Annex 7: Evidence tables

II. Diagnosis

II.1 Confirmation of ovulation

PICO Question: Should couples with mild infertility factors be included in the definition of UI?

Menstrual history + one Progesterone/ USS/ LH urinary measurement in luteal phase (NICE)

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Guermandi, E., Vegetti, W., Bianchi, M. M., Uglietti, A., Ragni, G. and Crosignani, P. Reliability of ovulation tests in infertile women. Obstet Gynecol. 2001; 97 (1): 92-6.	CS	101 infertile couples - regular 26-34 days and previous mid-luteal P test normal. Women were excluded if their serum FSH and LH concentrations in early follicular phase were higher than 10 mUI/mL and 12 mUI/mL, respectively, or if their prolactin exceeded 20 ng/mL in the midluteal phase. Exclusion criteria also included clinical signs of PCOS (acne, hirsutism, oligomenorrhea, obesity) or ultrasound evidence of polycystic ovaries according to the criteria of Adams et	Transvaginal ultrasound monitoring= gold standard; Urinary LH, BBT, Serum P	sensitivity, specificity, and accuracy in predicting or confirming ovulation	evidence of ovulation on USS: 96% (97/101 cycles). Urinary LH surge detected in 99% (100/101 cycles); agreement with USS: 97%; Sensitivity, specificity, and accuracy for LH readings were 1.00, 0.25, and 0.97, respectively BBT: 67 cycles in agreement with USS, 0.77 sensitivity, 0.33 specificity, and 0.74 accuracy for ovulation detection compared with USS. Serum P4 79%	Urinary LH best marker, P4 based on menstrual history performed worse	

		al,20 any ovarian or abdominal abnormalities that would interfere with adequate ultrasound investigation, and evidence or history of endocrine or other diseases that might influence the menstrual cycle.					

LUTEINIZING HORMONE (LH) URINARY MEASUREMENT

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Martinez, A. R., Bernardus, R. E., Kucharska, D. and Schoemaker, J. Urinary luteinizing hormone testing and prediction of ovulation in spontaneous, clomiphene citrate and human menopausal gonadotropin-stimulated cycles. A clinical evaluation. Acta Endocrinol (Copenh). 1991; 124 (4): 357-63.	CS	303 (but only 99 in spontaneous cycles that can be used)	Urinary LH (colour test), ultrasound	Agreement Urinary LH vs ultrasound	Positive test results, presumably reflecting the occurrence of a urinary LH surge above 50 IU/1, were observed in 97 (98%) spontaneous cycles The basal body temperature nadir correlated with the day of the positive test in 30% of spontaneous cycles.	Urinary LH testing with the LH Colour proved to be a simple and accurate method to detect the midcycle LH surge and predict ovulation.	

Bischof, P., Bianchi, P. G. and Campana, A. Comparison of a rapid, quantitative and automated assay for urinary luteinizing hormone (LH), with an LH detection test, for the prediction of ovulation. Hum Reprod. 1991; 6 (4): 515-8.	CS	32 spontaneously ovulating women.	Serum E2, ultrasound and urinary LH (by automated microparticle enzyme immunoassay for serum LH and by color assay)	Agreement quantitative and qualitative LH tests	follicular rupture was seen on day 1 or 2 after the LH peak. The time between the urinary LH peak and follicular rupture (as documented by daily ultrasound scans) varied between 9-51 h	Urinary LH testing was a simpler alternative to repetitive venopuncture	Comparison between qualitative and quantitative scores.
Gregoriou, O., Kassanos, D., Vitoratos, N., Papadias, C. and Zourlas, P. A. Clinical efficacy of LH-color: a new home ovulation test. Int J Gynaecol Obstet. 1990; 32 (2): 141-3.	CS	55 women. All patients had been previously investigated and were assumed to have normal ovulatory menstrual cycles. All had prior biphasic BBT charts with cycle lengths of 26 -32 days. All had been previously noted to have single midluteal serum progesterone determination of > 10 ng/ml and in-phase luteal phase endometrial biopsy. All had adequate midcycle cervical mucus and serum testosterone, DHEA sulfate, TSH, and prolactin within normal range.	USS vs LG urinary measurement at 0700 h and 1900 h by LH-Colour. Daily measurements of BBT were recorded and the predictor point of ovulation was the thermal nadir.	Agreement	100% agreement to detect ovulation. In 20 (36.36%) of the cases, the thermal nadir was noted on the day of decolouration, whereas in 22 (40%) and 13 (23.6%) patients the thermal nadir occurred on days - 1 and + 1 and on days -2anddays +2of the LH surge, respectively. The predictive value of LH-Colour was assessed in relation to the day of ovulation by echography. In 39 of the 55 cases (70.91%), ovulation occurred in the 24 h after the decolouration of the LH-Colour. Ultrasound showed the disappearance all of the dominant follicles	The good correlation found between the urinary LH surge and ultrasound, allows us to suggest the LH-Colour test as a reliable method in the study of infertile population and also as an adjunct to natural family planning. It is not to say that a urine test can replace the other methods that have been employed up to now, but the LH-Colour diminishes elaborate cycle monitoring and thus the inconvenience and cost for the patients as well as the workload of the physician.	

Guermandi, E., Vegetti, W., Bianchi, M. M., Uglietti, A., Ragni, G. and Crosignani, P. Reliability of ovulation tests in infertile women. Obstet Gynecol. 2001; 97 (1): 92-6.	CS	101 infertile couples - regular 26-34 days and previous mid-luteal P test normal. Women were excluded if their serum FSH and LH concentrations in early follicular phase were higher than 10 mUI/mL and 12 mUI/mL, respectively, or if their prolactin exceeded 20 ng/mL in the midluteal phase. Exclusion criteria also included clinical signs of PCOS (acne, hirsutism, oligomenorrhea, obesity) or ultrasound evidence of polycystic ovaries according to the criteria of Adams et al,20 any ovarian or abdominal abnormalities that would interfere with adequate ultrasound investigation, and evidence or history of endocrine or other diseases that might influence the menstrual cycle.	Transvaginal ultrasound monitoring= gold standard; Urinary LH, BBT, Serum P	sensitivity, specificity, and accuracy in predicting or confirming ovulation	evidence of ovulation on USS: 96% (97/101 cycles). Urinary LH surge detected in 99% (100/101 cycles); agreement with USS: 97%; Sensitivity, specificity, and accuracy for LH readings were 1.00, 0.25, and 0.97, respectively BBT: 67 cycles in agreement with USS, 0.77 sensitivity, 0.33 specificity, and 0.74 accuracy for ovulation detection compared with USS. Serum P4 79%	Urinary LH best marker, P4 based on menstrual history performed worse	

SERIAL BASAL BODY TEMPERATURE (BBT)

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Gregoriou, O., Kassanos, D., Vitoratos, N., Papadias, C. and Zourlas, P. A. Clinical efficacy of LH-color: a new home ovulation test. Int J Gynaecol Obstet. 1990; 32 (2): 141-3.	CS	55 women. All patients had been previously investigated and were assumed to have normal ovulatory menstrual cycles. All had prior biphasic BBT charts with cycle lengths of 26 -32 days. All had been previously noted to have single midluteal serum progesterone determination of > 10 ng/ml and in-phase luteal phase endometrial biopsy. All had adequate midcycle cervical mucus and serum testosterone, DHEA sulfate, TSH, and prolactin within normal range.	USS vs LG urinary measurement at 0700 h and 1900 h by LH-Colour. Daily measurements of BBT were recorded and the predictor point of ovulation was the thermal nadir.	Agreement	100% agreement to detect ovulation. In 20 (36.36%) of the cases, the thermal nadir was noted on the day of decolouration, whereas in 22 (40%) and 13 (23.6%) patients the thermal nadir occurred on days - 1 and + 1 and on days -2anddays +2of the LH surge, respectively. The predictive value of LH-Colour was assessed in relation to the day of ovulation by echography. In 39 of the 55 cases (70.91%), ovulation occurred in the 24 h after the decolouration of the LH-Colour. Ultrasound showed the disappearance all of the dominant follicles	The good correlation found between the urinary LH surge and ultrasound, allows us to suggest the LH-Colour test as a reliable method in the study of infertile population and also as an adjunct to natural family planning. It is not to say that a urine test can replace the other methods that have been employed up to now, but the LH-Colour diminishes elaborate cycle monitoring and thus the inconvenience and cost for the patients as well as the workload of the physician.	

Guermandi, E., Vegetti, W., Bianchi, M. M., Uglietti, A., Ragni, G. and Crosignani, P. Reliability of ovulation tests in infertile women. Obstet Gynecol. 2001; 97 (1): 92-6.	CS	101 infertile couples - regular 26-34 days and previous mid-luteal P test normal. Women were excluded if their serum FSH and LH concentrations in early follicular phase were higher than 10 mUI/mL and 12 mUI/mL, respectively, or if their prolactin exceeded 20 ng/mL in the midluteal phase. Exclusion criteria also included clinical signs of PCOS (acne, hirsutism, oligomenorrhea, obesity) or ultrasound evidence of polycystic ovaries according to the criteria of Adams et al,20 any ovarian or abdominal abnormalities that would interfere with adequate ultrasound investigation, and evidence or history of endocrine or other diseases that might influence the menstrual cycle.	Transvaginal ultrasound monitoring= gold standard; Urinary LH, BBT, Serum P	sensitivity, specificity, and accuracy in predicting or confirming ovulation	evidence of ovulation on USS: 96% (97/101 cycles). Urinary LH surge detected in 99% (100/101 cycles); agreement with USS: 97%; Sensitivity, specificity, and accuracy for LH readings were 1.00, 0.25, and 0.97, respectively BBT: 67 cycles in agreement with USS, 0.77 sensitivity, 0.33 specificity, and 0.74 accuracy for ovulation detection compared with USS. Serum P4 79%	Urinary LH best marker, P4 based on menstrual history performed worse	

Martinez, A. R., Bernardus, R. E., Kucharska, D. and Schoemaker, J. Urinary luteinizing hormone testing and prediction of ovulation in spontaneous, clomiphene citrate and human menopausal gonadotropin-stimulated cycles. A clinical evaluation. Acta Endocrinol (Copenh). 1991; 124 (4): 357-63.	CS	303 (but only 99 in spontaneous cycles that can be used)	Urinary LH (colour test), ultrasound	Agreement Urinary LH vs ultrasound	Positive test results, presumably reflecting the occurrence of a urinary LH surge above 50 IU/1, were observed in 97 (98%) spontaneous cycles The basal body temperature nadir correlated with the day of the positive test in 30% of spontaneous cycles.	Urinary LH testing with the LH Colour proved to be a simple and accurate method to detect the midcycle LH surge and predict ovulation.	

II.2 Oocyte/corpus luteum quality

PICO Question: What is the reliability of parameters detecting good oocyte/ corpus luteum quality?

Mid-luteal phase progesterone levels

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Hull, M. G., Savage, P. E., Bromham, D. R., Ismail, A. A. and Morris, A. F. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived form treated and untreated conception cycles. Fertil Steril. 1982; 37 (3): 355-60.	CS	138 cycles of 72 women with no physical cause for infertility were included as a subgroup.	midluteal serum progesterone level	conception spontaneous or with treatment	Lowest threshold was 8.5 ng/ml for conception cycles.	A midluteal serum P level above 9.4 ng/ml suggests better results.	The study design does not allow definitive conclusions.

ENDOMETRIAL BIOPSY

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Edi-Osagie, E. C., Seif, M. W., Aplin, J. D., Jones, C. J., Wilson, G. and Lieberman, B. A. Characterizing the endometrium in unexplained and tubal factor infertility: a multiparametric investigation. Fertil Steril. 2004; 82 (5): 1379-89.	CS	20 women with UI, 22 tubal factor, 21 fertile controls. Average age of 34, similar characteristics. Basal FSH <10 IU/L	Endometrial histology by Noyes criteria during the midluteal phase.	Endometrial maturation	UI group had similar maturation as fertile controls.		
Coutifaris, C., Myers, E. R., Guzick, D. S., Diamond, M. P., Carson, S. A., Legro, R. S., McGovern, P. G., Schlaff, W. D., Carr, B. R., Steinkampf, M. P. and et al. Reprint of: histological dating of timed endometrial biopsy tissue is not related to fertility status. Fertility and sterility. 2019; 112 (4): e116-e124.	RCT	287 ovulatory female partners of infertile couples, not necessarily UI. And 332 fertile women	Midluteal or late luteal endometrial biopsy, Noyes criteria.	Prevalence of out of phase endometrial biopsies	Prevalence of out of phase endometrial biopsy results were similar between fertile and infertile women in adjusted analyses. ROC curves showed less than 0.5 AUC values for endometrial biopsy to differentiate fertile and infertile women.		Male factor not assessed, not specific to UI, but in general suggests that endometrial dating does not help identifying infertile women.

II.3 Ovarian reserve

PICO QUESTION: SHOULD ONE OR MORE TESTS OF OVARIAN RESERVE BE INCLUDED IN THE DIAGNOSTIC WORK-UP?

AMH

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Casadei, L., Manicuti, C., Puca, F., Madrigale, A., Emidi, E. and Piccione, E. Can anti-Müllerian hormone be predictive of spontaneous onset of pregnancy in women with unexplained infertility? J Obstet Gynaecol. 2013; 33 (8): 857-61.	CS	83 women with unexplained infertility aged 35.9 ± 5.4 years (21 - 48 years), AMH 1.76 ± 1.47 ng/ml, 2.8 ± 2.4 years of infertility.	AMH-EIA Beckman Coulter A11893. underwent 6 months expectant management before ART.	Spontaneous pregnancy without live birth rate	14 women (17%) achieved spontaneous pregnancy. AMH had an AUC of 0.385 ± 0.07 (95% CI 0.25 - 0.52) spontaneous pregnancy	Serum AMH was not predictive of spontaneous pregnancy, women with AMH< 0.75 ng/ml had similar pregnancy rates with women who had higher AMH despite the former being older.	

Depmann M., Broer S. L., Eijkemans M. J. C., van Rooij I. A. J., Scheffer G. J., Heimensem J., Mol B. W., Broekmans F. J. M. Anti-Müllerian hormone does not predict time to pregnancy: results of a prospective cohort study. Gynecol Endocrinol. 2017 Aug;33(8):644-648.	CS	Prospective CS. Inclusion criteria were female age ranging between 18 and 46 years, the presence of two ovaries, no adnexal surgery in the past and the presence of a regular menstrual cycle (21–35 days).	A transvaginal ultrasound was performed for the assessment of the number of follicles measuring 2–10 mm. Blood samples were obtained for assessment of AMH and FSH.	Viable pregnancy of at least 11 weeks of gestational age	In the univariate analysis (Table 2), both the AFC and female age were significantly capable of predicting TTOP (p=0.02 and p=0.01 respectively). However, the C-statistic for both variables was poor (0.54 and 0.56, respectively). AMH was not significantly capable of predicting TTOP (HR 1.66, 95% CI 0.97–2.85, p values 0.18, C-statistic 0.55). In the multivariate Cox regression analysis (Table 2), where a correction for female age was performed, none of the variables analysed was significantly correlated with TTOP, nor did they reach a predictive accuracy level of any importance.		
Greenwood, E. A., Cedars, M. I., Santoro, N., Eisenberg, E., Kao, C. N., Haisenleder, D. J., Diamond, M. P. and Huddleston, H. G. Antimüllerian hormone levels and antral follicle counts are not reduced compared with community controls in patients with rigorously defined unexplained infertility. Fertil Steril. 2017; 108 (6): 1070-1077.	CS	277 women with unexplained infertility 32.3 ± 0.2 (25 - 40) years of age, randomly selected from the AMIGOS trial participants (Diamond et al. 2015, FS 2015;103:962) had to have cycle day 1 - 5 FSH <12 IU/L during the previous year, and >9 cycles/year. Male with >5 million/ml sperm. Compared with 226 ovulatory women from the OVA study (Rosen et al. FS 2012;97:238, community based ovarian ageing study), not seeking fertility	CD 2 - 4, for infertile women and controls (Shimadzu 4 - 8 MHz transvaginal)	AFC .	Analyses adjusted for age, race, BMI, smoking and study site revealed that infertility was not a predictor of AFC.		Large study with proper definitions of participants and analyses, suggest that women with UI do not have lower AMH levels than healthy women from the community, yet 54% of controls were nulligravid, risking

		treatment, aged 33.1 ± 0.3 years (25 - 40). Women with FSH >12 IU/L were excluded from the control group.					underestimation of a difference.
Hagen C. P., Vestergaard S., Juul A., Skakkebæk N. E., Andersson A., Main K. M., Hjøllund N. H., Ernst E., Bonde J.P., Anderson R.A., Jensen T. K. Low concentration of circulating antimüllerian hormone is not predictive of reduced fecundability in young healthy women: a prospective cohort study. Fertil Steril 2012;98(6):1602-8	CS	Prospective CS. 430 couples with no previous reproductive experience who intended to discontinue contraception to become pregnant were eligible for enrolment.	AMH concentrations were determined in a subgroup of 186 women	Fecundability ratio (monthly probability of conceiving)	Compared with the reference group of women with medium AMH levels, the unadjusted odds ratios of not becoming pregnant within the first six cycles for those with low AMH and high AMH were 1.35 (95 % CI 0.63–2.89) and 1.60 (0.76–3.39), respectively (Fig. 1 and Table 2). Compared with women with medium AMH, the monthly probabilities of conceiving (FR) for those with low and high AMH were 0.87 (95% CI 0.51–1.46) and 0.67 (95% CI 0.42–1.08), respectively (Table 2, unadjusted data in model 1). In the low AMH group, the adjusted FR was not different to the reference group, 0.81 (95% CI 0.44–1.40).		

Hvidman H. W., Bentzen J. G., Thuesen L. L., Lauritsen M. P., Forman J. L., Loft A., Pinborg A., Nyboe Andersen A.. Infertile women below the age of 40 have similar anti-Müllerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. Hum Reprod. 2016;31(5):1034-45	CS	Prospective CS with a historical control group. 382 infertile patients. Excluded: (i) patients referred for PGD, (ii) patients referred due to HIV or contagious hepatitis B or C infection and (iii) single and homosexual women, as they were per se not considered infertile. Furthermore, patients referred directly for oocyte donation (OD) or patients with PCOS were not included.	Study group: infertile women Control group: 350 non-users of hormonal contraception and no history of infertility A transvaginal ultrasonography was performed on CD 2–5. Blood samples were taken on CD 2-5.	Ovarian reserve parameters and age in infertile patients versus controls	The age-related depletion of the ovarian reserve was the same in the two cohorts; AMH levels decreased by 5.5% (95% CI: 4;7%) and AFC decreased by 5% (95% CI: 4;6%) per year age increase. Patients with unexplained infertility had similar AMH levels (age-adjusted: 28%, 95% CI: 223;10%) and AFC (age-adjusted: 25%, 95% CI: 216;7%) compared with other patients. In an age adjusted subgroup analysis comparing patients with unexplained infertility with controls, no differences in neither AMH levels (5%, 95% CI: 222;25%) nor AFC (22%, 95% CI: 214;11%) were observed.		
Nguyen, D. K., O'Leary, S., Gadalla, M. A., Roberts, B., Alvino, H., Tremellen, K. P. and Mol, B. W. The predictive value of anti-Müllerian hormone for natural conception leading to live birth in subfertile couples. Reprod Biomed Online. 2022; 44 (3): 557-564.	CS	Retrospective CS. exclusion criteria were couples who had anovulation, two-sided tubal blockage or total motile sperm count less than 1×10^6 (severe male factor) and couples with female age above 42 years.	AMH (ELISA)	natural conception leading to live birth within 12 months since consultation was recorded.	325 couples were eligible for inclusion in the final analysis. Thirty (9.2%) couples achieved natural conception, whereas 223 (68.6%) started ART treatment within 12 months. Forty-seven (14.5%) couples completed 12 months of follow-up without achieving natural conception and 25 couples (7.7%) were lost to follow-up. The estimated cumulative probability of achieving a natural conception leading to live birth for the cohort within 12 months since consultation was 20.9% (95% CI 12.9% to 28.2%). The unadjusted hazard ratio of		

					serum AMH was 0.94 (95% CI 0.82 to 1.08, P = 0.369), the adjusted HR was 0.85 (95% CI 0.71 to 1.00, P = 0.066).		
Steiner, A. Z., Pritchard, D., Stanczyk, F. Z., Kesner, J. S., Meadows, J. W., Herring, A. H. and Baird, D. D. Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age. Jama. 2017; 318 (14): 1367-1376.	CS	750 women recruited from community, 30 to 44 years of age, women with a risk factor or history of infertility were excluded such as breastfeeding women or those with a partner with known fertility problem, who had been trying ot conceive for 3 months or less.	serum on day 2 - 4, stored at -30 C, Ultrasensitive Ansh AMH kit, trying to conceive spontaneously	spontaneous conception attempt for 6 - 12 months	65% conceived in 6, 77% in 12 months, Cumulative probability of conception was not different for women with AMH <0.7 ng/ml, 0.7 - 8.4 ng/ml, or >8.4 ng(ml after adjusting for age, race, BMI, current smoking, recent contraceptive use.	AMH is not associated with spontaneous pregnancy	Not a population with UI but answers the Question, whether ORTs can predict fertility, despite the limitations.
Yücel, B., Kelekci, S. and Demirel, E. Decline in ovarian reserve may be an undiagnosed reason for unexplained infertility: a cohort study. Arch Med Sci. 2018; 14 (3): 527-531.	CS	148 women with UI (FSH >10 were excluded) and 112 women with male factor infertility, groups were similar for age, BMI, duration of infertility, and type of infertility (primary vs secondary)	serum collected on cycle day 2 - 4, stored at -20C, AMH-EIA Beckman Coulter A11893	women with UI had lower AMH levels then male factor group, 1.42 (0.4 - 6.2) vs 2.04 (0.64 - 8.2) ng(/ml, resp. Log regression with infertility as the dependent showed that AMH was significantly associated with UI, after adjusting for age.	women with UI had lower AMH levels than male factor group, 1.42 (0.4 - 6.2) vs 2.04 (0.64 - 8.2) ng(/ml, resp. Log regression with infertility as the dependent showed that AMH was significantly associated with UI, after adjusting for age.		poor quality study with regard to statistics.

Murto, T., Bjuresten, K., Landgren, B. M. and Stavreus-Evers, A. Predictive value of hormonal parameters for live birth in women with unexplained infertility and male infertility. Reprod Biol Endocrinol. 2013; 11 61.	CS	42 women with UI and 29 women with male infertility (abnormal semen analysis as per WHO criteria at the time), similar age and BMI	cycle day 2 - 5 AMH level Beckman Coulter, probability of live birth in 5 years, spontaneous or by fertility treatment	probability of live birth in 5 years, spontaneous or by treatment	Serum AMH levels were similar between UI and male factor groups, 2.7 (0.18 - 8.5) vs 2.95 (0.74 - 8.5) ng/ml, respectively, p = 0.98. AMH alone was a poor predictor.		small sample, very old study, yet results consistent with others.

