio [OR] 3·07, 95% CI 1·35-6·97; p = 0·009) • LBR: (29/50 [58·0%] vs. 17/49 [34·7%]; OR 2·60, 95% CI 1·15-5·86; p = 0·03) Women who received IVIG at 4-5 weeks of gestation vs placebo: • OPR: (OR 6·27, 95% CI 2·21-17·78; p < 0·001) • LBR: (OR 4·85, 95% CI 1·74-13·49; p = 0·003) These increases were not evident in women who received IVIG at 6 weeks of	A high dose of IVIG in very early pregnancy improved pregnancy outcome in women with four or more RPLs of unexplained aetiology	

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
						gestation. Four newborns in the IVIG group and none in the placebo group had congenital anomalies (p = 0·28).		

Additional references included as background information

Beer AE, Quebbeman JF, Ayers JW, Haines RF. Major histocompatibility complex antigens, maternal and paternal immune responses, and chronic habitual abortions in humans. Am J Obstet Gynecol 1981;141: 987-999.

Evers JLH. A nod is as good as a wink to a blind horse: round 2. *Human Reproduction* 2016;31: 1133-1134.

Hayes BD, Gosselin S, Calello DP, Nacca N, Rollins CJ, Abourbih D, Morris M, Nesbitt-Miller A, Morais JA, Lavergne V et al. Systematic review of clinical adverse events reported after acute intravenous lipid emulsion administration. Clin Toxicol (Phila) 2016;54: 365-404.

Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. Lancet 1985;1: 941-943.

18. WHICH THERAPEUTIC INTERVENTIONS COULD BE OFFERED TO ALL PATIENTS, IRRESPECTIVE OF A CAUSE, TO INCREASE LIVE BIRTH RATE?

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Hovdenak N, Haram K. European journal of obstetrics, gynecology, and reproductive biology. 2012;164(2): 127-32.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	**Maternal iron (Fe)** deficiency has a direct impact on neonatal Fe stores and birth weight, and may cause cognitive and behavioural problems in childhood. Fe supplementation is recommended to low-income pregnant women, to pregnant women in developing countries, and in documented deficiency, but overtreatment should be avoided. **Calcium (Ca) deficiency** is associated with pre-eclampsia and IUGR. Supplementation may reduce both the risk of low birth weight and the severity of pre-eclampsia. Gestational **magnesium (Mg) deficiency** may cause hematological and teratogenic damage. A Cochrane review showed a significant low birth weight risk reduction in Mg supplemented individuals. **Zn deficiency** in pregnant animals may limit fetal growth. Supplemental Zn may be prudent for women with poor gastrointestinal function, and in Zn deficient women, increasing birth weight and head circumference, but no evidence for beneficial effects of general Zn supplementation during pregnancy. **Low Se status** is associated with recurrent abortion, pre-eclampsia and IUGR, and although beneficial effects are suggested there is no EB recommendation for supplementation. An average of 20-30% of pregnant women suffer from any vitamin deficiency, and without prophylaxis, about 75% of these would show a deficit of at least one vitamin. **Vitamin B6 deficiency** is associated with pre-eclampsia, gestational carbohydrate intolerance, hyperemesis gravidarum, and neurologic disease of infants. Folate deficiency may lead to congenital malformations (neural tube damage, orofacial clefts, cardiac anomalies), anaemia and spontaneous abortions, and pre-eclampsia, IUGR and abruption placentae. Pregestational supplementation of folate prevents neural tube defects. A daily supplemental dose of 400 mug/day of folate is recommended when planning pregnancy. An insufficient supply of **vitamin B12** may cause reduced fetal growth. In vegetarian women, supplementation of vitamin B12 may be needed. **Vitamin A deficiency** is prevalent in the developing world, impairing Fe status and resistance to infections. The recommended upper limit for retinol supplements is 3000 IU/day. Vitamin A supplementation enhances birth weight and growth in infants born to HIV-infected women. Overdosing should be avoided. Low concentrations **of vitamin C** seem to increase the development of pre-eclampsia, and supplementation may be beneficial. Supplementation with **vitamin D** in the third trimester in vitamin D deficient women seems to be beneficial. The use of **vitamins E**, although generally considered "healthy", may be harmful to the pregnancy outcome by disrupting a physiologic oxidative gestational state and is consequently not recommended to prevent pre-eclampsia.			could be harmful	vit e may be harmful

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Hullender et al. Medical acupuncture 2013;25: 232-237.		**NA**	1 case DOR and RPL. 42-year-old	The patient received TCM treatment that involved weekly acupuncture and Chinese herbal therapy from June 2006 to May 2007.	live birth after 24 weeks of gestation.	After another miscarriage in September 2006, this patient conceived a viable pregnancy in December 2006, after 6 months of treatment. She continued treatment through 20 weeks and delivered a healthy son at 39.5 weeks of gestation.	Subfertile women with RPL may benefit from TCM treatment.	Case report
Li L, et al. Cochrane Database of Systematic Reviews. 2016;	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? ---------- ☒ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	RPL 9 RCTs (involving 861 women)	Chinese Herbal medicines (alone or combined with other intervention or other pharmaceuticals) Comparator: placebo, no treatment, other intervention (including bed rest and psycho-logical support), or other pharmaceuticals)	effectiveness and safety	Various Chinese herbal medicines were used in the different trials the methodological quality of the included studies was poor Chinese herbal medicines alone versus other pharmaceuticals alone: LBR not different between the two groups (RR 1.05; 95% CI 0.67 to 1.65; 1 trial, n=80) CHM and other pharma-ceuticals compared with pharmaceuticals alone: continuing pregnancy rate (RR 1.27 95% CI 1.10 to 1.48, 2 trials, 189 women) LBR (average RR 1.55; 95% CI 1.14 to 2.10; 6 trials, 601 women, Tau^2 = 0.10; I^2 = 73%) CHM + psychotherapy vs psychotherapy alone : higher LBR for combinations (RR 1.32; 95% CI 1.07 to 1.64; one trial, 90 women) 2 trials (341 women) reported no maternal adverse effects 1 trial (CHM vs other pharmaceuticals) reported that there were no abnormal fetuses (ultrasound) or after delivery.		

Bibliography	Study type	Study quality Funding + competing interest	PATIENTS No. Of patients Patient characteristics + group comparability	Interventions (+comparison) Include: Study duration / follow-up	Outcome measures Include: Harms / adverse events	Effect size	Authors conclusion	Comments
Yang GY, et al. BMC Complement Altern Med. 2013;13:320.	SR	Appropriate question ? Rigorous search ? Relevant studies included? Quality of studies? Methodology ? --- X ☐ **High quality (++)** ☐ **Acceptable (+)** ☐ **Unacceptable (-)**	Recurrent miscarriage	Chinese's herbal medicine 41 papers		potential positive effect however	Included trials of insufficient quality	further trials needed

Additional references included as background information
None