AFC

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Casadei, L., Manicuti, C., Puca, F., Madrigale, A., Emidi, E. and Piccione, E. Can anti-Müllerian hormone be predictive of spontaneous onset of pregnancy in women with unexplained infertility? J Obstet Gynaecol. 2013; 33 (8): 857-61.	CS	83 women with unexplained infertility aged 35.9 ± 5.4 years (21 - 48 years), AMH 1.76 ± 1.47 ng/ml, 2.8 ± 2.4 years of infertility.	Day 2 - 5 of cycle, sum of all follicles 2 - 9 mm in both ovaries, Hitachi 6.5 MHz vaginal probe	Spontaneous pregnancy without live birth rate	14 women (17%) achieved spontaneous pregnancy. AFC had an AUC of 0.418 ± 0.08 (95% CI 0.26 - 0.57) spontaneous pregnancy	AFC was not predictive of spontaneous pregnancy, AFC was highly correlated with AMH	
Depmann M., Broer S. L., Eijkemans M. J. C., van Rooij I. A. J., Scheffer G. J., Heimensem J., Mol B. W., Broekmans F. J. M. Anti-Müllerian hormone does not predict time to	CS	prospective CS. Inclusion criteria were female age ranging between 18 and 46 years, the presence of two ovaries, no adnexal surgery in the past and the presence of a	A transvaginal ultrasound was performed for the assessment of the number of follicles measuring 2–10 mm. Blood samples were	viable pregnancy of at least 11 weeks of gestational age	In the univariate analysis (Table 2), both the AFC and female age were significantly capable of predicting TTOP (p¼0.02 and p¼0.01 respectively). However, the C-statistic for both variables was poor (0.54 and 0.56, respectively).		

pregnancy: results of a prospective cohort study. Gynecol Endocrinol. 2017 Aug;33(8):644-648.		regular menstrual cycle (21–35 days).	obtained for assessment of AMH and FSH.		AMH was not significantly capable of predicting TTOP (HR 1.66, 95% CI 0.97–2.85, p values 0.18, C-statistic 0.55). In the multivariate Cox regression analysis (Table 2), where a correction for female age was performed, none of the variables analysed was significantly correlated with TTOP, nor did they reach a predictive accuracy level of any importance.		
Greenwood, E. A., Cedars, M. I., Santoro, N., Eisenberg, E., Kao, C. N., Haisenleder, D. J., Diamond, M. P. and Huddleston, H. G. Antimüllerian hormone levels and antral follicle counts are not reduced compared with community controls in patients with rigorously defined unexplained infertility. Fertil Steril. 2017; 108 (6): 1070-1077.	CS	277 women with unexplained infertility 32.3 ± 0.2 (25 - 40) years of age, randomly selected from the AMIGOS trial participants (Diamond et al. 2015, FS 2015;103:962) had to have cycle day 1 - 5 FSH <12 IU/L during the previous year, and >9 cycles/year. Male with >5 million/ml sperm. Compared with 226 ovulatory women from the OVA study (Rosen et al. FS 2012;97:238, community based ovarian ageing study), not seeking fertility treatment, aged 33.1 ± 0.3 years (25 - 40). Women with FSH >12 IU/L were excluded from the control group.	CD 2 - 4, for infertile women and controls (Shimadzu 4 - 8 MHz transvaginal)	AFC	Analyses adjusted for age, race, BMI, smoking and study site revealed that infertility was not a predictor of AFC .	Large study with proper definitions of participants and analyses, suggest that women with UI do not have lower AMH levels than healthy women from the community, yet 54% of controls were nulligravid, so this is low quality evidence and can be been excluded.	unfortunately 46% of controls were nulligravid.

Hvidman H. W., Bentzen J. G., Thuesen L. L., Lauritsen M. P., Forman J. L., Loft A., Pinborg A., Nyboe Andersen A.. Infertile women below the age of 40 have similar anti-Müllerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. Hum Reprod. 2016;31(5):1034-45	CS	prospective CS with a historical control group. 382 infertile patients. Excluded: (i) patients referred for PGD, (ii) patients referred due to HIV or contagious hepatitis B or C infection and (iii) single and homosexual women, as they were per se not considered infertile. Furthermore, patients referred directly for oocyte donation (OD) or patients with PCOS were not included.	study group: infertile women control group: 350 non-users of hormonal contraception and no history of infertility A transvaginal ultrasonography was performed on CD 2–5. Blood samples were taken on CD 2-5.	Ovarian reserve parameters and age in infertile patients versus controls	The age-related depletion of the ovarian reserve was the same in the two cohorts; AMH levels decreased by 5.5% (95% CI: 4;7%) and AFC decreased by 5% (95% CI: 4;6%) per year age increase. Patients with unexplained infertility had similar AMH levels (age-adjusted: 28%, 95% CI: 223;10%) and AFC (age-adjusted: 25%, 95% CI: 216;7%) compared with other patients. In an age-adjusted subgroup analysis comparing patients with unexplained infertility with controls, no differences in neither AMH levels (5%, 95% CI: 222;25%) nor AFC (22%, 95% CI: 214;11%) were observed.		

Rosen M. P., Johnstone E., Addauan-Andersen C., Cedars M. I.. lower antral follicle count is associated with infertility. Fertil Steril. 2011;95(6):1950-4	CS	case-control study. inclusion criteria for the infertile group included: 1) age 25–45 years; 2).regular ovulatory menstrual cycles between 22 and 35 days; 3) no endocrinopathies; and 4) with a diagnosis of unexplained infertility. Women with surgically diagnosed endometriosis, ovarian failure, tubal factor, isolated male factor, anovulation, or use of an oocyte donor or gestational surrogate were excluded. The control group for the primary analysis (community group) was composed of ovulatory women with regular menstrual cycles between 22 and 35 days in length, aged 25–45 years, and enrolled in the OVA (Ovarian Aging) study.	women presenting to the infertility clinic with unexplained infertility were compared with a sampling frame of women from the general community. AFC by TVS	relationship between AFC and infertility	The median AFC was lower and FSH significantly higher in the infertile women. The proportion of women with history of a live birth was significantly higher in the community compared with the infertile women (53% versus 8.2%; P<.0001). The infertile women have significantly lower AFCs for each age group except those women between 41–45 years of age. The difference in median AFC between groups was four for women 25–30 and 31–35 years of age and three for women 36–40 years of age.		
Yücel, B., Kelekci, S. and Demirel, E. Decline in ovarian reserve may be an undiagnosed reason for unexplained infertility: a cohort study. Arch Med Sci. 2018; 14 (3): 527-531.	CS	148 women with UI (FSH >10 were excluded) and 112 women with male factor infertility, groups were similar for age, BMI, duration of infertility, and type of infertility (primary vs secondary)	examination on cycle day 2 - 4, medison 7.5 MHz transvaginal probe, total follicle count between 2 - 10 mm	women with UI had lower AFC than male factor group, 9 (3 - 16) vs 10 (3 - 23) , resp., p =0.02. Log regression with infertility as the dependent showed that AFC was NOT	women with UI had lower AFC than male factor group, 9 (3 - 16) vs 10 (3 - 23) , resp., p =0.02. Log regression with infertility as the dependent showed that AFC was NOT significantly associated with UI, after adjusting for age.	poor quality study with regard to statistics.	poor quality study with regard to statistics.

				significantly associated with UI, after adjusting for age.			

DAY 3 FSH AND ESTRADIOL

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Steiner, A. Z., Pritchard, D., Stanczyk, F. Z., Kesner, J. S., Meadows, J. W., Herring, A. H. and Baird, D. D. Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age. Jama. 2017; 318 (14): 1367-1376.	CS	750 women recruited from community, 30 to 44 years of age, women with a risk factor or history of infertility were excluded such as breastfeeding women or those with a partner with known fertility problem, who had been trying ot conceive for 3 months or less.	serum on day 2 - 4, Immulyte Siemens FSH kit	spontaneous conception attempt for 6 - 12 months	65% conceived in 6, 77% in 12 months, Cumulative probability of conception was not different for women with FSH>10 IU/L, after adjusting for age, race, BMI, current smoking, recent contraceptive use (HR 1.22, 0.92 to 1.62.	FSH is not associated with spontaneous pregnancy	Not a population with UI but answers the Question, whether ORTs can predict fertility, despite the limitations.
Yücel, B., Kelekci, S. and Demirel, E. Decline in ovarian reserve may be an undiagnosed reason for unexplained infertility: a cohort study. Arch Med Sci. 2018; 14 (3): 527-531.	CS	148 women with UI (FSH >10 were excluded) and 112 women with male factor infertility, groups were similar for age, BMI, duration of infertility, and type of infertility (primary vs secondary)	examination on cycle day 2 - 4,	Hormone levels	women with UI had similar FSH with male factor group, 7.52 (4.21 - 9.88) vs 6.96 (5.1 - 9.37) , resp., p =0.07. Likewise estradiol levels were similar, 51.5 (27 - 86) pg/ml vs 43.5 (25 - 71) in UI and Male factor, respectively, p = 0.108.	poor quality study with regard to statistics. Method of inhibin measurement not reported.	

Clomiphene Citrate Challenge Test (CCCT)

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Scott, R. T., Leonardi, M. R., Hofmann, G. E., Illions, E. H., Neal, G. S. and Navot, D. A prospective evaluation of clomiphene citrate challenge test screening of the general infertility population. Obstet Gynecol. 1993; 82 (4 Pt 1): 539-44.	CS	general infertility population without oligo/anovulation or tubal reversal request. Eventually 236 consecutive women meeting criteria (no prior infertility assessment in addition to aforementioned). Mean 34 years of age 20 - 43 years.	dau 5 - 9 100 mg/Day CC, if FSH was > 10 IU/L on any occasion test was regarded abnormal. Becton dickinson WHO 2nd international reference.	women with abnormal CCCT conceived less often than those with normal results. Moreover women eventually diagnosed with UI had higher rate of abnormal CCCT.	52% of Women with UI (12/32) had abnormal CCCT as compared with 4.3% for tubal factor, 17.4% for oligo/anovulation, 8.7% for male factor, 4.3% for endometriosis, and 0% for pelvic adhesions.		Small number of women with UI, complicated design.

Ovarian volume, ovarian blood flow, inhibin B

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Steiner, A. Z., Pritchard, D., Stanczyk, F. Z., Kesner, J. S., Meadows, J. W., Herring, A. H. and Baird, D. D. Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive	CS	750 women recruited from community, 30 to 44 years of age, women with a risk factor or history of infertility were excluded such as breastfeeding women or those with a partner with known fertility problem, who had been trying ot	serum on day 2 - 4, stored at -30C, Ansh İnhibin B assay	spontaneous conception attempt for 6 - 12 months	65% conceived in 6, 77% in 12 months, Cumulative probability of conception was not associated with inhibin B levels, after adjusting for age, race, BMI, current smoking, recent contraceptive use (HR 0.999, 0.997 to 1.001, per 1 pg/ml increase in inhibin B level).	Inhibin B levels are not associated with probability of spontaneous conception	Not a population with UI but answers the Question, whether ORTs can predict fertility, despite the limitations.

Age. Jama. 2017; 318 (14): 1367-1376.		conceive for 3 months or less.					
Yücel, B., Kelekci, S. and Demirel, E. Decline in ovarian reserve may be an undiagnosed reason for unexplained infertility: a cohort study. Arch Med Sci. 2018; 14 (3): 527-531.	CS	148 women with UI (FSH >10 were excluded) and 112 women with male factor infertility, groups were similar for age, BMI, duration of infertility, and type of infertility (primary vs secondary)	examination on cycle day 2 - 4, medison 7.5 MHz transvaginal probe, total follicle count between 2 - 10 mm	women with UI had similar ovarian volume with male factor group, 6.2 (3.2 - 10.96) vs 6.06 (3.3 - 12.2) , resp., p =0.64. Likewise inhibin B levels were similar, 119 (40 - 145) pg/ml vs 120 (52 - 150) in UI and Male factor, respectively, p = 0.298.	women with UI had similar ovarian volume with male factor group, 6.2 (3.2 - 10.96) vs 6.06 (3.3 - 12.2) , resp., p =0.64. Likewise inhibin B levels were similar, 119 (40 - 145) pg/ml vs 120 (52 - 150) in UI and Male factor, respectively, p = 0.298.	poor quality study with regard to statistics. Method of inhibin measurement not reported.	
Murto, T., Bjuresten, K., Landgren, B. M. and Stavreus-Evers, A. Predictive value of hormonal parameters for live birth in women with unexplained infertility and male infertility. Reprod Biol Endocrinol. 2013; 11 61.	CS	42 women with UI and 29 women with male infertility (abnormal semen analysis as per WHO criteria at the time), similar age and BMI	cycle day 2 - 5 DSL Gen II ELISA for inhibin B, probability of live birth in 5 years, spontaneous or by fertility treatment	probability of live birth in 5 years, spontaneous or by treatment	Serum inhibin B levels were similar between UI and male factor groups, 37.1 (7.0 - 95.4) vs 47.5 (13 - 138.4) pg/ml, respectively, p = 0.208. Inhibin B alone was a poor predictor of live birth.	small sample, very old study, yet results consistent with others	

II.4 Tubal factor

PICO Question: What is the accuracy of commonly used tests of tubal patency?

Hystero-contrast-sonography (HyCoSy) vs. laparoscopy and dye

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Alcázar, J. L., Martinez, A., Duarte, M., Welly, A., Marín, A., Calle, A., Garrido, R., Pascual, M. A. and Guerriero, S. Two-dimensional hysterosalpingo-contrast-sonography compared to three/four-dimensional hysterosalpingo-contrast-sonography for the assessment of tubal occlusion in women with infertility/subfertility: a systematic review with meta-analysis. Hum Fertil (Camb). 2020; 1-13.	SR	30 studies, 1977 patients and 3885 tubes.	21 studies used 2D-HyCoSy to assess tubal occlusion, 6 of them used 3D/4D-HyCoSy 1 study used both techniques but in different set of patients and 2 of them used both techniques in the same patients. **Contrast solution**: 4 studies used saline solution, 11 used a galactose solution, 6 used sterile airsaline solution, 5 used sulphurhexafluoride, 2 studies used ExEm FoamTM, 1 study used perflutren lipid microsphere and 1 used air-saline and Exem FoamTM	sensitivity, specificity, LR+, LR-	**2D HyCoSy**: Pooled sensitivity, specificity, LR+, LR- were 86% (95% CI 80%–91%), and 94% (95% CI 90%–96%), 13.5 (95% CI 8.2–22.5), 0.14 (95% CI¼0.1–0.2), respectively. High heterogeneity was found for sensitivity (I^2=79.23%; Cochran Q=110.7; p<0.001) and for specificity (I^2=90.08%; Cochran Q=231.77; p<0.001). **3D/4D HyCoSy**: pooled sensitivity, specificity, LR+ and LR- for detecting tubal occlusion were 95% (95% CI=89%–98%), 89% (95% CI=82%–94%), 8.9 (95% CI=5.0–16.1), and 0.06 (95% CI=0.03–0.13), respectively. High heterogeneity was found for both sensitivity (I^2=76.98%; Cochran Q=34.96; p<0.01) and specificity (I^2=85.76%; Cochran Q=56.17; p<0.001). Both		

					methods had almost identical areas under the curve (0.96 for 2DHyCoSy and 0.97 for 3D/4D-HyCoSy)		
Wang, Y. and Qian, L. Three- or four-dimensional hysterosalpingo contrast sonography for diagnosing tubal patency in infertile females: a systematic review with meta-analysis. Br J Radiol. 2016; 89 (1063): 20151013.	SR	23 studies, 1153 females and 2259 tubes	16 and 7 studies reported the diagnostic accuracy of 3D and 4D HyCoSy for detecting tubal patency in infertile females. The contrast agent was Echovist in 3 studies, saline solution in 1 study and SonoVue in 19 studies.	sensitivity, specificity, LR+, LR-	pooled estimates of sensitivity and specificity were 0.92 (95% CI 0.90–0.94, with I^2=36.68) and 0.92 (95% CI 0.89–0.93 with I^2=38.99), respectively. The area under the ROC curve was 0.97 (95% CI 0.95–0.98)		
Chen, S., Du, X., Chen, Q. and Chen, S. Combined Real-Time Three-Dimensional Hysterosalpingo-Contrast Sonography with B Mode Hysterosalpingo-Contrast Sonography in the Evaluation of Fallopian Tube Patency in Patients Undergoing Infertility Investigations. Biomed Res Int. 2019; 2019 9408141.	CS	prospective CS. 739 female patients, of which 34 (62 tubes) had both hycosy and laparoscopy. The patients included in this study had no history of serious diseases and contraindications	4D-hycosy, B-mode hycosy, laparoscopy and dye	the predictive value of HyCoSy in assessing tubal patency, the sensitivity, specificity, positive and negative predictive values	Compared with the laparoscopy and dye test, tubal occlusion diagnostic accordance rates for 4D-HyCoSy were 88.7%(23+32)/62, with a kappa coefficient of 0.769 and a 76.9% agreement rate (Table 1). Distal occlusion diagnostic accordance rates for 4D-HyCoSy were 100% (8/8), with a k coefficient of 1.000 and a 100% agreement rate (Table 2). The sensitivity, specificity, PPV, and NPV of 4D-HyCoSy compared to laparoscopy were 88.4%, 88.8% 85.1%, and 91.4%, respectively. Twenty tubes were diagnosed as “patent” by 4D-HyCoSy although the B mode-HyCoSy procedure showed these tubes as passable but not smooth (Figure 2). Four tubes were misdiagnosed as proximal partial obstruction by		

					4D-HyCoSy, while subsequent B mode-HyCoSy indicated that these tubes were “patent”.		
Cimen, G., Trak, B., Elpek, G., Simsek, T. and Erman, O. The efficiency of hysterosalpingo-contrastsonography (HyCoSy) in the evaluation of tubal patency. J Obstet Gynaecol. 1999; 19 (5): 516-8.		Forty-seven patients, aged 19 to 41 years, affected with infertility. Patients, with a suspicion of acute or chronic pelvic inflammatory disease, those with galactosemia, age below 18 years and pregnant or who had any suspicion of pregnancy were excluded	HyCoSy was performed in the late proliferative phase (9± 11 days) of the cycle. In 18 patients laparoscopy was also performed and the results were compared with HyCoSy and R-HSG.	the predictive value of HyCoSy in assessing tubal patency, the sensitivity, specificity, positive and negative predictive values	sensitivity: 81.8%, specificity: 75%, PPV: 75%, NPV: 91.6%, concordance: 86%		
Liang, N., Wu, Q. Q., Li, J. H., Gao, F. Y., Sun, F. L. and Guo, C. X. Causes of misdiagnosis in assessing tubal patency by transvaginal real-time three-dimensional hysterosalpingo-contrast sonography. Rev Assoc Med Bras (1992). 2019; 65 (8): 1055-1060.		83 infertility patients (162 oviducts),	3D hyscosy and laparoscopy	The consistency of the test results was analysed using the Kappa value	With the results of the laparoscopic dye studies as the gold standard, the accuracy rate of TVS RT-3D-HyCoSy in diagnosing tubal patency was 88.9% (144/162), and the misdiagnosis rate was 11.1% (18/162). Furthermore, the sensitivity of diagnosing oviduct obstruction was 89.6% (86/96), the PPV was 91.5% (86/94), the specificity of diagnosing tubal patency was 87.9% (58/66), and the NPV was 85.3% (58/68). The accuracy of TVS RT-3D-HyCoSy was similar to that of these laparoscopic dye studies, the difference was not statistically significant, and the consistency between these two was good		

Malek-Mellouli, M., Gharbi, H. and Reziga, H. The value of sonohysterography in the diagnosis of tubal patency among infertile patients. Tunis Med. 2013; 91 (6): 387-90.	CS	Prospective CS. 40 consecutive women	hysterosalpingography, sonohysteroography and laparoscopy with dye test, within a period of 6 months.	agreement between laparoscopy and hycosy	Sonosalpingography showed patency in 51(63.7%) tubes, hysterosalpingography in 47 (58.7%) tubes, and laparoscopy in 52 (65%) tubes. The tubal patency found in 51 tubes by SHG was confirmed by laparoscopy in 44 tubes (positive predictive value, 87.9%). A uni- or bilateral tubal occlusion was observed in 28 patients by laparoscopy. In 8 tubes, occlusion suggested by sonosalpingography was not confirmed by laparoscopy and 7 tubes patent by sonosalpingography were found to be occluded by laparoscopy. There were 7 false positive and 8 false negative findings. The sensitivity of sonosalpingography in diagnosing tubal patency was 90% and the specificity 80%.		

Radić, V., Canić, T., Valetić, J. and Duić, Z. Advantages and disadvantages of hysterosonosalpingography in the assessment of the reproductive status of uterine cavity and fallopian tubes. Eur J Radiol. 2005; 53 (2): 268-73.	CS	prospective CS. 37 infertile women.	Hycosy with saline and contrast medium compared to laparoscopy and dye test. The surgeons at laparoscopy and hysteroscopy procedures were blinded to the results of the previous hysterosonosalpingography.	the predictive value of HyCoSy in assessing tubal patency, the sensitivity, specificity, positive and negative predictive values	The ultrasound saline contrast method for the assessment of the tubal status in comparison to laparoscopic findings of chromoperturbations showed 100% sensibility and negative predictive value, but also a low specificity of 66% and a positive predictive value of 57% (Table 2). The method found no false patent tube, 58 true patent and 77 nonpatent tubes. Of these 77 pathologic findings of nonpatent tubes by the ultrasound method, 30 tubes were proved patent by chromolaparoscopy. examination of tubal patency by the Echovist® yielded a better specificity (77%), and positive predictive value (70%) (Table 3) than examination with negative contrast. In addition to no false patent findings, it depicted 68 truly patent tubes, 20 false nonpatent and 47 true nonpatent tubes.		

Rezk, M. and Shawky, M. The safety and acceptability of saline infusion sonography versus hysterosalpingography for evaluation of tubal patency in infertile women. Middle east fertility society journal. 2015; 20 (2): 108-113.	CS	prospective CS. 104 infertile women. The inclusion criteria were: unexplained infertility, age between 20 and 40 years, infertility by at least 1 year. The exclusion criteria were serious semen abnormalities, FSH> 15 mIU/mL and contraindications for HSG or laparoscopy.	SIS, HSG, laparoscopy	the predictive value of SIS in assessing tubal patency, the sensitivity, specificity, positive and negative predictive values	SIS showed patency in 90 (86.5%) tubes, HSG in 85 (81.7%) tubes, and laparoscopy in 75 (72.1%) tubes. SIS and laparoscopy agreed in 15 out of 29 occluded tubes (concordance, 51.7%) while HSG and laparoscopy agreed in 11 out of 29 occluded tubes (concordance, 37.9%). The sensitivity, specificity, PPV, NPV were 52%, 95%, 79%, 84% for SIS. SIS was more acceptable than HSG as a screening test for tubal patency regarding the overall discomfort and the overall satisfaction rate.		
Shahid, N., Ahluwalia, A., Briggs, S. and Gupta, S. An audit of patients investigated by Hysterosalpingo-Contrast-Sonography (HyCoSy) for infertility. J Obstet Gynaecol. 2005; 25 (3): 275-8.	CS	retrospective CS. 171/186 case notes of patients, referred for HyCoSy as a part of investigation for sub-fertility were reviewed. 34 patients had both hycosy and laparoscopy and dye test	hycosy and laparoscopy and dye test	concordance of results between tests	15 patients had laparoscopy after hycosy. Of these 15 patients HyCoSy showed bilateral patent tubes in 8 patients. Laparoscopy and dye test confirmed these findings in 87.5% (n= 7) patients whereas one patient showed unilateral patent tube. The findings of bilaterally blocked tubes in one patient and unilateral patent tube in 6 patients on HyCoSy were confirmed on laparoscopy and dye test. 19 patients had laparoscopy before hycosy and conclusive results were shown by HyCoSy in 5 cases of inconclusive findings and in 4 cases where tubes filled with dye but there were no spill noted at		

					laparoscopy and dye test in spite of normal appearance of the tubes at laparoscopy.		
Zhou, L., Zhang, X., Chen, X., Liao, L., Pan, R., Zhou, N. and Di, N. Value of three-dimensional hysterosalpingo-contrast sonography with SonoVue in the assessment of tubal patency. Ultrasound Obstet Gynecol. 2012; 40 (1): 93-8.	CS	75 patients. Inclusion criteria included: 1) no vaginal bleeding and 2) no acute or subacute inflammation of the reproductive system. Women with unicornuate uterus or unilateral salpingectomy were not excluded;	3D-SonoVue-HyCoSy and lap and dye	concordance of results between tests	Thirty-four patients were diagnosed as having bilateral tubal occlusion by 3D SonoVue-HyCoSy, compared with 29 diagnosed by lap and dye. Fourteen patients were diagnosed as having unilateral tubal patency by 3D SonoVue-HyCoSy, compared with 19 diagnosed by lap and dye. Twenty-seven patients were diagnosed as having bilateral tubal patency by both 3D SonoVue-HyCoSy and lap and dye.		

HYSTEROSALPINGOGRAPHY (HSG) VS. LAPAROSCOPY AND DYE

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Broeze, K. A., Opmeer, B. C., Van Geloven, N., Coppus, S. F., Collins, J. A., Den Hartog, J. E., Van der Linden, P. J., Marianowski, P., Ng, E. H., Van der Steeg, J. W., Steures, P., Strandell, A., Van der Veen, F. and Mol, B. W. Are patient characteristics associated with the accuracy of hysterosalpingography in diagnosing tubal pathology? An individual patient data meta-analysis. Hum Reprod Update. 2011; 17 (3): 293-300.	SR	10 studies, 4521 women	HSG and laparoscopy	accuracy of HSG for tubal patency	Across the individual studies, sensitivity ranged between 46% and 100% and specificity ranged between 73% and 100% when diagnosing any tubal pathology. The unadjusted pooled accuracy of HSG showed a sensitivity of 70% (95% CI 0.66–0.74) and a specificity of 78% (95% CI 0.75–0.80). After imputation of missing laparoscopy results, these rates were 53% (95% CI 0.50–0.57) and 87% (95% CI 0.86–0.88) for sensitivity and specificity, respectively. In women with a low-risk clinical history, the sensitivity of HSG for detecting unilateral tubal pathology was 38% versus 61% in women with a high-risk history. For bilateral tubal pathology, sensitivity ranged between 0% and 100% and specificity ranged between 87% and 97% across the individual studies. The pooled estimates for sensitivity and specificity were 66% (95% CI 0.55–0.75) and 91% (95% CI 0.89–0.93), respectively. After		

					imputation of laparoscopy results, these rates were 46% (95% CI 0.41–0.51) and 95% (95% CI 0.94–0.95).		
Adelusi, B., al-Nuaim, L., Makanjuola, D., Khashoggi, T., Chowdhury, N. and Kangave, D. Accuracy of hysterosalpingography and laparoscopic hydrotubation in diagnosis of tubal patency. Fertil Steril. 1995; 63 (5): 1016-20.		All patients with factors, such as ovulatory failure or poor semen analysis, that may be contributory to their infertility were excluded from the study.	diagnostic HSG, followed by laparoscopy within a period of 6 months,	HSG and laparoscopy agreement	Whereas laparoscopy showed that both tubes were patent in 51.9% of cases, HSG identified both tubes as patent in 39.4% of cases. There was agreement between laparoscopy and HSG in only 31.7%. Similarly, agreement between the two methods in terms of bilateral tubal blockage was 16.3% of cases and, in terms of unilateral blockage, there was agreement in only 14.5% of cases. There was an overall agreement between the two techniques in 62.5% of cases.		
Agrawal, N. and Fayyaz, S. Can hysterolaparoscopic mediated chromopertubation obviate the need for hysterosalpingography for proximal tubal blockage?: An experience at a single tertiary care center. J Gynecol Obstet Hum Reprod. 2019; 48 (4): 241-245.	CS	prospective CS. 103 infertile patients. Infertile female patients, age between 19 and 33 years were registered to participate in the study after taking the informed written consent.	hysteroscopy and laparoscopy	diagnostic accuracy of HSG	In comparison to HSG with CPT (reference standard) for tubal blockage detection, it was found that HSG was true positive (TP) in 38 patients, true negative in 34 patients, false positive in 31 patients and FN in 0 patients. We found that for detection of tubal blockage, the sensitivity, specificity, PPV, NPV and accuracy of HSG was 100.00%, 52.31%, 36.89%, 57.07% and 67% respectively. Proximal tubal occlusion		

					detected on HSG and CPT showed a moderate agreement (weighted kappa – 0.447; 95% CI -0.312 to 0.583). Also when analysed independently tubal occlusion detection on HSG and CPT, it showed moderate agreement for primary infertile patients (weighted kappa – 0.474; 95% CI -0.294 to 0.654) and secondary infertile patients (weighted kappa – 0.411; 95% CI -0.206 to 0.616).		
Berker, B., Şükür, Y. E., Aytaç, R., Atabekoğlu, C. S., Sönmezer, M. and Özmen, B. Infertility work-up: To what degree does laparoscopy change the management strategy based on hysterosalpingography findings? J Obstet Gynaecol Res. 2015; 41 (11): 1785-90.	CS	retrospective CS. All patients who had both HSG and LS testing (n = 264) were included in the study. Patients with missing reports of either HSG or LS were not included. Patients with severe male factor infertility or severe ovarian dysfunction who proceeded to artificial reproductive technologies (ART without LS were excluded.	HSG and laparoscopy	diagnostic accuracy of HSG	diagnostic accuracy of HSG: The sensitivity, specificity, positive predictive and negative predictive values for any tubal pathology were 94%, 81.7%, 54.6%, and 98.3%, respectively. The sensitivity, specificity, positive predictive and negative predictive values for UTO were 72.2%, 84.5%, 26.5%, and 91.5%, respectively. The sensitivity, specificity, positive predictive and negative predictive values for BTO were 78.1%, 94.8%, 67.5%, and 96.9%, respectively. Generally, the validity (true positive +true negative /cohort×100) of the HSG test was 84.1% (47+175/264×100).		

Chang, Y. S., Lee, J. Y., Moon, S. Y. and Kim, J. G. Diagnostic laparoscopy in gynecologic disorders. Asia Oceania J Obstet Gynaecol. 1987; 13 (1): 29-34.		1267 patients	HSG and laparoscopy	concordance of HSG and laparoscopic findings	In 982 (77.5%) of these patients there was complete agreement between HSG and Laparoscopy while 177 patients (17.0%) had a false positive HDG and 108 patients (25.9%) a false negative HSG		
Dabekausen, Y. A., Evers, J. L., Land, J. A. and Stals, F. S. Chlamydia trachomatis antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. Fertil Steril. 1994; 61 (5): 833-7.	CS	prospective CS. 211 consecutive women, of which 34 had both HSG and laparoscopy	C. trachomatis antibody testing, HSG, laparoscopy.	HSG and laparoscopy agreement	In 24/34 patients the HSG and laparoscopy results corresponded, but in 10 patients a discrepancy was found. The probability of tubal factor infertility with an abnormal HSG was 59%. The LR+ for HSG was 2.6 and LR- 0.5 (OR 4.8, 95% CI 1.0-21.8)		
Foroozanfard, F. and Sadat, Z. Diagnostic value of hysterosalpingography and laparoscopy for tubal patency in infertile women. Nurs Midwifery Stud. 2013; 2 (2): 188-92.	CS	prospective CS. 62 infertile women. Inclusion criteria were no prior pelvic surgery, no history of pelvic infection, normal bimanual pelvic examination, normal semen parameters of partner, no ovulatory dysfunction, and excluding criteria were surgical procedures that had occurred between the performance after HSG, women who did not return for laparoscopy evaluation, technical problems related	Laparoscopy was performed three month after HSG (13). The HSG was performed by radiologist. The procedure was performed between days 6 and 12 of the menstrual cycle at least 48 hours after menses had ceased.	sensitivity, specificity of HSG	Forty three cases had normal HSG, among them 81.4% had normal laparoscopy. In the nineteen cases with abnormal HSG (unilateral or bilateral no patency), 47.4 % of patients showed abnormal results on laparoscopy. The sensitivity of HSG on bilateral tubal patency or no bilateral tubal patency was 92.1% and its specificity was 85.7%. The PPV and the NPV were 97.2% and 66.6% respectively. Furthermore, results of HSG were false-negative in 33.3% of patients, false-positive in 2.8% and		

		to HSG and women who became pregnant after hysterosalpingography.			accuracy was 91.1%. The sensitivity and specificity of HSG on bilateral tubal patency and any abnormality of patency (unilateral or bilateral tubal no patency) were 77.8% and 52.9% respectively, the PPV and the NPV were 81.4 % and 47.4% respectively. Furthermore, results of HSG were false-negative in 52.6% of patients, false-positive in 18.6% (Table 3) and accuracy was 71%.		
Gündüz, R., Ağaçayak, E., Okutucu, G., Karuserci Ö, K., Peker, N., Çetinçakmak, M. G. and Gül, T. Hysterosalpingography: a potential alternative to laparoscopy in the evaluation of tubal obstruction in infertile patients? Afr Health Sci. 2021; 21 (1): 373-378.	CS	This retrospective study included 208 infertile patients. Patients with uterine factors, male factors, smokers, premature ovarian failure, patients with chronic diseases, and history of abdominal surgery were excluded in the study. Patients with distal tubal obstructions on HSG and L/S were included in the study, proximal tubal obstruction as it may be secondary to transient tubal spasms (20% of cases) or amorphous debris or minimal adhesions (40% of cases)6 were excluded in the study.	HSG; and also received laparoscopy for showing either pathology or >6 months infertility after HSG	concordance of HSG and laparoscopic findings	HSG and L/S results were compatible in 147 (70.6%) of the 208 patients whose tubes were found to be either patent or obstructed. HSG was found to have a specificity of 64.6%, a sensitivity of 81.3%, a positive predictive value of 56.4%, and a negative predictive value of 86% in the detection of tubal obstruction.		

Hamed, H. O., Shahin, A. Y. and Elsamman, A. M. Hysterosalpingo-contrast sonography versus radiographic hysterosalpingography in the evaluation of tubal patency. Int J Gynaecol Obstet. 2009; 105 (3): 215-7.	CS	Prospective CS. 88 infertile women, of which 57 women had all 3 procedures. The women and their husbands were younger than 40 years, the women had regular cycles with normal ovulation, and the men had normal semen. Exclusion criteria were pelvic infections and organic lesions	Hycosy, HSG and laparoscopy. The HyCoSy and HSG procedures were performed in this order and in the same week at the Department of Radiology. The operator who did the HSG procedure was unaware of the HyCoSy results.	performance of HSG and hycosy, compared to laparoscopy	**HyCoSy:** sensitivity of 76.1% and a specificity of 79.4%, with a PPV of 71.4% and NPV of 83.1%. The finding of HyCoSy and laparoscopy and the dye test was the same for 89 tubes, for a compatibility rate of 78.1%. **HSG:** sensitivity of 81.8% and a specificity of 77.1%, with a PPV of 69.2% and a NPV of 87.1%. The compatibility rate between the diagnosis of HSG and laparoscopy was 79.9% (Table 3).		
Hiroi, H., Fujiwara, T., Nakazawa, M., Osuga, Y., Momoeda, M., Kugu, K., Yano, T., Tsutsumi, O. and Taketani, Y. High incidence of tubal dysfunction is determined by laparoscopy in cases with positive Chlamydia trachomatis antibody despite negative finding in prior hysterosalpingography. Reprod Med Biol. 2007; 6 (1): 39-43.	CS	retrospective CS. 314 patients	HSG with water-soluble iodinated contrast material and laparoscopy	sensitivity, specificity of HSG	sensitivity and specificity for tubal patency were 0.63 and 0.79, respectively, calculated with laparoscopic findings as the gold standard. For peritubal adhesion, sensitivity and specificity were 0.65 and 0.61, respectively. NPV for occlusion was 82% in patients with at least one background factor, and 93% in patients without any background factors. 35 patients were diagnosed with fallopian tubes which were observed to be patent by HSG, but not observed to be patent by chromopertubation under laparoscopy		

Ismajovich, B., Wexler, S., Golan, A., Langer, L. and David, M. P. The accuracy of hysterosalpingography versus laparoscopy in evaluation of infertile women. Int J Gynaecol Obstet. 1986; 24 (1): 9-12.	CS	215 women.	HSG and laparoscopy. HSG was performed during the proliferative phase using a water soluble contrast medium. Laparoscopy was performed in the secretory phase, either 6 months after a normal HSG or 1 to 2 months after the abnormal HSG.	concordance of HSG and laparoscopic findings	Thirty-two women (25%) had normal HSG and peritubal adhesions on laparoscopy. Thirty-four (28%) women who had normal pelvic organs on laparoscopy had tubal disease diagnosed on HSG. Forty-seven (22%) women had pelvic pathology undiagnosed by HSG (Table II).		
Keltz, M. D., Gera, P. S. and Moustakis, M. Chlamydia serology screening in infertility patients. Fertil Steril. 2006; 85 (3): 752-4.	CS	prospective CS. 210 infertile patients.	Chlamydia antibody IgG by microimmunofluorescence, A titre of >1:32 was considered a positive result. HSG in all patients for tubal patency, laparoscopy when clinically needed.	correlation between chlamydial serology, HSG, and laparoscopic findings	84/210 (40%) were CAT positive. CAT positivity, both low and high, was 74.0% sensitive and 93.0% specific at detecting tubal disease. PPV 94.8% and NPV 69.8%. HSG was 78% sensitive and 82% specific for finding tubal disease at laparoscopy. CAT+HSG: 97.3% sensitivity.		
Loy, R. A., Weinstein, F. G. and Seibel, M. M. Hysterosalpingography in perspective: the predictive value of oil-soluble versus water-soluble contrast media. Fertil Steril. 1989; 51 (1): 170-2.	CS	77 consecutive patients with primary and secondary infertility. Both groups were comparable in age.	HSG; OSCM was used in 33 patients and WSCM was used in 44 patients compared to laparoscopy. The mean interval between HSG and laparoscopy was 4.5 months for the OSCM group and 3.5 months for the WSCM group.	concordance of HSG and laparoscopic findings	HSG. Eleven of 12 patients with tubal occlusion were identified by HSG using OSCM (sensitivity = 92%) as compared with 5 of 8 patients (sensitivity= 63%) using WSCM ($P < 0.01$). The specificities were 67% and 75% for OSCM and WSCM, respectively (not significant		

Ngowa, J. D., Kasia, J. M., Georges, N. T., Nkongo, V., Sone, C. and Fongang, E. Comparison of hysterosalpingograms with laparoscopy in the diagnostic of tubal factor of female infertility at the Yaoundé General Hospital, Cameroon. Pan Afr Med J. 2015; 22 264.	CS	cross-sectional study. 208 women.	HSG and laparoscopy	sensitivity, specificity, PPV, NPV	There was a moderate sensitivity (51.0%; 95% IC. 37.5-64.4) and a high specificity (90.0%; 95% IC.74.4-96.5) of HSG in the diagnosis of bilateral proximal tubal occlusion. However, there was a high PPV (89.3 %; 95% IC. 72.8-96.3) and a moderate NPV (52.9%; 95%IC. 39.5-65.9) of HSG in the diagnosis of bilateral proximal tubal occlusion. Concerning distal tubal patency, HSG had a high sensitivity (86.8%; 95%IC. 76.7-92.9) and a low specificity (42.2%; 95% CI. 29.0-56.7) in the diagnosis of bilateral or unilateral tubal occlusion. However, HSG had a moderate PPV (69.4%; 95% IC. 58.9-78.2) and a moderate NPV (67.9%; 95%IC. 49.3-82.0).		
Rezk, M. and Shawky, M. The safety and acceptability of saline infusion sonography versus hysterosalpingography for evaluation of tubal patency in infertile women. Middle east fertility society journal. 2015; 20 (2): 108-113.	CS	prospective CS. 104 infertile women. The inclusion criteria were: unexplained infertility, age between 20 and 40 years, infertility by at least 1 year. The exclusion criteria were serious semen abnormalities, FSH> 15 mIU/mL and contraindications for HSG or laparoscopy.	Saline infusion sonography (SIS) and hysterosalpingography (HSG) were performed in all cases. Laparoscopy was performed within one week from the screening tests.	concordance of HSG and laparoscopic findings	HSG showed patency in 85 (81.7%) tubes, and laparoscopy in 75 (72.1%) tubes. HSG and laparoscopy agreed in 11 out of 29 occluded tubes (concordance, 37.9%). HSG: Sensitivity 38%, specificity 96%, PPV 79%, NPV 80%. SIS was more acceptable than HSG as a screening test for tubal patency regarding the overall discomfort and the overall satisfaction rate.		

Rice, J. P., London, S. N. and Olive, D. L. Reevaluation of hysterosalpingography in infertility investigation. Obstet Gynecol. 1986; 67 (5): 718-21.	CS	143 women. Patients who had undergone elective tubal ligation were not included.	HSG and laparoscopy with chromopertubation	concordance of HSG and laparoscopic findings	The diagnosis of tubal patency was confirmed by laparoscopy in 63 (85.1%) of the 74 patients. The remaining 11 (14.9%) patients had tubal occlusion by laparoscopy.		
Tan, J., Deng, M., Xia, M., Lai, M., Pan, W. and Li, Y. Comparison of Hysterosalpingography With Laparoscopy in the Diagnosis of Tubal Factor of Female Infertility. Front Med (Lausanne). 2021; 8 720401.		retrospective cohort study with 1276 patients. All the enrolled patients had a regular menstrual cycle, and routine semen examination of the husband was normal. We excluded patients who had an ovarian cyst, uterine malformation, endometriosis, or any other type of organic lesion that could be found by routine ultrasonography. 20.97% (n = 181) of patients had a history of previous pelvic surgery.	HSG was performed. If the results of HSG were normal or not patent, but the patients did not become pregnant in the 12 months after examination, we performed a laparoscopic procedure. If the results of HSG were occlusion or hydrosalpinx, but the patients desired to conceive, naturally, they chose to perform the laparoscopic examination.	concordance of HSG and laparoscopic findings	performance of HSG in the diagnosis of right tube patency or occlusion compared to laparoscopy as the gold standard. There was a high sensitivity (73.65%), specificity (83.21%), positive predictive value (50.93%), and negative predictive value (92.08%). The Kappa value was as high as 0.47, 95% CI (0.399, 0.541), p < 0.001. The corresponding sensitivity, specificity, positive predictive value, and negative predictive value of HSG in diagnosing left tube patency or occlusion were 78.98, 87.72, 56.19, and 95.44%, respectively. The Kappa value was 0.574, 95% CI (0.505, 0.0.643), p < 0.001.		

Tshabu-Aguemon, C., Ogoudjobi, M., Obossou, A., King, V., Takpara, I. and Alihonou, E. HYSTEROSALPINGOGRAPHY AND LAPAROSCOPY IN EVALUATING FALLOPIAN TUBES IN THE MANAGEMENT OF INFERTILITY IN COTONOU, BENIN REPUBLIC. J West Afr Coll Surg. 2014; 4 (2): 66-75.	CS	retrospective CS. 96 patients explored for tubal infertility. Exclusion criteria were infertility of less than two years.	HSG followed by laparoscopy and methylene blue test	concordance of HSG and laparoscopic findings	The concordance of HSG–laparoscopy in tubal obstruction was 46.84%. The concordance HSG -laparoscopy showed 12.5% of proximal tubal obstruction. HSG showed 11.46% of distal tubal obstruction and 6.25% of tubes showing patency at HSG were found to be occluded at laparoscopy. Laparoscopy revealed adhesive bands undetected with HSG in 33.33% of cases, pelvic endometriosis undetected with HSG in 6.25% of cases, and patent tubes but with inflammatory features in 11.46% of cases.		
Tvarijonaviciene, E. and Nadisauskiene, R. J. The value of hysterosalpingography in the diagnosis of tubal pathology among infertile patients. Medicina (Kaunas). 2008; 44 (6): 439-48.		prospective cross-sectional study. 149 infertile women. Inclusion criteria: 1) Infertility diagnosis according WHO definition. 2) Woman's age 19–42 years. 3) Confirmed ovulatory cycles and/or normal ovarian reserve. 4) Absence of severe sperm pathology. 5) Patient's consent to the study. Exclusion criteria: 1) Women younger 19 and older 42 years. 2) Diminished ovarian reserve. 3) Severe sperm	The HSGs were performed by staff gynaecologist and staff radiologist. The results of HSGs were evaluated by one of the three staff radiologists. Laparoscopy and dye test (LS) was performed within one–three months after HSG by staff gynaecologists	Sensitivity, specificity, LH+, LH–, pretest and posttest probabilities of HSG in diagnosis of general tubal pathology, tubal occlusion, and peritubal adhesions were calculated, regarding LS as the reference standard.	For 2 (1.3%) patients, febrile morbidity after the procedure was registered. Following HSG, 63.8% (95/149) of patients were diagnosed with general tubal pathology. Following LS, 39.5% (59/149) of women were found with general tubal pathology. Accuracy of HSG versus laparoscopy for tubal patency: 84.1% (73.3-94.9) sensitivity, 59.1% (49.6-68.5) specificity, 2.1 (1.6-2.7) LR+, 0.3 (0.1-0.5) LR-, post-test probability for positive result: 47.4% (39.0-55.0) and post-test probability		

		pathology. 4) Previous HSG related to infertility. 5) Previous diagnostic laparoscopy related to infertility. 6) Previous laparoscopic or abdominal tubal surgery related to infertility. 7) Contraindications for HSG or laparoscopy. 8) Absence of the patient's consent.			for negative result: 11.4% (6.0-16.0)		

CHLAMYDIA ANTIBODY TESTING VS. LAPAROSCOPY AND DYE

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Mol, B. W., Dijkman, B., Wertheim, P., Lijmer, J., van der Veen, F. and Bossuyt, P. M. The accuracy of serum chlamydial antibodies in the diagnosis of tubal pathology: a meta-analysis. Fertil Steril. 1997; 67 (6): 1031-7.	SR	2,729 patients with subfertility in 23 studies	Chlamydia antibody titer and laparoscopy as part of subfertility work-up. CAT: 5 studies used immunoperoxidase (IP) assay (16-18,21,29), 15 studies used immunofluorescense (IF) or microimmunofluorescence (MIF) (7-12, 19, 20, 22-28), 2 studies used ELISA (14 15), and 1 study used both MIF and ELISA (13). The cutoff values for test	Sensitivity and specificity of Chlamydia antibody titers in the diagnosis of tubal pathology using laparoscopy with chromopertubation as the reference standard.	The sensitivity of Chlamydia antibody testing for tubal pathology-varied between 0.21 and 0.90, with the specificity varying between 0.29 and 1, substantial heterogeneity between studies. The discriminative capacity of Chlamydia antibody testing however, was significantly different between studies using MIF or IF, studies using ELISA, and studies using IP as assay for Chlamydia		

			positivity of most studies varied between 1:8 and 1:64 except 1 study that used a cut-off value of 1:640 (28).		antibody testing. Performance of CAT varied significantly with the way of tubal pathology verification.		
Akande, V. A., Hunt, L. P., Cahill, D. J., Caul, E. O., Ford, W. C. and Jenkins, J. M. Tubal damage in infertile women: prediction using chlamydia serology. Hum Reprod. 2003; 18 (9): 1841-7.		cross-sectional study. 1006 infertile women	laparoscopy for tubal patency and CAT, IgG was measured using the whole-cell inclusion immunofluorescence test.	CAT and laparoscopy findings	The antibody titres in women with tubal damage were significantly higher than in women without tubal damage. Women with tubal damage but no tubal occlusion had significantly lower median antibody levels than those with at least one tube occluded (1:512 vs. 1:1024; $P < 0.001$). A linear relationship between serum CAT and the likelihood of tubal damage was observed		
Babay, Z. A. and Al-Meshari, A. The role of Chlamydia trachomatis infection in female infertility. Ann Saudi Med. 1993; 13 (5): 423-8.		158 consecutive females undergoing evaluation for infertility were screened, 75 were enrolled for tubal patency testing. Controls: 50 women attending the postnatal clinic	laparoscopy, endocervix and peritoneal samples for C. trachomatis culture		Infertile group: 37/86 pregnancies (43%) in Chlamydia positive mothers ended in miscarriage, while in the Chlamydia negative mothers, 3 pregnancies (10%) ended in miscarriage. Control group: 90/116 (77.6%) of pregnancies in Chlamydia positive controls while 12/116 (10.3%) Chlamydia negative controls ended in miscarriage. Cervical chlamydia culture was positive in 49/75 (65.3%) infertile patients and in 22/50 (44%) postnatal controls. 33/49 (67.3%) of culture positive infertile patients had tubal blockage and of these, 12 (67.3%)		

					patients had severe pelvic adhesions. Of the culture negative infertile patients 5/26 (19.1%) had blocked tubes and two of these had severe adhesions.		
Coppus, S. F., Opmeer, B. C., Logan, S., van der Veen, F., Bhattacharya, S. and Mol, B. W. The predictive value of medical history taking and Chlamydia IgG ELISA antibody testing (CAT) in the selection of subfertile women for diagnostic laparoscopy: a clinical prediction model approach. Hum Reprod. 2007; 22 (5): 1353-8.	CS	retrospective CS. 207 consecutive women referred for evaluation of subfertility by laparoscopy	laparoscopy and CAT by ELISA	prognostic value of CAT	prevalence of tubal pathology was 30.4% (63/207). **Prediction model:** CAT alone: sensitivity 37% (95% CI 26–49), specificity 88% (95% CI 82–93). Clinical history+CAT: AUC to 0.70 (95% CI 0.62–0.78)	The number of laparoscopies that has to be performed to detect one woman with tubal pathology is comparable when using history, CAT or history and CAT and much lower than without any workup.	
den Hartog, J. E., Land, J. A., Stassen, F. R., Slobbe-van Drunen, M. E., Kessels, A. G. and Bruggeman, C. A. The role of chlamydia genus-specific and species-specific IgG antibody testing in predicting tubal disease in subfertile women. Hum Reprod. 2004; 19 (6): 1380-4.	CS	Prospective CS. 313 subfertile women. Patients who had undergone previous pelvic surgery (except for an uneventful appendectomy or Caesarean section) were excluded. Of these 313 women, subfertile women without distal tubal pathology served as controls.	Serology for antibodies to C. trachomatis , C. pneumoniae and C. psittaci (by MIF) and antibodies to chlamydia lipopolysaccharide (LPS, by ELISA). Laparoscopy for tubal patency testing	predictive value of CAT for distal tubal pathology	59/254 (18.8%) had distal tubal pathology. The prevalence of species-specific IgG antibodies to C. trachomatis was significantly higher in women with distal tubal pathology (54.2%), as compared to women without distal tubal pathology (7.9%). C. trachomatis: sensitivity 54.2%, specificity 92.1%, OR 13.9 (95% CI 6.6-29.2)		

den Hartog, J. E., Land, J. A., Stassen, F. R., Kessels, A. G. and Bruggeman, C. A. Serological markers of persistent C. trachomatis infections in women with tubal factor subfertility. Hum Reprod. 2005; 20 (4): 986-90.	CS	retrospective CS. 313 subfertile women, only patients having a laparoscopy were included in this study.	CAT: IgG by MIF; titre of ≥32 was considered positive; IgA by EIA, threshold index of ≥1.4 was considered positive. Patients with a negative CAT and an otherwise normal fertility work-up underwent a HSG to evaluate the tubal status. If the HSG showed abnormalities, or if they did not conceive within 6 months after the HSG, a laparoscopy with tubal testing was performed. Patients with a positive CAT underwent a laparoscopy with tubal testing immediately after the fertility work-up.	prognostic value of CAT	59 (18.8%) met the definition of distal tubal pathology (extensive peri-adnexal adhesions and/or distal occlusion of at least one tube), whereas 254 women (81.2%) did not have distal tubal pathology and served as controls. IgG and IgA antibodies to C. trachomatis, IgG antibodies to cHSP60 and a positive hs-CRP test were found significantly more often in women with distal tubal pathology as compared to women without distal tubal pathology. C. trachomatis IgG test was the best predictor of tubal pathology (OR 13.9, 95% CI 7.0-27.5).		
Logan, S., Gazvani, R., McKenzie, H., Templeton, A. and Bhattacharya, S. Can history, ultrasound, or ELISA chlamydial antibodies, alone or in combination, predict tubal factor infertility in subfertile women? Hum Reprod. 2003; 18 (11): 2350-6.	CS	prospective CS. 207 consecutive women referred for tubal evaluation	Medical history, transvaginal ultrasound or C. trachomatis antibody testing (acute lower tract infection by EIA and confirmed by direct immunofluorescence; serum by ELISA) and laparoscopy and dye to determine tubal factor infertility	CAT and laparoscopy findings	CAT was negative in 167 (81%) women, equivocal in seven (3%) women, and positive in 33 (16%) women. 63 (30%) of the study population were diagnosed with tubal factor infertility by laparoscopy. Performance of CAT in predicting TFI: accuracy 73%, sensitivity 37%, specificity 88%, LR+ 3.1, LR- 0.7		

Ng, E. H., Tang, O. S. and Ho, P. C. Measurement of serum CA-125 concentrations does not improve the value of Chlamydia trachomatis antibody in predicting tubal pathology at laparoscopy. Hum Reprod. 2001; 16 (4): 775-9.	CS	prospective CS. 110 consecutive women attending infertility clinic.	CAT (by micro-immunofluorescence) and CA-125 (EIA) serology, laparoscopy and dye test, endocervical swab for C. trachomatis. CA-125 concentration of > 35 IU/ml were considered positive and CAT values of >1:32 were considered positive	CAT, CA-125 positivity and laparoscopic findings	2/110 (1.8%) endocervical swab was positive for C. trachomatis. 28/110 women tested CAT positive (25.5%). 11/110 had positive CA-125 and only one woman tested positive for both CAT and CA-125. 31/110 women had tubal pathology (28.2%), of which 17 with positive CAT and 14 with negative CAT p<0.05, CAT in predicting tubal pathology: sensitivity 54.8%, specificity 86.1%, LR+: 3.94, LR-0.53, OR 7.51 (OR 2.90-19.45		
Rantsi, T., Land, J. A., Joki-Korpela, P., Ouburg, S., Hokynar, K., Paavonen, J., Tiitinen, A. and Puolakkainen, M. Predictive Values of Serum Chlamydia trachomatis TroA and HtrA IgG Antibodies as Markers of Persistent Infection in the Detection of Pelvic Adhesions and Tubal Occlusion. Microorganisms. 2019; 7 (10):	CS	retrospective CS. 116 subfertile women. Laparoscopy was performed in women with positive CAT of tubo-ovarian abnormalities by USS, in severe dysmenorrhea, endometriosis or cysts	all women underwent laparoscopy with methylene blue dye test, C. trachomatis TroA, HtrA and MOMP antibodies by EIA. Optical density of >0.4 was considered positive	seroprevalence of TroA, HtrA and MOMP IgG, sensitivity, specificity, accuracy, PPV and NPV	28/79 women had tubal factor infertility. Serology: 28/79 (35.4%) positive for TroA IgG, 27/79 (34.2%) HtrA IgG and 32/79 (40.5%) MOMP IgG. Women with TFI had more often TroA IgG (60.7% vs. 21.6%, p < 0.001) and HtrA IgG antibodies (57.1% vs. 21.6%, p = 0.001) than women without TFI. **Accuracy**: TroA 72.2%, sensitivity of 60.7% and specificity of 78.4%, PPV 60.7%, NVP 78.4%. HtrA specificity 78.4%, sensitivity 57.1%. MOMP: specificity 66.7% and sensitivity 53.6%. All 3: specificity 88.2%, sensitivity 35.7%.		

Singh, S., Bhandari, S., Agarwal, P., Chittawar, P. and Thakur, R. Chlamydia antibody testing helps in identifying females with possible tubal factor infertility. Int J Reprod Biomed. 2016; 14 (3): 187-92.	CS	prospective CS. 200 consecutive women. There was no statistical difference in mean age of patients with positive and negative titres for chlamydial antibody.	all women underwent diagnostic laparoscopy and Chlamydia serum IgG antibodies were determined by ELISA	laparoscopy findings and Chlamydia trachomatis antibody titers were compared	only 5% (10/200) of women were seropositive for anti-chlamydial IgG antibody. only 30% of patients with positive antibody titre had primary infertility in contrast to 64.73% with negative titres. Association of seropositivity with type of infertility appears to be statistically significant. The positive predictive value of CAT test is 100%, while negative predictive value is 78.95% for diagnosing tubal disease. CAT test was positive in 10/50 patients of tubal disease so sensitivity was 20%, while the test had 100% specificity as it was negative in all 150 patients with normal tubes		
Sönmez, S., Sönmez, E., Yasar, L., Aydin, F., Coskun, A. and Süt, N. Can screening Chlamydia trachomatis by serological tests predict tubal damage in infertile patients? New Microbiol. 2008; 31 (1): 75-9.	CS	prospective CS. 152 women presenting in the fertility clinic; control group: women right after delivery. No statistical difference between CAT positive and CAT negative cases.	all patients underwent laparoscopy and CT titers were measured in serum by IFA (positive if titer >1/10)	laparoscopy findings and Chlamydia trachomatis antibody titers were compared	36 antibody positive cases and 68 antibody negative cases in the study group. CT positivity was similar in the study (34.6%) and control groups (22.5%). Sensitivity for CT positivity for tubal damage was 40%, specificity was 69.49%, PPV was 50%, and NPV was 60.29%.	We found a linear correlation between high titers and severe tuboperitoneal adhesions.	

Tanikawa, M., Harada, T., Katagiri, C., Onohara, Y., Yoshida, S. and Terakawa, N. Chlamydia trachomatis antibody titres by enzyme-linked immunosorbent assay are useful in predicting severity of adnexal adhesion. Hum Reprod. 1996; 11 (11): 2418-21.	CS	prospective CS. 131 women attending fertility clinic. Age and duration of infertility were similar between CAT positive and CAT negative patients	C. trachomatis IgG and A was detected in serum by ELISA. A diagnostic laparoscopy was performed in all patients	sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and likelihood ratio for adnexal adhesions were calculated	51/131 (39%) of patients tested positive for CAT. Tubal occlusion on at least one side in 24/51 (47%) patients with positive CAT and in 20/80 (25%) patients with negative CAT. Abnormal tubal appearance on at least one side: in 25/51 (49%) patients with positive CAT and 19/80 (24%) patients with negative CAT. **Adnexal adhesions**: predictive value of IgG: sensitivity 68.2%, specificity 78.8%, PPV 57.7% and NPV 87.9%. predictive value of IgA: sensitivity 68.2%, specificity 82.7%, PPV 62.5%, NPV 86.9%. The LR+ for the IgG and IgA antibody titres by ELISA 5=1.11 were 3.2 for IgG and 3.9 for IgA. The LR+ of IgG and IgA 5=2.0 was 7.7 and 5.1 respectively, indicating a patient with adnexal adhesion to be 7.7 and 5.1 times more likely to have a positive test result (antibody titre 5=2.0) than a patient without adnexal adhesion.		

van Dooremalen, W. T. M., Verweij, S. P., den Hartog, J. E., Kebbi-Beghdadi, C., Ouburg, S., Greub, G., Morré, S. A. and Ammerdorffer, A. Screening of Chlamydia trachomatis and Waddlia chondrophila Antibodies in Women with Tubal Factor Infertility. Microorganisms. 2020; 8 (6):	CS	retrospective CS. 891 women attending fertility clinic	CAT was detected in blood by pELISA Medac, women tested positive were offered laparoscopy with methylene blue dye testing. CAT-negative patients underwent HSG and in case of abnormal findings, laparoscopy was offered.	CAT status, HSG and laparoscopy results	119/890 women tested positive for CAT (13.4%). C. trachomatis antibodies were present significantly more often in the TFI+ compared to the TFI- group, respectively, 41.9% vs. 9.6% ($p < 0.0001$; OR: 6.8; 95% CI 4.28–10.76). In the severe TFI group, the prevalence of C. trachomatis (43.9%) was similar to that of the total TFI+ group (41.9%). Tthe prevalence of W. chondrophila antibodies was similar in both the TFI+ and TFI- group (p: 0.457; OR: 0.8; 95% CI: 0.55–1.30), with 39.2% testing positive in the TFI- group and 35.2% and 31.8% in the TFI+ and sTFI group, respectively.		
Veenemans, L. M. and van der Linden, P. J. The value of Chlamydia trachomatis antibody testing in predicting tubal factor infertility. Hum Reprod. 2002; 17 (3): 695-8.	CS	prospective 295 female infertility patients, unselected. 18 excluded	Chlamydia antibody titre with the C. trachomatis-spot IF test. In patients with a positive CAT test, a laparoscopy with chromotubation was performed. In patients with a negative CAT test, a HSG was performed. If the HSG was abnormal, laparoscopy was performed. patients with a normal HSG who didn't conceive after 6 months also underwent laparoscopy	The diagnostic value of CAT was compared with HSG in tubal pathology, using likelihood ratios (LR)	84/277 patients tested positive for CAT, of which 78 had laparoscopy. 28/78 had tuboperitoneal abnormalities (35.9%) and 50/78 had none (64.1%). 67 patients with a negative CAT had laparoscopy, of which 7 (10.4%) had tuboperitoneal abnormalities. the LR+ of CAT was 1.8 (a patient with TFI is 1.8 times more likely to have a positive result than a patient without TFI), and the LR- was 0.4 (a patient with TFI is 0.4 times as likely to have a negative test as a patient without the disease). ROC was 1:32	Laparoscopy with tubal patency testing remains the most accurate method of diagnosing tuboperitoneal pathology.	

II.5 Uterine factor

PICO Question: Which diagnostic procedures should be performed to confirm a normal uterine structure/anatomy, uterine wall/myometrium?

3D Ultrasound vs. 2D ultrasound

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Caliskan, E., Ozkan, S., Cakiroglu, Y., Sarisoy, H. T., Corakci, A. and Ozeren, S. Diagnostic accuracy of real-time 3D sonography in the diagnosis of congenital Mullerian anomalies in high-risk patients with respect to the phase of the menstrual cycle. J Clin Ultrasound. 2010; 38 (3): 123-7.	CS	Prospective cohort study. total number of patients 108 with suspected congenital mullerian defects at HSG, or suspected to have, one group, one centre	1 gynaecologist performed the 2DUS, the 2nd gynaecologist performed the real-time 3DUS results were compared and correlated with the definitive diagnosis obtained by MRI, laparoscopy, or hysteroscopy		sensitivity, specificity, positive-predictive values, negative-predictive values, false-positive and false-negative rates of 2DUS and real-time 3DUS for detecting CMDs, in the follicular and luteal phases	3DUS is an accurate method that can be used for the diagnosis of CMDs	
Jurkovic, D., Geipel, A., Gruboeck, K., Jauniaux, E., Natucci, M. and Campbell, S. Three-dimensional ultrasound for the assessment of uterine anatomy and detection of congenital anomalies: a comparison with	CS	total number of patients 61 with a history of recurrent miscarriage or infertility and who had previously been investigated by hysterosalpingography, one group, one centre	2DUS images were obtained in 60 (98.3%) and 3DUS images in 58 (95.1%;)cases.		Comparison between hysterosalpingography and US showed that five false-positive diagnoses of arcuate uterus and three of major uterine anomalies were made on 2DUS, 3US agreed with HSG in all cases of arcuate uterus and major congenital anomalies.	The ability to visualize both the uterine cavity and the myometrium on 3DUS facilitated the diagnosis of uterine anomalies and enabled easy differentiation between subseptate and bicornuate uteri.	

hysterosalpingography and two-dimensional sonography. Ultrasound Obstet Gynecol. 1995; 5 (4): 233-7.							
Ludwin, A., Pityński, K., Ludwin, I., Banas, T. and Knafel, A. Two- and three-dimensional ultrasonography and sonohysterography versus hysteroscopy with laparoscopy in the differential diagnosis of septate, bicornuate, and arcuate uteri. J Minim Invasive Gynecol. 2013; 20 (1): 90-9.	CS	total number of patients 117 with a history of recurrent abortions or infertility and a 2DVUS initial diagnosis of a septate, bicornuate, or arcuate uterus prospective clinical study, university hospital and private hospital and clinic.	2D-TVS, 3D-TVS, 2D-SIS, and 3D-SIS performed by experienced examiners and hysteroscopy with laparoscopy to establish the final diagnosis		Specificity, Sensitivity 3D-SIS showed perfect diagnostic accuracy (100.0%) in general detection of uterine abnormalities, compared with initial 2D-TVS (77.8%), expert 2D-TVS (90.6%), 2D-SIS (94.0%), and 3D-TVS (97.4%).	Although 3D-SIS was identical to HSC/LPSC, with the highest accuracy, there was no significant difference in diagnostic value between 3D-TVS with 2D-SIS and 3D-SIS or between expert 2D-TVS and 3D-TVS with 2D-SIS. The high diagnostic value of US tools questions the need for endoscopy in the differential diagnosis of the most common congenital uterine anomalies	

PICO Question: Which additional diagnostic procedures should be performed to confirm an anatomically normal uterine cavity?

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Fatemi, H. M., Kasius, J. C., Timmermans, A., van Disseldorp, J., Fauser, B. C., Devroey, P. and Broekmans, F. J. Prevalence of unsuspected uterine cavity abnormalities diagnosed by office hysteroscopy prior to in vitro fertilization. Hum Reprod. 2010; 25 (8): 1959-65.	RCT	Sub-analysis of an RCT. 678 asymptomatic subfertile women with normal 2D US women under the age of 43 years with no prior hysteroscopy examination nor prior IVF/ICSI attempt to conceive (Belgian statute book, 2003).Women with any of the predefined abnormalities at TVS followed the regular routine and underwent a therapeutic hysteroscopy to resolve the uterine cavity pathology prior to starting the infertility treatment.	In case no menorrhagia or metrorrhagia was present and TVS did not show abnormalities, women were indicated for a screening hysteroscopy on an outpatient basis	intrauterine abnormalities, defined as endometrial polyps, submucous myomas, intrauterine adhesions or uterine septa.	The frequency of one or more abnormalities per patient was 11% (Fig. 2). Endometrial polyps were identified in 41 cases (6%). Most detected polyps (63%) were smaller than 0.6 cm, in only three cases it concerned a polyp .1.0 cm. Submucous myomas were found in six cases (1%), all with an estimated diameter between 0.5 and 2.0 cm. Also 15 cases with intrauterine adhesions (2%) and 14 cases with a septum (2%) were diagnosed. In two cases more than one abnormality was identified.		

Almog, B., Shalom-Paz, E., Shehata, F., Ata, B., Levin, D., Holzer, H. and Tan, S. L. Saline instillation sonohysterography test after normal baseline transvaginal sonography results in infertility patients. Is it justified? Gynecol Endocrinol. 2011; 27 (4): 286-9.	CS	retrospective CS. 294 women with a baseline TVS as part of the infertility work-up	All TVS results (positive and negative) were further investigated by SIS. Positive SIS results were further investigated by hysteroscopy. The **study group** (n=124): patients with a completely negative findings on baseline TVS (endometrial line≤5 mm). The **control group** (n=170): patients with any abnormality on baseline TVS scan.	Abnormalities included highly suggestive findings for ILs (such as polyps, echogenic and thick endometrium, submucous fibroid distorting the cavity, septum) and out of cavity lesions (such as intramural and sub serosal fibroids, adenomyosis).	Table I. Results of SIS, hysteroscopy and pathology in the study group and control. Study group ($n=124$) / Control group ($n=170$) / p Age: 35.6.2 ± 4.9 / 36.6 ± 4.8 / NS Positive SIS results (%): 13 (10.4) / 62 (36.4) / <0.05 Positive hysteroscopic results (%): 3 (23.0) / 42 (67.7) / <0.05 Positive pathology results: 0 (0) / 35 (83.3) / <0.05 PPV* (%): 0 / 56.4 NS, not significant. *positive predictive value of SIS considering pathology reports as gold standard.		
Bakas, P., Hassiakos, D., Grigoriadis, C., Vlahos, N., Liapis, A. and Gregoriou, O. Role of hysteroscopy prior to assisted reproduction techniques. J Minim Invasive Gynecol. 2014; 21 (2): 233-7.	CS	prospective CS. 217 women. Inclusion criteria were primary or secondary infertility, age ,40 years, body mass index ,30, follicle-stimulating hormone level ,10 IU/L, and regular menstrual cycle every 26 to 35 days. Exclusion criteria were known presence of endometriosis or adenomyosis and history of recurrent	diagnostic hysteroscopy after normal TVS and HSG	incidence of intrauterine anomalies that were undetected during HSG or TVS	**Table 2** Hysteroscopic findings in women with and without previous ART attempts Variable / Previous ART trial (n = 95) / No previous ART trial (n = 122) / p value Hysteroscopic finding, No. (%) Endometrial polyp: 14 (14.7) / 12 (9.8) / >.05 Uterine septum: 13 (13.6) / 15 (12.3) / >.05 Submucosal myoma: 10 (10.5) / 2 (1.6) / >.05 Synechiae: 3 (3.2) / 0 / >.05 Total: 40 (42) / 29 (23.7) / .006 ART = assisted reproduction technique.		

		miscarriage. The diagnostic workup included medical history, gynaecologic examination, TVS, HSG, semen analysis, and hormone profile (FSH, luteinizing hormone, estradiol, prolactin, thyroid-stimulating hormone, and anti-mullerian hormone at days 2 to 4 of menses).					
Makled, A. K., Farghali, M. M. and Shenouda, D. S. Role of hysteroscopy and endometrial biopsy in women with unexplained infertility. Arch Gynecol Obstet. 2014; 289 (1): 187-92.	CS	prospective CS. 100 women with unexplained infertility	diagnostic hysteroscopy after normal TVS and HSG	incidence of intrauterine anomalies that were undetected during HSG or TVS	Diagnostic hysteroscopy showed endometrial polyps in 31 of the infertile patients (31 %). Of these patients, only 18 (18 %) were correctly diagnosed by TVS. Seven of the missed patients were diagnosed with hyperplasia, while six patients had no abnormality.		

Yang, J. H., Chen, M. J. and Yang, P. K. Factors increasing the detection rate of intrauterine lesions on hysteroscopy in infertile women with sonographically normal uterine cavities. J Formos Med Assoc. 2019; 118 (1 Pt 3): 488-493.	CS	retrospective CS. 1726 infertile women.	normal uterine cavities on 2D-TVS, who subsequently underwent office hysteroscopic examinations.	diagnosis of intrauterine lesions were visible, including endometrial polyp, IUA, Caesarean scar defect, tortuous cervical canal, unicornuate uterus, endometritis, myoma compression, and uterine septum, endometritis	intrauterine lesions in 260 women (15.1%) and normal uterine cavities in 1466 women (84.9%). The types of abnormal hysteroscopic findings were endometrial polyps (n=105, 6.1%), IUAs (n=99, 5.7%), Caesarean scar defects (n=25, 1.5%), tortuous cervical canals (n=9, 0.5%), unicornuate uteri (n=8, 0.5%), endometritis (n=8, 0.5%), myoma compressions (n=4, 0.2%), and uterine septa (n=2, 0.1%)		

II.6 Laparoscopy

PICO QUESTION: SHOULD WOMEN UNDERGO A LAPAROSCOPY BEFORE BEING DIAGNOSED WITH UI?

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Tanahatoe, S. J., Lambalk, C. B. and Hompes, P. G. The role of laparoscopy in intrauterine insemination: a prospective randomized reallocation study. Hum Reprod. 2005; 20 (11): 3225-30.	RCT	154 women with unexplained infertility > 1 y (mean 2.9 y) , age 31-34 year. Academic Hospital	Intervention Diagnostic laparoscopy before start IUI (DLSF) or DLS after IUI, (IUIF) ic 6 cycles. Surgical treatment of mild/moderate adhesions and/or endometriosis was performed, in case of severe pelvic pathology the treatment consisted of secondary surgery or direct IVF. Surgeons were not blinded.	Analysis according intention to treat. Primary outcome measure was the number of abnormal laparoscopies leading to a change of treatment versus total number of performed laparoscopies. The study was powered on an assumed difference of 25% more abnormal laparoscopies in the IUIF group. Pregnancy was not an outcome measure. Follow-up stopped after 6 IUI cycles in DLSF or after ongoing pregnancy and in IUIF group after	Laparoscopies performed in group 1 DLSF N=64/77 and group 2 N= 23/77 IUIF. No abnormalities at laparoscopy in 52% DLSF and 44% IUIF (P=0.63 and OR 1.4 (95% CI 0.5-3.6). Abnormalities 45% vs 56 % and intervention (ie surgical treatment in 48% and 56% respectively: adhesiolysis in 4% group 1 vs 0%, evaporation endometriosis in 44% vs 52%, and fimbriolysis in 0 vs 4%). Pregnancies 44% vs 49%: Natural 12 vs 16 and IUI pregnancy 22 vs 22 (P 0.63 OR 1.2 (95% CI: 0.7-2.3). Dropouts before DLS in fig 1 (discontinuation treatment and/or pregnancy before IUI). There was no significant difference in the waiting period between DLS in DLSF group and start IUI in the IUIF group.	Laparoscopy performed after 6 cycles of IUI for unexplained infertility, did not detect more abnormalities with clinical consequences compared with those performed prior to IUI treatment. The impact of the laparoscopic detection and treatment of pelvic pathology prior to IUI seems negligible in terms of pregnancy outcome.	Not specified if IUI or OS+IUI. The outcome of the study suggests that a diagnostic laparoscopy should not be done routinely after a basic fertility work up which includes patent tubes at HSG. Abnormal findings such as adhesions and endometriosis otherwise missed will be detected , but it is questionable if treatment of then detected pelvic disease will improve pregnancy rates after IUI.

				clinical pregnancy or if pregnancy did not occur after 6 completed IUI cycles.			Adequately powered, large RCT's are required to answer this question (a power calculation by the author's suggests that at least 1000 patients are required).
Lavy, Y., Lev-Sagie, A., Holtzer, H., Revel, A. and Hurwitz, A. Should laparoscopy be a mandatory component of the infertility evaluation in infertile women with normal hysterosalpingogram or suspected unilateral distal tubal pathology? Eur J Obstet Gynecol Reprod Biol. 2004; 114 (1): 64-8.	CS	retrospective CS. 86 patients in whom both HSG and laparoscopy were completed were included in the present study. Patients who underwent laparoscopy 12 months or more after HSG was performed were excluded from the study.	Laparoscopy following either normal or abnormal HSG	changes of treatment plan	Of the 63 patients with ‘‘combined normal’’ HSG, three patients were found to have bilateral tubal occlusion on laparoscopy that caused a change in the original treatment regimen and referral to IVF. This represents a false negative rate of 4.8% with regard to the original treatment plan.		

Tanahatoe, S., Hompes, P. G. and Lambalk, C. B. Accuracy of diagnostic laparoscopy in the infertility work-up before intrauterine insemination. Fertil Steril. 2003; 79 (2): 361-6.	CS	retrospective chart review. 495 patients	laparoscopy following normal HSG	The end point of this study is the number of diagnostic laparoscopies leading to a change in treatment decision where IUI was initially indicated.	Laparoscopy did not change the initial treatment decision in 371 (75%) patients, but did in 124 (25%) patients. The latter treatment decisions included direct laparoscopic surgery of the abnormal findings in 103 (20.8%) cases, fertility-increasing operation by laparotomy in 13 (2.6%) cases, and treatment with IVF in 8 (1.6%) cases.		

II.7 Cervical/ vaginal factor

PICO Question: What is the need for female lower genital tract investigations?

Post-coital test

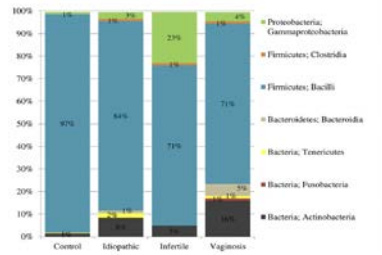
Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Oei, S. G., Helmerhorst, F. M. and Keirse, M. J. When is the post-coital test normal? A critical appraisal. Hum Reprod. 1995; 10 (7): 1711-4.	SR	53 study reports 11 studies fulfilled inclusion criteria 4007 women Criteria: (i) the studies should relate to infertile couples; (ii) the reports should provide sufficient data on the materials and methods used; (iii) the test results categorized as normal or abnormal (or positive or negative) should be expressed in numbers of motile spermatozoa per HPF; and (iv) the occurrence of pregnancy must be reported for the total group of women with both normal and abnormal PCT.	For each study, they calculated the sensitivity, specificity, predictive values of normal and abnormal test results and likelihood ratios for normal and abnormal results	Table II, page 1712 Table III, page 1712 Table IV. Test properties of the post-coital, page 1713 Prevalence Sensitivity Specificity Predictive value of normal result Predictive value of abnormal result Likelihood ratio for normal result Likelihood ratio for abnormal result	The predictive values of normal and abnormal PCT were 0.37-0.92 and 0.58-0.85 respectively. Sensitivity was 0.10- 0.90 and specificity 0.30-0.97. Likelihood ratios for normal and abnormal PCT were 0.77 and 1.85 respectively.	The discriminating ability of the PCT is poor, and altering definitions of normality hardly enhances its predictive power. As long as the value of the PCT for the assessment and treatment of so-called 'cervical factor infertility' remains unclear, a cut-off point with high specificity and a high likelihood ratio for an abnormal test result is recommended.	

Oei, S. G., Helmerhorst, F. M., Bloemenkamp, K. W., Hollants, F. A., Meerpoel, D. E. and Keirse, M. J. Effectiveness of the postcoital test: randomised controlled trial. Bmj. 1998; 317 (7157): 502-5.	RCT	total number: 444 couples intervention group 227; control group 217 a university and two non-university teaching hospitals	In the intervention group the postcoital test was planned 14-16 days before menstruation and 6-18 hours after intercourse. Treatment for negative postcoital test results was in accordance with standard clinical practice. Follow-up 24 months	Treatment was given more often in the intervention group than in the control group (54% v 41%). Cumulative pregnancy rates at 24 months in the intervention group (49% (42% to 55%)) and the control group (48% (42% to 55%)) were similar. Reproducibility is questionable	Figure, page 504 Cumulative pregnancy rates for 227 couples in intervention group, which included postcoital test, and 217 couples in control group which excluded the test.	Routine use of the postcoital test in infertility investigations leads to more tests and treatments but has no significant effect on the pregnancy rate.	
Hessel, M., Brandes, M., de Bruin, J. P., Bots, R. S., Kremer, J. A., Nelen, W. L. and Hamilton, C. J. Long-term ongoing pregnancy rate and mode of conception after a positive and negative post-coital test. Acta Obstet Gynecol Scand. 2014; 93 (9): 913-20.	CS	2476 couples with unexplained infertility PCT was performed in 1624 couples three fertility clinics retrospective study	the protocol for ultrasound timing of PCT is included, Table 1, page 915 Main outcome measures: pregnancy rate after three years	pregnancy rates	**Table 1.** Fecundity Rates for the Postcoital Test by Peak Periovulatory Serum Estradiol Levels PCT (sperm/HPF) / Estradiol (pg/mL): 81–200 / 201–500 / 501–1500 / 1501–3433 0: 0/5 / 2/18 (11%) / 13/73 (18%) / 5/24 (21%) 1–10: 0/2 / 5/27 (19%) / 13/77 (17%) / 10/45 (22%) >10: 0/8 / 3/12 (25%) / 6/43 (14%) / 4/21 (19%) *P*: .61 / .86 / .96 χ^2: 0.994 / 0.30 / 0.088 PCT = postcoital test; HPF = high-power field. Spontaneous and ongoing pregnancy rates after a positive post-coital test were 37.7 and 77.5%compared with 26.9 and 68.8% after a negative test ($p < 0.001$).	The post-coital test plays a significant role in prognostic models for prediction of spontaneous pregnancy in couples with, until then, unexplained infertility. In addition, the post-coital test is particularly useful in male factor infertility, where a positive test was associated with a higher spontaneous pregnancy rate.	
Oei, S. G., Bloemenkamp, K. W., Helmerhorst, F. M., Naaktgeboren, N. and Keirse, M. J. Evaluation of	CS	224 couples, who underwent a PCT as part of routine fertility work-up 24 were excluded	The PCT was performed according to the method described by Hull et	Cumulative pregnancy rates in relation to results of	The predictive values of normal and abnormal PCTs were 0.54 and 0.58 overall and 0.74 and 0.47 if only untreated women were considered.	The PCT has poor predictive power. This and the psychological impact on subfertile	

the postcoital test for assessment of 'cervical factor' infertility. Eur J Obstet Gynecol Reprod Biol. 1996; 64 (2): 217-20.		one fertility clinic retrospective study	al.	the PCT follow-up 18 months	Sensitivity and specificity were, respectively, 0.47 and 0.65 for all women and 0.54 and 0.68 for untreated women only. Likelihood ratios for normal and abnormal PCTs were 0.83 and 1.32 overall and 0.67 and 1.72 in untreated women.	couples attest to the need for more rigorous study designs in evaluating this test.	
Glazener, C. M., Ford, W. C. and Hull, M. G. The prognostic power of the post-coital test for natural conception depends on duration of infertility. Hum Reprod. 2000; 15 (9): 1953-7.	Rest	reanalysis of data 207 couples originally studied between 1982 and 1983	PCT	relationship between the result of the PCT and the chance of conception	In couples with less than 3 years and positive PCT, 68% conceived within 2 years compared with 17% of those with negative result. After 3 years, corresponding rates were 14% and 11%.	use of the PCT will enable clinicians to allocate scarce, expensive and invasive resources effectively	

VAGINAL MICROBIOTA TESTING

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Amato, V., Papaleo, E., Pasciuta, R., Viganò, P., Ferrarese, R., Clementi, N., Sanchez, A. M., Quaranta, L., Burioni, R., Ambrosi, A., Salonia, A., Clementi, M., Candiani, M. and Mancini, N. Differential Composition of Vaginal Microbiome, but Not of Seminal	CS	prospective cohort study. 25 couples with UI undergoing IUI	microbiota composition was analysed by 16S rRNA gene amplification and compared to sequences from healthy subject using a reference database	hierarchical clustering for the relative abundance of lactobacillus species, comparison of taxonomic data with pregnancy outcome	women with UI: increase in the diversity of taxa. Pregnancy rate: 5/23 and a reduction in Lactobacillaceae together with an increase in Bifidobacteriaceae, NS compared to healthy controls. a significant lower Shannon index was found in pregnant women compared to non-pregnant women (0.8 ± 0.9 vs. 1.5 ± 1.1)		

Microbiome, Is Associated With Successful Intrauterine Insemination in Couples With Idiopathic Infertility: A Prospective Observational Study. Open Forum Infect Dis. 2020; 7 (1): ofz525.							
Campisciano, G., Florian, F., D'Eustacchio, A., Stanković, D., Ricci, G., De Seta, F. and Comar, M. Subclinical alteration of the cervical-vaginal microbiome in women with idiopathic infertility. J Cell Physiol. 2017; 232 (7): 1681-1688.	CS	96 women: 27 infertile women attending the ART clinic and 69 fertile ones; Four groups: 1- women with idiopathic infertility (14), 2- with a diagnosed infertility (n 13), fertile women with BV (39) and fertile healthy women (30); To identify bacterial species suitable as biomarkers	Biological samples were collected 5–7 days before the menstrual period and before programmed in vitro fertilization practice. BV was diagnosed using the Nugent score criteria. In parallel, the diagnosis was assessed also by culture isolation. A real time quantitative PCR and sequencing ware performed;	Prevalence of BV	The analysis revealed a significant beta-diversity variation ($p < 0.001$) between the 4 groups. L. iners, L. crispatus, and L. gasseri distinguished idiopathic infertile women from the other groups. In these women, a microbial profile similar to that observed in bacterial vaginosis women has been detected.  **FIGURE 1** Comparison of microbiome taxonomic profiles. Comparison between cervical–vaginal microbiomes from the four groups included in the study. The phylum-level taxonomic classification was based on the relative abundance of normalized samples	The quantitative assessment and identification of specific microorganisms of the cervical–vaginal microflora could increase the accuracy of available tools for the diagnosis of infertility and improve the adoption of therapeutic protocols.	

Campisciano, G., Iebba, V., Zito, G., Luppi, S., Martinelli, M., Fischer, L., De Seta, F., Basile, G., Ricci, G. and Comar, M. Lactobacillus iners and gasseri, Prevotella bivia and HPV Belong to the Microbiological Signature Negatively Affecting Human Reproduction. Microorganisms. 2020; 9 (1):	CS	prospective observational study. 47 Infertile couples undergoing the use of ART (25 IU, 22 explained infertility)	vaginal lavages, follicular fluids, embryo culture mediums, and seminal fluids were tested;	Microbial composition of seminal fluid and vaginal lavage	Concerning the unexplained infertility group, there was a different microbial composition between the seminal fluids and the vaginal lavages. Lactobacilli were dominant in the vaginal lavages, and the most abundant species was L. Iners, which is linked to a decreased fertility rate. Prevotella was increased in the seminal fluids of the explained infertility group, along with HPV-positive seminal fluids. **Table 1.** Alpha diversity. The bacterial diversity values are given as the mean and the 95% confidence interval (CI). All of the pairwise comparisons were performed using a Kruskal–Wallis test ($p < 0.001$). ECM: embryo culture medium. **CHAO1** Explained Infertility / Unexplained Infertility / *p* Value Vaginal lavages: 42 (95% CI = 36–48) / 42 (95% CI = 35–49) / 0.4 Follicular Fluids: 59 (95% CI = 53–65) / 63 (95% CI = 49–77) / 0.3 Seminal Fluids: 94 (95% CI = 79–109) / 130 (95% CI = 101–159) / 0.08 ECM: 24 (95% CI = 19–29) / 38 (95% CI = 27–49) / 0.1 **SHANNON** Explained Infertility / Unexplained Infertility / *p* Value Vaginal lavages: 1 (95% CI = 0.7–1.3) / 1.4 (95% CI = 1.1–1.7) / 0.1 Follicular Fluids: 2.6 (95% CI = 2.2–3) / 2.6 (95% CI = 2.2–3) / 0.7 Seminal Fluids: 3.7 (95% CI = 3.4–4) / 4 (95% CI = 3.7–4.3) / 0.09 ECM: 2.7 (95% CI = 2.4–3) / 3 (95% CI = 2.7–3.3) / 0.4	Their results support the concept that the assessment of the reproductive tract microbiome adds a new microbiological perspective to human reproduction. Male and female genital tracts show peculiar microbiomes that can impair the fertility rate. The seminal microbiome used for IVF needs to be taken into consideration.	
Patel, N., Patel, N., Pal, S., Nathani, N., Pandit, R., Patel, M., Patel, N., Joshi, C. and Parekh, B. Distinct gut and vaginal microbiota profile in women with recurrent implantation failure and unexplained infertility. BMC Womens Health. 2022; 22 (1): 113.		UE was diagnosed if a cause remains undefined after our routine fertility tests with the following criteria: infertility of more than 1 year, normospermic male partner, normal menstrual rhythm with regular ovulation, bilateral tubal patency verified through the hysterosalpingogram or laparoscopy, and normal hormonal tests (i.e., thyroid, prolactin, AMH) [23, 24]. Exclusion criteria included	Study group: n=10, women with UI. Control group: n=11 fertile women Participants collected the faecal samples in a sterile plastic container with a tight closing lid. To collect the vaginal samples, using a sterile swab stick, clinicians thoroughly wiped the posterior fornix of	α-diversity and β-diversity. differences in microbial community	Firmicutes accounted for the vast majority of the vaginal bacteria, with higher relative abundance in UI than controls (69.7 vs 53). Fusobacteria (18% vs.0.14) and Bacteriodetes (4.1% vs. 0.92) were relatively more abundant in the controls than in the UI group. Within the genus of Lactobacillus, L. jensenii and L. vaginalis were only detected in the UI group.	Given the small sample size, we could not detect a significant statistical difference between groups.	

		diabetes, polycystic ovary syndrome and endometriosis, diarrhoea, ongoing pregnancy, addiction (e.g., drugs, alcohol, tobacco etc.) and the use of antibiotics within at least two weeks before sample collection.	the vagina of the participants				
Sezer, O., Soyer Çalışkan, C., Celik, S., Kilic, S. S., Kuruoglu, T., Unluguzel Ustun, G. and Yurtcu, N. Assessment of vaginal and endometrial microbiota by real-time PCR in women with unexplained infertility. J Obstet Gynaecol Res. 2022; 48 (1): 129-139.		cross-sectional study. 52 women. The diagnosis of unexplained infertility was made after excluding common causes of infertility using standard fertility studies, including semen analysis, evaluation of ovulation, and tubal patency testing.	study group: 26 women with UI control group: 26 controls with a history of healthy delivery An expert gynaecologist collected vaginal and endometrial samples of 52 women during the regular vaginal speculum examination following at least 3 days of sexual abstinence, in the middle of the second half of their natural menstrual cycles (between 9 and 12th day), with sterile swabs without further intervention.	detection of Lactobacillus spp., Candida spp., Mycoplasma hominis, Mycoplasma genitalium, Enterobacteriaceae family, Staphylococcus spp., Streptococcus spp., Eubacterium spp., Peptostreptococcus spp., Atopobium vaginae	**unexplained vs fertile** lactobacilli-impaired microbiota proportion: 76.9% vs 26.9% (p<0.05). Mycoplasma hominis flora increment or pathogenic microorganism growth rate 34.6% vs 7.7% (<0.05). lactobacilli/TBM mean proportion in the vaginal samples 38.2% vs 76.3% (p<0.05). Average Staphylococcus ssp. (p = 0.003), C1 (p = 0.013), C2 (p = 0.008), C3 (p < 0.001), C4 (p = 0.046), Peptostreptococcus spp. (p = 0.004), Atopobium vaginae ssp. (p = 0.019), and Mycoplasma hominis (p = 0.016) growth rates were significantly higher in the unexplained infertility patients		

Tomusiak, A., Heczko, P. B., Janeczko, J., Adamski, P., Pilarczyk-Zurek, M. and Strus, M. Bacterial infections of the lower genital tract in fertile and infertile women from the southeastern Poland. Ginekol Pol. 2013; 84 (5): 352-8.	CS	161 women; infertility >1 year, asymptomatic. Women and their partners had been thoroughly investigated to exclude other factors which may have played a role in problems with conception, such as anatomical and hormonal abnormalities, endometriosis and abnormal sperm parameters. Women receiving antibiotic therapy or up to three weeks after the treatment were excluded from the study.	**Study group**: n=161 women with UI. **Control group**: n=60 with no history of fertility problems and at least one child, comprised the control group. The material was obtained from the posterior vaginal fornix and the cervical canal (swabs; PCR), as well as urine (first-catch urine specimens containing epithelial cells; strand displacement technology).	detection of C. trachomatis, N. gonorrhoeae, M. genitalium, M. hominis, U. urealyticum, G. vaginalis, E. coli, S. agalactiae, E. faecalis.	**Infertile vs fertile women.** U. urealyticum found in 9% vs 8% (NS). M. hominis found in 4% vs 0%. (p=0.05). C. trachomatis 0% vs 3% (p<0.05). None of the women tested positive for N. gonorrhoeae or M. genitalium. Normal bacterial vaginal flora was confirmed in 80 women (79%) treated for infertility and 51 women (85%) from the control group. BV was confirmed (based on pH, Nugent score and quantitative culture results) in 7 women (7%) treated for infertility, and none from the control group.		

II.8 Male genito-urinary anatomy

PICO Question: Should men undergo additional diagnostic procedures to confirm normal genito-urinary anatomy before being diagnosed with UI?

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Lotti, F., Frizza, F., Balercia, G., Barbonetti, A., Behre, H. M., Calogero, A. E., Cremers, J. F., Francavilla, F., Isidori, A. M., Kliesch, S., La Vignera, S., Lenzi, A., Marcou, M., Pilatz, A., Poolamets, O., Punab, M., Peraza Godoy, M. F., Rajmil, O., Salvio, G., Shaeer, O., Weidner, W., Maseroli, E., Cipriani, S., Baldi, E., Degl'Innocenti, S., Danza, G., Caldini, A. L., Terreni, A., Boni, L., Krausz, C. and Maggi, M. The European Academy of Andrology (EAA) ultrasound study on	CS, multi-centre, international observational study (11 centres)	study population is healthy fertile men n=248 (partner pregnant or with a baby). Aim of the study: To report and discuss the scrotal organs CDUS reference ranges and characteristics in HFM and their associations with clinical, seminal, and biochemical parameters. The inclusion criteria: 1. healthy, fertile men. 2. age ≥ 18 years; 3. capacity to give consent for study participation. "Fertile men" were defined as (a) partners of a pregnant woman in the second or third trimester of pregnancy or (b) men with a child	Scrotal colour Doppler ultrasound (CDUS). The parameters to be analysed and the methods used to evaluate them were standardized and reported at www.andrologyacademy.net/eaa-studies. Intra- and inter-operator comparability of scrotal CDUS parameters: intra- and inter-operator comparability of the male genital tract-CDUS parameters were assessed on seven males of infertile couples. Intra-operator comparability was assessed for the main quantitative and qualitative scrotal CDUS parameters considering the results of three evaluations for each parameter (Table 1). Inter-operator comparability was derived from the measures and observations obtained by six different sonogiraphists for the main	A number of CDUS parameters in each category: 1. testis and scrotal sac, 2. Pampiniform plexus and varicocele, 3. Epididymis and proximal vas deferens. Main CDUS are indicated in table 1, but study results expand on more detailed parameters. Study reports reference ranges for CDUS parameters and makes correlations between scrotal	I wrote results only for correlation between scrotal CDUS and seminal parameters as this can be considered indirectly linked to male infertility (considering that semen analysis is the gold standard for male fertility evaluation. I. Mean TV was positively associated with 1. sperm concentration (r=0.315, p<0.0001 unadjusted, r=0.274 p<0.0001 after adjustment for confounding factors: age, waistline, lifestyle, cFT levels, and # EAA Centers) and 2. total count (r=0.219, p=0.001 unadjusted, r=0.278 p<0.0001 after adjustment for confounding factors). II Subjects with testicular inhomogeneity showed a	No association between scrotal CDUS parameters and time to pregnancy, number of children or history of miscarriage was observed. The present findings in fertile men will help in better understanding the pathophysiology of sperm abnormalities and male infertility, underlying modifications in their management.	Study attempts to bring reference values for CDUS parameters in a fertile cohort of men, but does not answer directly the PICO question. Instead it correlates CDUS parameters with semen parameters.

healthy, fertile men: Scrotal ultrasound reference ranges and associations with clinical, seminal, and biochemical characteristics. Andrology. 2021; 9 (2): 559-576.		less than one year old, achieved through natural conception. Healthy men were defined as subjects with no personal history of previous or current systemic diseases or treatments with a recognized negative effect on semen parameters.	quantitative and qualitative param- eters, respectively (Table 1). The comparability of quantitative and qualitative parameters was expressed using the coefficient of variation (CV) [(standard deviation (σ) / mean (μ)) x 100] and the concordance rate (CR) [(number of concordant observations/number of operators) x 100)], respectively. CV < 10 is considered acceptable.	CDUS and: 1. clinical parameters, 2. physical examination (PE) parameters, 3. biochemical parameters, 4. seminal parameters. I report results for correlation between CDUS and seminal parameters as rest of outcomes not relevant to the PICO	lower sperm vitality compared with the rest of the sample (Fig. 4 C), while those with any parenchymal calcification had lower sperm concentration and total count (Fig 4 D, E). Intratesticular artery PSV was positively as- sociated with sperm normal morphology (r=0.226, p=0.017 unadjusted, Adj.r=0.240 p<0.008). III. Epididymal head size was positively associated with sperm normal morphology (r=0.385, p<0.0001, Adj. r=0.233, p=0.002) and vas deferens mean sizes was positively associated with progressive motility (r=0.214, p=0.004 Adj. r=0.235, p=0.001). IV. Subjects with MAR test ≥ 1% showed a higher prevalence of epididymal tail echotexture inhomogeneity (OR=5.75[1.35-24.1], p=0.017), and a higher mean size of vas deferens and of epididymal body and tail (Figure 5), as compared with the rest of the sample.		

II.9 Male additional tests

PICO Question: Is there added value of additional tests in the male with normal WHO semen analysis?

Anti-sperm antibodies

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Ayvaliotis, B., Bronson, R., Rosenfeld, D. and Cooper, G. Conception rates in couples where autoimmunity to sperm is detected. Fertil Steril. 1985; 43 (5): 739-42.	Rest	n=108, Couples divided in 4 sub-categories: (1) no other cause of infertility was found in either partner, ie UI n=35; (2) the woman was apparently normal, but in the face of a significant male factor (semen volume, < 2 ml; sperm concentration, < 20 million/ml; motility, < 45%; oval heads, < 45%); (3) a female factor leading to infertility was present (inadequate luteal phase, as documented by two endometrial biopsies; oligoovulation, i.e., cycle length more than 45 days; endometriosis, periadnexal adhesions, and immunities to sperm); and (4) both the man and woman were	IBT of sperm washed of seminal fluid(Couples were categorized into those where 50% or more of spermatozoa in the ejaculate were antibody-bound (high level) and those where < 50% were antibody-bound (low level)	natural pregnancy rate (follow up between 6 to 46 months). Comparison of PR is within each of the 4 categories, between couples with high and those with low ASA	Category 1, UI: PR in sub-group '> 50% sperm antibody-bound' is 4/26 (15.3%); PR in sub-group <50% sperm antibody bound is 6/9 (66.7%), significantly different p<0.005	The chance of conception was greatest in those couples where antibody binding was < 50%; i.e., most sperm were free of detectable surface-bound immunoglobulins. Th	Overall number of study group is very small (108) and only a subgroup of patients (35) are with UI, rest are either female, male or mixed aetiology; no report of baseline characteristic; no attempts to adjust for confounding factors; Strong detection bias: no precise definition of outcomes & no clear method to determine outcomes

		abnormal. aim: determine pregnancy rates in infertile couples where surface-bound immunoglobulins had been demonstrated on the husband's spermatozoa. Each of those categories further divide in low and high ASA.					
Barbonetti, A., Castellini, C., D'Andrea, S., Minaldi, E., Totaro, M., Francavilla, S. and Francavilla, F. Relationship between natural and intrauterine insemination-assisted live births and the degree of sperm autoimmunisation. Hum Reprod. 2020; 35 (6): 1288-1295.	CS	n=84 men of IUI couples recruited by call (inclusion criteria: having undergone post-coital test, PCT, exclusion criterion: having an untreatable cause of female infertility and assessment of ovulatory function and tubal patency of the female partner. All males have immunological infertility (positive MAR test). Couples divided in 2 groups: Group A (100% MAR, n=44) and B (moderate 50-99% MAR, n=40). Comparison controls within each group (IUI vs natural conception); occurrence of natural pregnancies and the effectiveness of IUI were analysed in connection with the degree of sperm autoimmunisation, also accounting for the post-coital test outcome. In	IgG MAR in semen (positivity ≥50%)	occurrence of natural pregnancies and the effectiveness of IUI were analysed in connection with the degree of sperm autoimmunisation, also accounting for the PCT outcome; LBR, Predictive value of MAR% positivity for LBR	Group A: natural LBR 2/44 (4.5%), LBR after IUI 14/38 (36.8%), LBR after ICSI 7/15 (46.7%). Group B: natural LBR 12/40 (30%), LBR after IUI 7/26 (26.9%), LBR after ICSI 5/6 (83.3%). Predictor of natural live birth: % MAR test positivity: β (95% CI): −0.06 (−0.10, −0.02) p value= 0.007	A 100%-positive IgG-MAR test can represent the sole cause of a couple's infertility, which could be successfully treated with IUI. On the other hand, a lower degree of positivity (50-99%) may only represent a contributing factor to a couple's infertility, and so the decision to treat or wait also depends on the evaluation of conventional prognostic factors including the PCT outcome.	Initial strong bias towards including patients in the study who have post-coital test (PCT) done. Though subgroups were comparable at baseline, the inclusion criteria and patients selected could have biased the overall results. The range of age among female patients was large (23-44) which will confound factors. Sub-categorisation of patients in 2 groups is also biased and inappropriate as thresholds for the 2 groups are too close (50-99% and 100%); no precise definition of outcomes & no

		group A: couples receiving IUI 38/44 (83.3%), couples receiving ICSI 15/44 (34%). Group B: 26/40 (65%), couples receiving ICSI 6/40 (15%)					clear method to determine outcome
Bozhedomov, V. A., Nikolaeva, M. A., Ushakova, I. V., Lipatova, N. A., Bozhedomova, G. E. and Sukhikh, G. T. Functional deficit of sperm and fertility impairment in men with antisperm antibodies. J Reprod Immunol. 2015; 112 95-101.	DS	1060 infertile men with normal sperm and 107 fertile men. Female partners had full investigation with no abnormalities and therefore UI.	Semen analysis according to WHO (2000), MAR test, acrosome reaction (AR) by exposure to ionophore A23187 and flow cytometry, DNA fragmentation by the sperm chromatin dispersion method (Halosperm; reference level <20%), ROS by chemiluminiscence with luminol (tests results of the fertile control group was considered normal).	Semen analysis, MAR, acrosome reaction (AR), DNA fragmentation, ROS	ASA -IgG increased; MAR>50% in 15.6%; AR decreased in ASA positive men 2.1x lower; DNA fragmentation increased in ASA positive men; ROS levels higher in ASA positive men	Normozoospermic men with infertility have ASA 8.4x more commonly than fertile men.	Immune dysfunction with ASA positive men more likely in unexplained infertility
Lähteenmäki, A. In-vitro fertilization in the presence of antisperm antibodies detected by the mixed antiglobulin reaction (MAR) and the tray agglutination test (TAT). Hum Reprod. 1993; 8 (1): 84-8.	CS	IVF couples with male autoimmunity as a cause for infertility n=33; normal semen parameters only in subgroups of studied cohort. Another subgroup analysed to look at how sperm motility affects fertilisation, which is also ASA-ve. Some of the couples also had identified female infertility.	IgG MAR in semen (If 10-39% of motile spermatozoa were covered by latex particles, the test was interpreted as weakly positive. A positive reaction occurred when > 39% of the motile spermatozoa were incorporated in	The MAR values were divided into three categories and fertilisation and pregnancy rate (per embryo transfer) compared in those groups (Weakly positive, >0 and <40%; Positive, >40 and	fertilisation rate as per MAR category (Weakly positive, >0 and <40% ; Positive, >40 and <90% ; Strongly positive, >90%): 42/35/17 where category 2 and 3 significantly differ (p=0.0005). Pregnancy rate as per MAR category: 43/45/33 not significantly different	Only the strongly positive MAR group (values > 90%) revealed a significant reduction in fertilization rate compared to the other MAR groups. The pregnancy rate per embryo transfer was not directly associated with either sperm MAR	All couples undergo ART treatment (IVF). Hence, not appropriate cohort to look at predictive value of ASA test, though the 'control group' withing this study is patients in which ASA test was not done. selection bias: patient cohort

			mixed agglutinates. If there were >90% of motile spermatozoa in these agglutinates, the test was considered strongly positive). 16 men were further evaluated by direct (If the total binding was > 17% the test was considered positive) and 22 by indirect immunobead test (IBT).	<90% ; Strongly positive, >90%)			are not comparable at baseline; not adjusted for confounding factors female age (big age range), more than one cycle /couple, duration of infertility; no precise definition of outcomes & no clear method to determine outcome
Lähteenmäki, A., Reima, I. and Hovatta, O. Treatment of severe male immunological infertility by intracytoplasmic sperm injection. Hum Reprod. 1995; 10 (11): 2824-8.	CS	Study Group A, n=29 undergoing ICSI (anti-sperm antibodies in the male, by mixed antiglobulin reaction, MAR assay; many of these men with low motile sperm count); some of the female partners have secondary infertility, anovulation or oligoovulation; Group A subdivided in 2 (AI: at least 1 previous IVF attempt n=22, AII: no previous IVF attempts n=7); Control Group B (ICSI couples in general n=20, male infertility, MAR negative); females with normal tubal patency and endocrinology; divided in 2 sub-groups BI: at least 1 previous IVF	Sperm MAR tests for immunoglobulin (Ig) G (group A, n = 29; group C, n = 37) and IgA antibodies (group A, n = 26; group C, n = 22) (FertiPro, Gentbrugge, Belgium) were carried out according to the instructions of the manufacturer. The test result was considered to be positive when >10% of motile spermatozoa were attached to the latex particles. Serum samples in groups A and C were checked	miscarriage, clinical pregnancy, live birth rate (LBR)	Clinical pregnancy (%): Group A : total 13/28 (46) AI: 9/22 AII: 4/6, five miscarriages; Group B: 6(30), Group C: 11/37 (30), no miscarriages. The couples in group A had higher antibody levels in the male partner than those in group C, but differences were not significant.	Fertilization rate in group C (conventional IVF) was significantly lower than in groups A and B. In addition, group C patients more often had only single-embryo transfers, which had a significant effect on the outcome. The effects that anti-sperm antibodies have at the level of gamete interaction can be circumvented by direct ICSI. Post-fertilization failures may still have an effect on the outcome of this treatment of severe male immunological	all couples are assigned to ART treatments for known infertility, (male & female factor in some of the females in one of control groups, group B and positive group, group A) or female only factor (Control group C), hence huge selection bias that will affect outcomes; lack of appropriate controls; study seems to be not blinded; no precise definition of outcomes & no clear method to

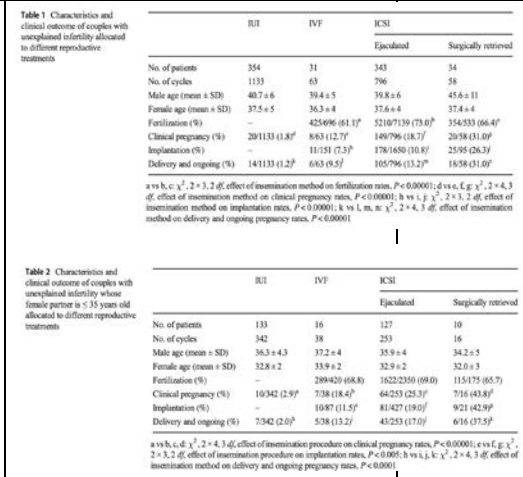
		attempt n=13, BII: no previous IVF attempts n=7); Second Control Group, undergoing conventional IVF C (n=37, males with anti-sperm antibodies detected by MAR, tray agglutination test, TAT, and/or flow cytometry, CM); women with impaired tubal patency or ovulatory problems. Mild endometriosis in all groups ignored; Setting: single centre	by TAT according to the method described by Friberg (1974). Agglutination of the washed donor spermatozoa at a serum dilution of 3=1:16 was considered positive. Flow cytometry has been described in detail elsewhere (Rasanen et al., 1992). When >5% of the live spermatozoa were covered with antibodies, the assay result was considered positive.			infertility. ICSI offers a good chance of fertilization for couples with male immunological infertility.	determine outcome; no statistical attempts to adjust for confounders (e.g., female age, previous unsuccessful ART attempts, abnormal semen parameters present in some subgroups)
Pagidas, K., Hemmings, R., Falcone, T. and Miron, P. The effect of antisperm autoantibodies in male or female partners undergoing in vitro fertilization-embryo transfer. Fertil Steril. 1994; 62 (2): 363-9.	CS	n=31, Control: IVF tubal infertility (n=312), Group A: IVF +ve ASA in female sera (n=15); Group B: IVF +ve ASA on sperm (n=16); all with normal semen characteristics. Group A and B subdivided in 2 categories, pregnancy with high % ASA (≥ 50%) and pregnancy in sub-category with low % ASA (<50%). sub-group A high % ASA (≥ 50%) n=8, sub-group A low % ASA (<50%) n=8	IBT (IgA, M, G), A specimen was classified as positive when >10% of the motile sperm showed positive binding	pregnancy rate	overall pregnancy in group A: 9/15 and in group B: 7/16. Pregnancy per subcategory. Pregnancy rate in sub-group B with high % ASA (≥ 50%) was 38% and in low % ASA (<50%) was 50%	In conclusion, fertilization rates or failure to con- ceive in our study could not be related to the pro- portion of antibody-coated spermatozoa or by the antibody class (isotype) detected by the immuno-bead test because the IVF-ET parameters were similar among the study groups and the controls. In addition, neither the regional specificity (or	Control group defined by the study is not appropriate as it would introduce bias (female factor). Real comparison is between Group A and B, but these groups have small size; In reality, group A would serve as 'control ' group because it is ASA+ve only in female sera; no precise definition of outcomes & no

						localization of the antibody) as defined by localization of the immunobead on the sperm surface, nor the antibody titer could be correlated with success or failure of IVF-ET procedure.	clear method to determine outcome
Rajah, S. V., Parslow, J. M., Howell, R. J. and Hendry, W. F. The effects on in-vitro fertilization of autoantibodies to spermatozoa in subfertile men. Hum Reprod. 1993; 8 (7): 1079-82.	CS	n=36 IVF couples; Group 1, n=16: couples with ASA positive male partners (either in sera or on sperm) with normal semen parameters characteristics Control group 2, n=20: IVF female factor, with no ASA in either semen or sera	MAR (the test was scored + , + + or+++when up to 20%,80% or >80% of spermatozoa were adhering to the erythrocytes); direct IBT (The test was regarded as positive if 20% or more of motile spermatozoa were attached to one or more beads)	fertilisation and pregnancy rate	fertilisation rate (per eggs collected): Group 1 (53/105, 50.5%) Group 2: 93/128, 72.2%) difference significant p=0.001;. Pregnancy rate (per embryo transfer): Group 1: 46.1% Group 2: 33.3% difference not significant	Antisperm antibodies in the male interfere with sperm—egg fusion and subsequent fertilization but once fertilization has occurred, the pregnancy rate remains the same.	potential selection bias: no clear inclusion criteria applied; groups are expected to not be comparable at clinical baseline level because of aetiology of infertility (Control Group 2 is female factor; no adjustment for confounders (big age range for males and females in both groups; duration of infertility); small sample size in both groups (Group 1: 16 couples, Group 2: 20 couples)

Vazquez-Levin, M. H., Notrica, J. A. and Polak de Fried, E. Male immunologic infertility: sperm performance on in vitro fertilization. Fertil Steril. 1997; 68 (4): 675-81.	CS	IVF couples, Control, n=9: tubal infertility; study group n=7: females with tubal infertility and men with significant levels of sperm bound ASA (at least 20% of the sperm were swimming with adhered particles between the clumps of erythrocytes)	IgG MAR (The reaction observed under the microscope was considered to be positive if at least 20% of the sperm were swimming with adhered particles between the clumps of erythrocytes.)	pregnancy rate	study group: 1/9 (11%); control group: 4/9 (44%), differences not statistically significant	The fertilization rate and early embryonic cleavage of human embryos was found to be reduced significantly in patients with high levels of surface-bound antisperm antibodies. Moreover, embryonic quality and the PR may be compromised by the presence of significant levels of surface-bound antisperm antibodies.	potential selection bias at level of inclusion criteria: no clear inclusion criteria applied and no rationale provided as to choice for analysing these groups; groups are expected to not be comparable at clinical baseline level because of aetiology of infertility (Control group is female factor); no provision of baseline characteristics, hence no adjustment for potential confounding factors; mall sample size in both groups (control group: 9 couples, study group: 7 couples). The study had no appropriate length of follow-up (up to pregnancy rate but no LBR reported)

DNA FRAGMENTATION TEST

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Borges, E., Jr., Zanetti, B. F., Setti, A. S., Braga, Dpaf, Provenza, R. R. and Iaconelli, A., Jr. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. Fertil Steril. 2019; 112 (3): 483-490.	prospective CS	First ICSI couples with female factor; inclusion criteria: couples with primary infertility undergoing their first ICSI cycle as a result of non–male factor infertility indications, which exclusively had fresh ET at day 5. The exclusion criteria were as follows: presence of any altered seminal parameter according to the cut-off values established, history of male factor infertility, any alteration detected during male partner workup, paternal smoking habit, previous conventional IVF cycle, ICSI cycle with vitrified/ thawed or donated oocytes, surgical sperm retrieval, cryopreserved sperm, vitrified/thawed ET, or preimplantation genetic tests. Couples with a history of pregnancy loss	sperm chromatin dispersion (SCD) test; Threshold values : low fragmentation (%30% SDF, n=433) and high fragmentation (>30% SDF, n=42)	1. comparison in fertilisation rate, embryo quality, implantation rate and pregnancy rate between couples with high and low DNA fragmentation index, DFI (as categorical variable) 2. As continuous variable, influence of DNA fragmentation on ICSI outcomes. Definitions of outcomes: Clinical pregnancy was diagnosed when fetal heartbeat was detected. Implantation rate was calculated as the number of gestational sacs divided by the number of	Higher miscarriage rate was observed in cycles with SDF above the cut-off (P=.018) ;No influence of continuous SDF was observed on laboratory and clinical parameters (Supplemental Table 3)		comparison groups discrepant in terms of numbers (low DFI n=433 vs high DFI n=42); Couples not UI (female factor) though authors provide analysis showing that female factor infertility did not influence laboratory and clinical outcomes. Selection bias present. male patient subgroup with high DFI has statistically significant longer abstinence period

		were also excluded from the analysis. Cycles were divided according to SDF rate into two groups: low fragmentation (%30% SDF, n=433) and high fragmentation (>30% SDF, n=42)		embryos transferred. Pregnancy rates were calculated per ET. Miscarriage was defined as a pregnancy loss before 20 weeks.			
O'Neill, C. L., Parrella, A., Keating, D., Cheung, S., Rosenwaks, Z. and Palermo, G. D. A treatment algorithm for couples with unexplained infertility based on sperm chromatin assessment. J Assist Reprod Genet. 2018; 35 (10): 1911-1917.	CS, retrospective	couples with unexplained infertility (male normal semen parameters and female with regular ovulation, tubal patency, and a normal uterine cavity unable to conceive after 1 year) and poor IUI outcome (n=354) included in a treatment algorithm depending on the outcomes of their DNA fragmentation test (SCSA or TUNEL). The algorithm is as follows: if sperm DNA frag results normal couples were allocated to IVF, if abnormal, they were allocated to ICSI with ejaculated sperm. Of the ICSI couples if no pregnancy was achieved, ICSI with surgically retrieved sperm was offered ; Outcomes: Fertilization rate, implantation rate,	SCSA and TUNEL. Threshold: for SCSA < 25% and for TUNEL ≤ 15% was considered normal	comparison of fertilisation rate (for IVF and ICSI groups) clinical pregnancy and delivery between IUI initial results and following IVF and ICSI (with ejaculated and surgically retrieved sperm)			selection bias: female age is confounding factor. Possible performance bias (different ovarian stimulation protocols) and detection bias (no precise definition of outcomes). Later authors mention that their inclusion criteria for females was <35yrs, though they also analysed couples above that age. No even number of couples allocated to treatments.

		pregnancy characteristics, and delivery rates					
Repalle, D., Saritha, K. V., Bhandari, S. Sperm DNA fragmentation negatively influences the cumulative live birth rate in the intracytoplasmic sperm injection cycles of couples with unexplained infertility. Clin Exp Reprod Med 2022; 49(3): 185-195	CS	prospective CS; couples (n=145) with unexplained infertility (normal semen analysis and no obvious female factor); inclusion & exclusion criteria: Couples undergoing their first ICSI cycle. The diagnosis of unexplained infertility was based on the following criteria: (1) normal ovarian reserve with an antral follicle count ≥ 8 and anti-Müllerian hormone levels ≥ 1.5 ng/mL, (2) normal tubal patency and uterine function evaluated by diagnostic laparoscopy and hysteroscopy, and (3) normal semen parameters for the male partner according to WHO 2010 criteria. None of the female partners were ≥ 41years of age in this study population. Female partners with < 5 mature metaphase II oocytes and male partners with normal semen parameters (WHO 2010 criteria) altered on the day of transvaginal oocyte recovery (TVOR) or egg collection were	Acridine orange; Threshold values: low fragmentation (SDF ≤%30, n of patients =97) and high fragmentation (SDF >30% , n of patients=48)	primary outcome: CLBR; other outcomes: implantation rate; cumulative pregnancy rate; miscarriage rate; predictive value of DNA frag for CLBR and miscarriage rate, but in sub-group analysis (positive vs negative live birth group), but not in low vs high DNA frag group	semen parameters do not differ between high and low DNA frag group, only the DNA frag results differed (Table 2). Subgroup analysis (fresh vs frozen embryo transfers) shows higher implantation rate, clinical pregnancy rate and LBR in the low DNA frag group for fresh embryo transfers, but not in frozen transfers (Table 3); Subgroup analysis in negative vs positive live birth groups (Table 4) shows that potential confounders (day of embryo transfer and fresh vs frozen embryo transfer) do not affect live birth rate and as such they don't affect the prognostic value of DNA frag results on CLBR and miscarriage, but that's based on analysis of negative and positive live birth groups, not the initial 2 groups of low and high DNA frag. I still think that there is a bias introduced by the different number of patients in low and high DNA frag group. Subgroup analysis between positive and negative live birth groups shows DNA frag as independent predictor for CLBR and miscarriage rate when adjusted for Female partner's age, embryo	In conclusion, SDF negatively influenced the CLBR, and a high SDF was associated with a higher miscarriage rate in the ICSI cycles of couples with unexplained infertility. These findings suggest that there is a need to evaluate SDF prior to ART cycles in couples with unexplained infertility to enable better counselling.	**Selection bias:** confounders: 1. abstinence period (not mentioned), 2. previous failed assisted conception (IUI cycles), 3. discrepant number of patients in both groups, study does not account for number of embryos transferred per cycle or number of embryo transfers as potential confounder considering the n difference. 4. Subgroup analysis shows stat difference in CLBR between low and high DNA frag group only in fresh cycles (here the discrepancy in the total number of transfers between the groups is 2-fold, Authors look at day of embryo transfer and type of transfer as

		excluded. Participants with life-threatening diseases such as cancer or chronic kidney disease were also excluded from the study. Control for confounders: day of embryo transfer and type of transfer (fresh vs frozen); Embryo utilization (the ratio of the number of embryos transferred and the number of embryos frozen to the total number of embryos formed); patients were later divided in 2 groups on live birth outcomes (positive and negative live birth group)			utilization rate, high-quality embryo rate, but not male age (Table 6).		confounding factors on the prognostic value of DNA frag but in a subgroup of positive and negative live birth groups. They don't account for the bias on these two confounders coming from the number of patients within the two comparison groups (high and low DNA frag group). 5. Male age as potential confounder **Performance bias: confounder:** number of embryo transfers between groups

II.10 Additional tests for systemic conditions

PICO Question: Should there be additional evaluations of possible systemic cause of UI in the couple?

Auto-immunity

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Anti-sperm antibodies in serum							
Mardesic, T., Ulcova-Gallova, Z., Huttelova, R., Muller, P., Voboril, J., Mikova, M. and Hulvert, J. The influence of different types of antibodies on in vitro fertilization results. Am J Reprod Immunol. 2000; 43 (1): 1-5.		44 couples referred for IVF treatment in whom the presence of antibodies was the only detectable cause of infertility	indirect MAR test for IgG, IgA, IgM and IgE. AZA were detected by passive hemagglutination test and ELISA	pregnancy rate	In 44 treated couples, 19 pregnancies occurred after IVF (43.2%). In 22 couples, the fertilization rate was lower than in patients with infertility of other etiology, but was satisfactory without ICSI (118:270, fertilization rate 43.7%) and ten pregnancies were achieved (45.5%). Standard IVF was possible in ten out of 15 cases (66.6%) with ASA (fertilization rate 37.6%) and in ten out of 11 couples (91%) with APA (fertilization rate 46.6%), but only in two women (11.1%) with AZA.		

Menge, A. C., Medley, N. E., Mangione, C. M. and Dietrich, J. W. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. Fertil Steril. 1982; 38 (4): 439-46.		698 human couples with primary or secondary unexplained infertility	detecting serum antibodies against sperm-the tray agglutination test (TAT)	pregnancy rate	In the study 14.8% of the men and 19.6% of the women had sperm-agglutinating antibodies. The incidence of pregnancy was influenced significantly by the presence of circulating spermagglutinating and -immobilizing antibodies in both sexes (Table 1). In men the pregnancy rate dropped significantly from 42.7% to 7.1% at agglutinin titers> 1:16. in women at titers ≥ 1:16 the incidence of pregnancy was only 4.0%, compared with 46.2% in the negative group		
Monem, F. M. and Moalla, H. A. Antisperm antibodies and unexplained infertility in Syria. An unsolved problem? Saudi Med J. 2003; 24 (8): 912-3.		group 1: 30 men and 24 women with UI group 2: controls, 45 fertile men and women	antisperm antibodies (ASA) (immunoglobulin (Ig) A, IgM, and IgG antibody classes) in their serum by indirect immunofluorescence and ELISA	presence of antibodies and association with UI	IIF: 22/54 patients positive and 3/45 controls ELISA: 20/54 positive and 4/45 controls There was a strong correlation between UI and antisperm antibodies		
Yasin, A. L., Yasin, A. L. and Basha, W. S. The Epidemiology of Anti-Sperm Antibodies Among Couples with Unexplained Infertility in North West Bank, Palestine. J Clin Diagn Res. 2016; 10 (3): Qc01-3.		42 couples with UI	ASA by ELISA	presence of antibodies and association with UI	The prevalence of ASA was 14.3% (6/42) among all couples, 9.5% (4/42) among males and 4.8% (2/42) among females. 22 couples managed with IVF-ICSI, and it was found that no relation between ASA status and the successfulness of IVF-ICSI exists		

Coeliac disease							
Tersigni, C., Castellani, R., de Waure, C., Fattorossi, A., De Spirito, M., Gasbarrini, A., Scambia, G. and Di Simone, N. Celiac disease and reproductive disorders: meta-analysis of epidemiologic associations and potential pathogenic mechanisms. Hum Reprod Update. 2014; 20 (4): 582-93.	SR	Unexplained infertility well defined n=586. Controls included n=6096	Clinical and biochemical diagnosis of CD	Prevalence of CD in unexplained infertility versus controls	Unexplained infertility OR for CD was 5.06 (CI 2.13-11.35)		Recommend screening for CD in unexplained infertility. More pregnancy complications in CD too including miscarriage, IUGR, LBW and preterm delivery
Karaca, N., Yılmaz, R., Aktun, L. H., Batmaz, G. and Karaca, Ç. Is there any relationship between unrecognized Celiac disease and unexplained infertile couples? Turk J Gastroenterol. 2015; 26 (6): 484-6.	CS	68 patients unexplained infertility, included males; after exclusion 65 couples studied	CD by Antigliadin antibodies (IgG and IgA), antiendomysial (IgG and IgA) and tissue transglutamininase antibodies (IgG and IgA) and total IgA followed by gastroscopy+biopsy if positive serological tests. Histopathological examination of biopt		7.9% positive for autoantibodies; only one female and one male positive for celiac disease		Very small study in Turkish population
Thyroid antibodies							
Abalovich, M., Mitelberg, L., Allami, C., Gutierrez, S., Alcaraz, G., Otero, P. and Levalle, O. Subclinical hypothyroidism and	CS	retrospective cohort study. 244 women with infertility (14 unexplained) and 155 controls	TSH and T4	TSH and T4	Subclinical hypothyroidism (SH) found in 13.9% infertile and 3.9% fertile. **In UI:** 0% subclinical hypothyroidism, 3/14 (21.4%) diagnosed with thyroid autoimmunity	Recommend measuring TSH in all infertile women	Marginal value as only 14 unexplained infertility patients

thyroid autoimmunity in women with infertility. Gynecol Endocrinol. 2007; 23 (5): 279-83.							
Kilic, S., Tasdemir, N., Yilmaz, N., Yuksel, B., Gul, A. and Batioglu, S. The effect of anti-thyroid antibodies on endometrial volume, embryo grade and IVF outcome. Gynecol Endocrinol. 2008; 24 (11): 649-55.	CS	case control study. 79 patients unexplained infertility n=31 thyroid pathology, n=23 normal thyroid function but positive anti-thyroid peroxides ot positive anti thyroidgloulin antibodies n=15 euthyroid with treatment, positive anti-TPO or anti-Tg antibodies. All going through IVF	thyroid function tests TAA and thyroid ultrasonography)	Embryo quality, clinical and biochemical pregnancy rates	No differences except clinical pregnancy rate less in last group. Clinical pregnancy 41% vs 30% vs 13%	Anti-TPO titre above a cut-off point affects clinical pregnancy rate	Small and unconvincing study
Poppe, K., Glinoer, D., Van Steirteghem, A., Tournaye, H., Devroey, P., Schiettecatte, J. and Velkeniers, B. Thyroid dysfunction and autoimmunity in infertile women. Thyroid. 2002; 12 (11): 997-1001.	CS	case control study. 73 unexplained infertility cases	TSH, FT4, TPO-Ab	TSH and FT4 levels, TPO antibodies	TSh 1.3 mIU/L vs 1.1; Ft4 12 vs11; TPO-Ab 14%vs8% RR 1.68 (0.27-2.73)	No increase in thyroid abnormalities in unexplained infertility	No evidence of increased thyroid autoimmunity in unexplained infertility
Other auto-immune tests							
Bellver, J., Soares, S. R., Alvarez, C., Muñoz, E., Ramírez, A., Rubio, C., Serra, V., Remohí, J. and Pellicer, A. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous	CS/D	prospective cohort study. 31 patients with unexplained infertility	Protein C resistance, IgM, IgG anticardiolipin antibodies, homocysteine, Factor V Leiden, prothrombin, MTHFR, TSH, thyroxine, anti-thyroid peroxidase	Only positives against controls were ATPO 29% vs 12.5%; ATG 25.8% vs 9.4%; both together 32.3% vs 15.6%; all other non significant			Low numbers but well conducted

abortion. Hum Reprod. 2008; 23 (2): 278-84.			and anti-thyroglobulin measured				
Hovav, Y., Almagor, M., Benbenishti, D., Margalioth, E. J., Kafka, I. and Yaffe, H. Immunity to zona pellucida in women with low response to ovarian stimulation, in unexplained infertility and after multiple IVF attempts. Hum Reprod. 1994; 9 (4): 643-5.	CS	15 patients unexplained infertility compared with other infertility and 20 fertile women	Zona pellucida antibodies	Zona pellucida antibodies	Zero positive in case or controls	Not relevant for unexplained infertility	Low numbers and no other papers on this
Kovács, M., Hartwig, M., Aleksza, M., Tihanyi, M., Nagy, T., Vajda, G., Daru, J. and Gasztonyi, B. Antiphospholipid antibodies in relation to sterility/infertility. Hum Immunol. 2012; 73 (7): 726-31.	CS	100 patients with unexplained infertility	Antiphospholipids, anticardiolipin, ANA, ENA, anti-TPO, aPS, aPT, ab2glycoprotein, aANX, ASA	Presence of Antiphospholipids, anticardiolipin, ANA, ENA, anti-TPO, aPS, aPT, ab2glycoprotein, aANX, ASA	27% positive aCL, 4 of these has previously diagnosed APS and others no clinical features	Recommend testing	High percentage positive but no controls
Aoki, K., Dudkiewicz, A. B., Matsuura, E., Novotny, M., Kaberlein, G. and Gleicher, N. Clinical significance of beta 2-glycoprotein I-dependent anticardiolipin antibodies in the reproductive autoimmune failure syndrome: correlation	Rest	65 unexplained infertility patients	IgG autoantibodies to 6 phospholipid antigens by ELISA. B2 -GPI-dependent and independent antibodies studied.	Presence of phospholipid antigens	Anticardiolipin antibody 12.3% vs 3.1% p<0.05; 2 or more aPS, aCL, aPI 6.2% vs 0%; no difference for aPS, aPI, B2-GPI dependent or independent anticoardiolipin antibody	Worth measuring anticardiolipin antibody	Small study from 1995

with conventional antiphospholipid antibody detection systems. Am J Obstet Gynecol. 1995; 172 (3): 926-31.							
Luborsky, J., Llanes, B., Davies, S., Binor, Z., Radwanska, E. and Pong, R. Ovarian autoimmunity: greater frequency of autoantibodies in premature menopause and unexplained infertility than in the general population. Clin Immunol. 1999; 90 (3): 368-74.	Rest	53 people with unexplained infertility. 12 normally cycling women as controls and 53 blood bank specimens	Ovarian antibodies by ELISA. Other organ autoantibodies tested.	Ovary and thyroid autoantibodies more common.	Ovarian antibodies 33-61%vs 17%; thyroid antibodies 47-66% vs 34%	Ovarian and thyroid antibodies more common in unexplained infertility	Controls not ideal and blood bank specimens had no history
Luborsky, J., Llanes, B., Roussev, R. and Coulam, C. Ovarian antibodies, FSH and inhibin B: independent markers associated with unexplained infertility. Hum Reprod. 2000; 15 (5): 1046-51.	Rest	52 women with unexplained infertility. Controls 12 cycling women	Ovarian antibodies	Presence of ovarian antibodies	Ovarian antibodies positive while FSH levels normal	In unexplained infertility ovarian antibodies are an independent marker of potential ovarian failure and may precede changes in regulatory hormones	No a prevalence study and controls debateable
Palacio, J. R., Iborra, A., Gris, J. M., Andolz, P. and Martínez, P. Anti-endometrial autoantibodies in women with a diagnosis of infertility. Am J	Rest	5 unexplained infertility 6 controls	Anti-endometrial antibodies	Presence of anti-endometrial antibodies	40-60% were positive depending on cell line	Anti-endometrial antibody may be common	Numbers too small to be convincing

Reprod Immunol. 1997; 38 (2): 100-5.							
Radojcić, L., Ma+A21:H21rjanović, S., Vićovac, L. and Kataranovski, M. Anticardiolipin antibodies in women with unexplained infertility. Physiol Res. 2004; 53 (1): 91-6.	Rest	42 unexplained infertility and 27 fertile women	Anticardiolipin antibodies; antithyroglobulin antibodies	Presence of Anticardiolipin antibodies; antithyroglobulin antibodies	aCL positive in 23.8%; anti-TG antibodies in 21.4%		
Witkin, S. S., Bongiovanni, A. M., Berkeley, A., Ledger, W. J. and Toth, A. Detection and characterization of immune complexes in the circulation of infertile women. Fertil Steril. 1984; 42 (3): 384-8.	Rest	39 unexplained and 38 control women	Circulating immune complexes, immunoglobulins, sperm related antigens, sperm agglutination	Presence of Circulating immune complexes, immunoglobulins, sperm related antigens, sperm agglutination	CICs positive in 38% vs 3% with all containing igG, half activating complement.4/39 had antisperm antibodies, half causing sperm agglutination.	Limitation of assays noted. Some may be antisperm antibodies. May indicate underestimate of undetected antisperm antibodies.	I am not sure of the validity of the assays

THROMBOPHILIA

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Bellver, J., Soares, S. R., Alvarez, C., Muñoz, E., Ramírez, A., Rubio, C., Serra, V., Remohí, J. and Pellicer, A. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Hum Reprod. 2008; 23 (2): 278-84.	CS	31 patients unexplained infertility 32 controls	Protein C, protein S, antithrombin III, lupus anticoagulant, APCR, IgM, IgG, ACA, homocysteine, Factor V, prothrombin G20210a, MTHFR, TSH, FT4, TPO, ATG	Prevalence of analyte	APCR more common (15.4%), lupus (11.5%) and combined thrombophilia (19.2%) higher but not statistically. Anti=TPO, Anti-TG both statistically increased	Anti-thyroid antibodies more common	Small numbers
Casadei, L., Puca, F., Privitera, L., Zamaro, V. and Emidi, E. Inherited thrombophilia in infertile women: implication in unexplained infertility. Fertil Steril. 2010; 94 (2): 755-7.	CCS	case-control study. 100 unexplained, 200 controls	Factor V, prothrombin, MTHFR mutations	Factor V, prothrombin, MTHFR mutations	No differences between groups. MTHFR OR 1.28 (95% CI 0.68-2.4); Factor V (OR 1 (95% CI 0.36-2.75); prothrombin OR 0.85 (95% CI 0.22-3.37)		Good study

Steinvil, A., Raz, R., Berliner, S., Steinberg, D. M., Zeltser, D., Levran, D., Shimron, O., Sella, T., Chodick, G., Shalev, V. and Salomon, O. Association of common thrombophilias and antiphospholipid antibodies with success rate of in vitro fertilisation. Thromb Haemost. 2012; 108 (6): 1192-7.	CS	retrospective cohort study. 594 women with unexplained infertility undergoing IVF, 637 fertile, 17337 no history of thrombosis.	Factor V Leiden, prothrobin G20210A, APC, Ig-anti-cardiolipin, beta2 glycoprotein antibodies, lupus anticoagulant with Russell viper venom time, APT	Prevalence of analyte	APCR and/orFVL7.9% vs. 3.8%, OR 2.18, 95% CI 1.28-3.72; prothrombin 3.1% vs. 4.2%, OR 0.73, 95% CI 0.39-1.37; lupus/anticardiolipin 3.3% vs. 4.7%, OR 0.70, 95% CI 0.38-1.28	None of the three thrombophilia's significantly associated with number of IVF cycles or lower fertility success rates. Rather women with positive APCR and/or Factor V leiden had higher live birth rates.	Big well conducted study
Behjati, R., Modarressi, M. H., Jeddi-Tehrani, M., Dokoohaki, P., Ghasemi, J., Zarnani, A. H., Aarabi, M., Memariani, T., Ghaffari, M. and Akhondi, M. A. Thrombophilic mutations in Iranian patients with infertility and recurrent spontaneous abortion. Ann Hematol. 2006; 85 (4): 268-71.	CCS	case-control study 36 unexplained infertility, 62 healthy fertile women	Factor V Leiden, MTHFR, prothrombin mutations	Factor V Leiden, MTHFR, prothrombin mutations	Factor V (31%) higher in unexplained and no difference others	Mild difference in factor V, nil in others	Poor study

Coulam, C. B. and Jeyendran, R. S. Thrombophilic gene polymorphisms are risk factors for unexplained infertility. Fertil Steril. 2009; 91 (4 Suppl): 1516-7.	CCS	92 unexplained infertility, 60 fertile controls	MTHFR, Factor V, prothrombin, factor XIIIV34L, b fibrinogen, PAI, HPA, MTHFR C677T and MTHFR A1298C	MTHFR different between groups	MTHFR C677T 22% vs 0%, p=0.01	Difference in C677T but not A1298C - needs testing	Minor difference and does not test hetero-vs homozygosity
Fatini, C., Conti, L., Turillazzi, V., Sticchi, E., Romagnuolo, I., Milanini, M. N., Cozzi, C., Abbate, R. and Noci, I. Unexplained infertility: association with inherited thrombophilia. Thromb Res. 2012; 129 (5): e185-8.	CCS	case control study. 230 unexplained infertility, 240 fertile	Prothrombin, Factor V, protein S and C, antithrombin	General thrombophilia and prothrombin increased, factor V not; no live birth or pregnancy data	General thrombophilia 13% vs 7.1%; FVL 4.8% vs 3.8% ; PT (5.7% vs. 2.1%, OR 2.82, 95% CI 1.02-8.03). ; PC+PS+AT 2.6% vs 1.2%		Age difference significant; like all above studies no evidence of effect on pregnancy chance or outcome; recognise expensive and not common
Kydonopoulou, K., Delkos, D., Rousso, D., Ilonidis, G. and Mandala, E. Association of plasminogen activator inhibitor-type 1 (PAI-1) -675 4G/5G polymorphism with unexplained female infertility. Hippokratia. 2017; 21 (4): 180-185.	CCS	retrospective case control study. 115 Greek women unexplained infertility; 107 fertile	PAI-1 4G -675 allele	Prevalence of gene	5G/5G 22.6% vs 39.3%; 4G/5G 48.7% vs 41.1%; 4G/4G 28.7vs 19.6%	4G/5G associated with female infertility when dominant model followed	Difficult to see their conclusion from the data

Milenkovic, J., Milojkovic, M., Mitic, D., Stoimenov, T. J., Smelcerovic, Z., Stojanovic, D., Vujic, S. and Bojanic, N. Interaction of thrombophilic SNPs in patients with unexplained infertility-multifactor dimensionality reduction (MDR) model analysis. J Assist Reprod Genet. 2020; 37 (6): 1449-1458.	CCS	prospective case control study. 105 unexplained and 120 controls	Factor V Leiden, prothrombin, MTHFR, PAI-1 4G/5G	Prevalence data - no pregnancy outcomes.	MTHFR C677T CC 19.1% vs 40.8%, Ct 60%vs45.8%, 20.9% vs 13.3% p<0.002; others not significant. Interaction of MTHFR plus FVL significant p<0.013.	MTHFR C677T polymorphism plus FVL G1691A associated with unexplained infertility	Association rather than causation

OXIDATIVE STRESS

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Aktan, G., Doğru-Abbasoğlu, S., Küçükgergin, C., Kadıoğlu, A., Ozdemirler-Erata, G. and Koçak-Toker, N. Mystery of idiopathic male infertility: is oxidative stress an actual risk? Fertil Steril. 2013; 99 (5): 1211-5.	CS	prospective cohort study. 28 men in unexplained infertility plus 14 fertile donors	DNA fragmentation by TUNEL assay and the intracellular formation of ROS by oxidation of the cell-permeable dye 20,70-dichlorodihydrofluorescein diacetate (DCFH2-DA) to fluorescent 20,70-dichlorofluorescein (DCF)., MDA, PC and NT levels in semen and seminal plasma	Prevalence data - no pregnancy outcomes.	Tunel 72 vs 4.2%; ROS 56 vs 4.7%; MDA 8.6 vs 5.2%; PC 0.78 vs 0.46% NT 234 vs 148% all significant in UI vs fertile while FRAP not significant	Idiopathic infertility males, while having normal semen parameters, have oxidative stress features	Small numbers and no other clinical information

Desai, N., Sharma, R., Makker, K., Sabanegh, E. and Agarwal, A. Physiologic and pathologic levels of reactive oxygen species in neat semen of infertile men. Fertil Steril. 2009; 92 (5): 1626-31.	CCS	Case-control study. 54 men partners of unexplained infertility couples, 51 healthy fertile male volunteers	WBC, ROS by cheminiluminiscence with luminol	Prevalence	Concentration higher in controls, ROS 0.35 vs 0.01 p<0.001. Using cut-off of 0.0185 PPV 82.4$ vs NPV 77.8%	ROS measured by luminol based chemiluminesence highly specific and sensitive test in males	Convincing study but semen anlyses different
Saleh, R. A., Agarwal, A., Nada, E. A., El-Tonsy, M. H., Sharma, R. K., Meyer, A., Nelson, D. R. and Thomas, A. J. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril. 2003; 79 Suppl 3 1597-605.	CS	prospective cohort study. 23 men from unexplained couples, 16 controls	ROS using luminol, TAC, SCSA DNA damage	Prevalence, clinical pregnancy	ROS-TAC score 47 (45,51) UI vs 43 (32,49) p<0.009; DFI 23 (15,32) vs 15 (11,21) p=0.02; ROS negatively correlated with fertilisation (r=-0.59) and embryo quality (r=-0.89); DFI negatively correlated with fertilisation (r=-0.70) and embryo quality (r=-0.70)	Males have higher DNA damage than controls as well as oxidative stress. Although not separated in unexplained couples, relate to lower pregnancy outcomes	Good techniques but clinical comparisons less well done
Venkatesh, S., Shamsi, M. B., Dudeja, S., Kumar, R. and Dada, R. Reactive oxygen species measurement in neat and washed semen: comparative analysis and its significance in male infertility assessment. Arch Gynecol Obstet. 2011; 283 (1): 121-6.	CCS	Case-control study. 17 men with normal sperm in unexplained and 43 fertile controls	SA, ROS by luminol	Prevalence data - no pregnancy outcomes.	NROS unexplained vs controls (0.79 (IQR 0.41-2.01) vs. 0.03 (IQR 0.014-0.11) 104 RLU/min/20 million sperms; WROS 2.35 (IQR 0.91-23.1) vs. 0.24 (IQR 0.12-0.38) 104 RLU/min/20 million sperms)	ROS measurement useful in unexplained	No pregnancy data, small numbers

Faure, C., Leveille, P., Dupont, C., Julia, C., Chavatte-Palmer, P., Sutton, A. and Levy, R. Are superoxide dismutase 2 and nitric oxide synthase polymorphisms associated with idiopathic infertility? Antioxid Redox Signal. 2014; 21 (4): 565-9.	CCS	case-control study. 35 women and 34 men from unexplained infertility couples and compared to 34 men and 35 women fertile controls	DNA studies MnSOD, MPO, Gpx1, catalase, eNOS	Prevalence data - no pregnancy outcomes.	MnSOS men 2.94 (1.14-7.60) higher; women eNOS 1.91 (1.03-3.54)	Genetic susceptibility to oxidative stress is a risk factor for male infertility	Multiple comparisons - hard to justify data
Mayorga-Torres, B. J. M., Camargo, M., Cadavid Á, P., du Plessis, S. S. and Cardona Maya, W. D. Are oxidative stress markers associated with unexplained male infertility? Andrologia. 2017; 49 (5):	CCS	case-control study. 23 men unexplained infertility, 54 donors, 34 fertile controls	SA, ROS (flow using dichlorofluorescein diacetate), lipid peroxidation, mitochondrial membrane potential, DNA fragmentation	Comparison of prevalence	SA similar, ROS unexplained vs fertile (121.2±29.9 vs. 71.7±8.7) ; all other not significant. DFI only different against general population not fertile men	Oxidative stress important	Good data but unclear interpretation
Oborna, I., Wojewodka, G., De Sanctis, J. B., Fingerova, H., Svobodova, M., Brezinova, J., Hajduch, M., Novotny, J., Radova, L. and Radzioch, D. Increased lipid peroxidation and abnormal fatty acid profiles in seminal and blood plasma of normozoospermic males from infertile	CCS	case-control study. 12 normospermic males with idiopathic infertility compared with 17 fertile controls	Lipid peroxidation (TBARS assay), fatty acid analysis	Comparison of prevalence	TBARS and AA higher. DHA not different	Systemic oxidative stress may result in lipid peroxidation and altered fatty acid profile leading to infertility	Unexplained part of larger group. Results not shown but differences stated

couples. Hum Reprod. 2010; 25 (2): 308-16.							
Pekel, A., Gönenç, A., Turhan, NÖ and Kafalı, H. Changes of sFas and sFasL, oxidative stress markers in serum and follicular fluid of patients undergoing IVF. J Assist Reprod Genet. 2015; 32 (2): 233-41.	Rest	31 unexplained infertility in women with 40 male infertility as control group undergoing IVF.	sFas, sFasL, MDA, SOD, TAC in serum and follicular fluid	Comparison of prevalence	Serum Fas 2.85 lower in unexplained than control 2.90; serum sFasL lower (3.24) but FF higher (3.87) in unexplained compared with endometriosis. MDA FF lower, SOD higher. FF TAC lower than controls but higher in blood.	Serum and FF sFas lower in unexplained infertility implying increased apoptosis. Antioxidant levels lower	Hard to find the data - merely stated rather than present in tables. No fertile control - reject paper
Taken, K., Alp, H. H., Eryilmaz, R., Donmez, M. I., Demir, M., Gunes, M., Aslan, R. and Sekeroglu, M. R. Oxidative DNA Damage to Sperm Cells and Peripheral Blood Leukocytes in Infertile Men. Med Sci Monit. 2016; 22 4289-4296.	CCS	prospective case-control study. 30 unexplained infertility men and 22 healthy volunteers fertile	MDA, NO, DNA isolation and hydrolyzation	Comparison of prevalence	Sperm parameters different but in normal range; seminal MDA higher 9.68 vs 6.63; serum MDA higher 12.55 vs 7.7; seminal NO not different; serum NO higher 19.3 vs 11,2; serum 8-OHdG/106dG higher 1.55 vs 1.03, leukocyte 8-OHdG/106dG higher 1.25 vs 0.77	Oxidative condition have potential pathogenetic role in reduction of sperm motility and count	Include
Veena, B. S., Upadhya, S., Adiga, S. K. and Pratap, K. N. Evaluation of oxidative stress, antioxidants and prolactin in infertile women. Indian J Clin Biochem. 2008; 23 (2): 186-90.	CCS	case-control study. 13 unexplained infertility compared with controls	Serum MDA by thiobarbituric acid reaction, LDH, FRAP by colorimetric method as measures of antioxidant status.	Comparison of prevalence	Serum nitrite lower unexplained vs controls 3.0 vs 5.0; LDH higher 83vs 68; MDA higher 3.92 vs 2.82	Oxidative damage increased in unexplained	Small numbers and no pregnancy information

Verit, F. F., Verit, A., Kocyigit, A., Ciftci, H., Celik, H. and Koksal, M. No increase in sperm DNA damage and seminal oxidative stress in patients with idiopathic infertility. Arch Gynecol Obstet. 2006; 274 (6): 339-44.	CCS	case-control study. 30 men from unexplained partnership and 20 fertile donors	Sperm DNA damage using comet; TAS in semen; TOS semen; oxidative stress index	Comparison of prevalence	TAO, TOS, OSI, sperm DNA damage no different	No differences	reasonable paper
Zhang, J., Mu, X., Xia, Y., Martin, F. L., Hang, W., Liu, L., Tian, M., Huang, Q. and Shen, H. Metabolomic analysis reveals a unique urinary pattern in normozoospermic infertile men. J Proteome Res. 2014; 13 (6): 3088-99.	Rest	71 men from unexplained partnership and 47 fertile controls	Urinary metabolome performed looking at 37 biomarkers re energy production, antioxidation and hormone regulation.		Able to distinguish between groups using multiple analytes	Should use this to distinguish	Complicated paper with many different pathways
Lazzarino, G., Pallisco, R., Bilotta, G., Listorti, I., Mangione, R., Saab, M. W., Caruso, G., Amorini, A. M., Brundo, M. V., Lazzarino, G., Tavazzi, B. and Bilotta, P. Altered Follicular Fluid Metabolic Pattern Correlates with Female Infertility and Outcome Measures of In Vitro Fertilization. Int J Mol Sci. 2021; 22 (16):		135 women with different infertility diagnosis, 35 controls		follicular fluid metabolites	27/55 metabolites were different between infertile women and controls		

Şentürk, R., Tola, E. N., Bozkurt, M. and Doğuç, D. K. The role of oxidant status on the etiopathogenesis of unexplained infertility and intracytoplasmic sperm injection - embryo transfer success: a case-control study. J Obstet Gynaecol. 2021; 1-7.		case-control study. Exclusion criteria were endocrinopathy, chronic disease or medication use, ovarian pathology, hypogonadotropic hypogonadism, and having a history of pelvic surgery on the ovary/uterus. Couples who had received any form of vitamin supplementation within 3 months before the commencement of treatment were also excluded.	study group: 20 primary UI patients control group: 20 women having ICSI for male factor infertility	primary outcome: follicular fluid and serum TAS, TOS levels and OSI. secondary outcome: embryo quality, implantation, clinical pregnancy and living birth rate.	FF-TOS and FF-OSI of the UI patients were statistically higher than the control group (p=0.04, p=0.02, respectively). The systemic TOS and OSI were also significantly increased in the UI group compared to the control group (p=0.019, p=0.01, respectively). No significant difference in implantation, clinical PR or LBR		

GENETIC/GENOMIC TESTS

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Rull, K., Grigorova, M., Ehrenberg, A., Vaas, P., Sekavin, A., Nõmmemees, D., Adler, M., Hanson, E., Juhanson, P. and Laan, M. FSHB -211 G>T is a major genetic modulator of reproductive physiology and health in childbearing age women. Hum Reprod. 2018; 33 (5): 954-966.	Rest	36 idiopathic infertility couples, 169 controls	FSHB -211, FSHRc2039, FSHR-29 variants and association with FSH, LH, AMH	Hormone levels	Unexplained infertility exhibited double T allele frequency (23.6% vs 12.4%) and greater than 3X excess of TT homozygotes (5.6% vs 1.8%) for FSHB-211 G>t on increased LH/FSH ratio.	This allele is more common in unexplained infertility	Little relevance to clinical outcome

Sahmani, M., Sakhinia, E., Farzadi, L., Najafipour, R., Darabi, M., Mehdizadeh, A., Shahnazi, V., Shaaker, M. and Noori, M. Two common polymorphisms in the peroxisome proliferator-activated receptor γ gene may improve fertilization in IVF. Reprod Biomed Online. 2011; 23 (3): 355-60.	CS	prospective cohort study. 98 patients with unexplained infertility undergoing IVF. Unable to ascertain controls ? Population based data?	Genotype His447His and Pro12Ala polymorphisms of PPAR gamma gene	Frequency of polymorphisms Fertilization rate	No relationship pregnancy rate and SNPs. T allele of His447His associated with higher fertilisation. Also Pro12 Ala had higher fertilisation		No real clinical outcome
Salas-Huetos, A., Blanco, J., Vidal, F., Grossmann, M., Pons, M. C., Garrido, N. and Anton, E. Spermatozoa from normozoospermic fertile and infertile individuals convey a distinct miRNA cargo. Andrology. 2016; 4 (6): 1028-1036.	CCS	8 males from unexplained couples and 10 fertile men	736 human miRNAs measured using Nano-RNA chip from sperm RNA	Frequency of miRNAs	115 miRNAs ubiquitous in all normospermic infertile individuals while 59 miRNAs were not detected. 57 miRNAs differentially expressed; 20 regulated by host promoter that in 3 cases comprised genes involved in fertility.	Specific sperm miRNA expression in normospermic fertile individuals	Evolving area but may have diagnostic relevance. Small sample.
Suganya, J., Kujur, S. B., Selvaraj, K., Suruli, M. S., Haripriya, G. and Samuel, C. R. Chromosomal Abnormalities in Infertile Men from Southern India. J Clin Diagn Res. 2015; 9 (7): Gc05-10.	CCS	180 men with all wives described as normal; 28 normal sperm count	Karyotype	Karyotype performed	All normal karyotype	No value if sperm count normal but numbers of men low	Small sample

Vani, G. T., Mukesh, N., Rama Devi, P., Usha Rani, P. and Reddy, P. P. Methylenetetrahydrofolate reductase C677T polymorphism is not associated with male infertility in a South Indian population. Andrologia. 2012; 44 Suppl 1 252-9.	CCS	case-control study. 206 men with unexplained infertility and 230 healthy individuals	MTHFR polymorphism in blood	C and Y allele frequencies	CT and TT homozygotes against control 1.36 (0.83-2.22). CT genotype 1.19 ().71-1.97)	No value in measuring this	No value in measuring MTHFR in blood of males
Witkin, S. S., Bierhals, K., Linhares, I., Normand, N., Dieterle, S. and Neuer, A. Genetic polymorphism in an inflammasome component, cervical mycoplasma detection and female infertility in women undergoing in vitro fertilization. J Reprod Immunol. 2010; 84 (2): 171-5.	CS	prospective cohort study. 243 females undergoing IVF; 19 unexplained infertility	NALP3 polymorphism in interleukin 1 (CIAS1 7 unit repeat)	Frequency of polymorphisms	Frequency was 18.4% in unexplained vs 28.9% female infertility and 17% male infertility	Absence of CIAS1 12 unit repeat and presence of 7 unit repeat reduces NALP3 gene transcription associated with female infertility and cervical mycoplasma infection.	Not relevant to unexplained with the numbers presented
Papanikolaou, E. G., Vernaeve, V., Kolibianakis, E., Assche, E. V., Bonduelle, M., Liebaers, I., Van Steirteghem, A. and Devroey, P. Is chromosome analysis mandatory in the initial investigation of normovulatory women seeking infertility treatment? Hum Reprod. 2005; 20 (10): 2899-903.		1206 normo-ovulatory subfertile women. Inclusion criteria were: (i) infertility duration of >12 months; (ii) regular menstrual cycles (21–35 days). Besides a full medical history and general clinical examination, the diagnostic work-up of these couples included the following: a complete endocrine investigation of the hypothalamo-hypophyseogonadal axis	cytogenetic analysis (FISH)	chromosome abnormalities (CA)	The cause of infertility was not associated with the prevalence of CAs in the patients analysed. However, a significantly higher (P = 0.04) prevalence of CAs was observed in women with secondary infertility (1.25%) compared to those with primary infertility (0.25%)		

		including ovulation confirmation; thyroid function and prolactin status; evaluation of semen characteristics according to the criteria of Kruger et al. (1986); minor pelvic ultrasound examination; hysterosalpingography; and when indicated, hysteroscopy and/or laparoscopy.					
Trková, M., Kapras, J., Bobková, K., Stanková, J. and Mejsnarová, B. Increased micronuclei frequencies in couples with reproductive failure. Reprod Toxicol. 2000; 14 (4): 331-5.		50 couples with unexplained infertility. Exclusion criteria included work-related exposure to mutagenic agents, anticancer therapy, viral infections, use of a medical treatment for at least 3 months, and previous exposure to diagnostic X ray.	chromosome analysis in 50 couples with UI and 15 fertile couples by karyotyping (G-banding)	chromosome abnormalities (CA)	Micronucleated cells evaluated by couple Parameter / Infertile/abortion / Abortion / Infertile / Controls Number of couples: 50 / 31 / 19 / 10 Micronucleated cells/1000 cells; mean ± SD (range): 29.88 ± 8.35 (18–53) / 30.23 ± 9.34 (18–52) / 29.32 ± 6.63 (23–53) / 21.20 ± 4.26 (12–27) *P* value (compared to controls): <0.0001 / 0.0005 / <0.000		

Ertosun, M. G., Araci, D. G., Peker, A., Uzuner, S. Y., Toylu, A., Ozekinci, M., Usta, M. F., Clark, O. A. Investigation of the relationship between reproductive disorders and chromosomal abnormalities in a large-scale, single-center 10-year retrospective study. J Gynecol Obstet Hum Reprod 2022; 51(9): 102467	CS	4345 individuals with reproductive disorders undergoing genetic analysis. Unexplained infertility included but no detail on tests performed to make this diagnosis. UI was 11% of the total patients	Conventional karyotype testing	chromosome abnormalities (CA)	Abnormalities in 3% UI compared with 2.2% ART failure and 1.6% recurrent miscarriage. No statistical analysis. No recommendation re testing in UI specifically.	General recommendation for karyotype testing in infertility but no recommendation for UI specifically	karyotype testing cannot be preferentially recommended other than for general infertility

VITAMIN D DEFICIENCY

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Butts, S. F., Seifer, D. B., Koelper, N., Senapati, S., Sammel, M. D., Hoofnagle, A. N., Kelly, A., Krawetz, S. A., Santoro, N., Zhang, H., Diamond, M. P. and Legro, R. S. Vitamin D Deficiency Is Associated With Poor Ovarian Stimulation Outcome in PCOS but Not Unexplained Infertility. J	RCT	900 subjects with unexplained infertility treated with letrozole or clomiphene citrate. 647 had banked serum. 607 patients were PCOS and probably anovular; 647 AMIGOS and unexplained	25 hydroxy vitamin D. 4 cycles of ovarian stimulation	live birth rate, miscarriage rate	34.7% had a pregnancy and rates were comparable with study treatment. (1.07, 0.73-1.58). Vitamin D deficiency had a higher miscarriage rate 1.82, 0.92-3.61 p=0.09. Cumulative live birth same 32% vs 29% (1.1, 0.7-1.7)	Vitamin D deficiency may have a role in PCOS but not shown for unexplained infertility	Good study but no statistical significance

Clin Endocrinol Metab. 2019; 104 (2): 369-378.							
Lopes, V. M., Lopes, J. R., Brasileiro, J. P., Oliveira, I., Lacerda, R. P., Andrade, M. R., Tierno, N. I., Souza, R. C. and Motta, L. A. Highly prevalence of vitamin D deficiency among Brazilian women of reproductive age. Arch Endocrinol Metab. 2017; 61 (1): 21-27.	CCS	retrospective case-control study. 26 women with unexplained infertility, 90 other infertility, reference group	25 hydroxyvitamin D prevalence of deficiency	Unexplained (23.3 ng/ml) identical to other infertility and no difference to reference group (23.8 ng/ml)	women with UI and women with male factor infertility (23.3 ± 8.6 vs. 26.2 ± 9.2 ng/ml)	Vitamin D deficiency high in infertility but same as control	No evidence for deficiency
Rudick, B., Ingles, S., Chung, K., Stanczyk, F., Paulson, R. and Bendikson, K. Characterizing the influence of vitamin D levels on IVF outcomes. Hum Reprod. 2012; 27 (11): 3321-7.	CS	retrospective cohort study. 188 infertile women for IVF. 22 had unexplained infertility	Pregnancy rate by vitamin D status	No difference with other infertility classes for vitamin D deficiency or pregnancy outcomes. In all infertility Asian who were depleted had higher pregnancy rates.	No specific effect on unexplained infertility but deficiency associated with lower pregnancy rates in non-Hispanic whites but not in Asians	Contributes little to unexplained infertility data.	
Güngör, K., Güngör, N. D., Başar, M. M., Cengiz, F., Erşahin, S. S. and Çil, K. Relationship between serum vitamin D levels semen parameters and sperm DNA damage in men with unexplained infertility. Eur Rev Med Pharmacol Sci. 2022; 26 (2): 499-505.		58 UI infertile couples. Detection of pathology in any of the semen parameters, presence of known etiological factors such as cryptorchidism or history of reproductive tissue surgery, history of chemotherapy or radiotherapy or severe oligoasthenoteratozoospermia, patients who received	study group: 58 men with UI control group: 50 age and BMI matched fertile men with at least 2 children	vit D levels sperm DNA damage	Compared with the fertile group, male patients with unexplained infertility had significantly lower vit D levels (27.00 ng/mL (12.63-39.30) vs.23.66 ng/mL (7.50-55.00), p<0.004). sperm DNA damage, it was found in 31.50% (9.0-71.0) of infertile men and 26.00% (11.0-54.0) of fertile men. DNA damage was found to be significantly higher in the		

		hormonal treatment or vitamin D supplementation at last six months were excluded. couples with IVF/ICSI decision were excluded from the study			unexplained infertile group (p<0.002).		
Ko, J. K. Y., Shi, J., Li, R. H. W., Yeung, W. S. B. and Ng, E. H. Y. 100 YEARS OF VITAMIN D: Effect of serum vitamin D level before ovarian stimulation on the cumulative live birth rate of women undergoing in vitro fertilization: a retrospective analysis. Endocr Connect. 2022; 11 (2):	CS	retrospective CS. Women undergoing their 1st IVF cycle. Those undergoing donor oocyte IVF, in vitro maturation, pre-implantation genetic testing and women whose archived serum sample could not be retrieved were excluded	vitamin D levels between vitamin D deficient, insufficient and replete groups	CLBR/initiated cycle clinical pregnancy rate (per cycle started and per transfer in the fresh cycle); (v) ongoing pregnancy rate (per transfer in the fresh cycle); (vi) miscarriage rate (in the fresh cycle) and (vii) live birth rate (per transfer in the fresh cycle).	the CLBR in the vitamin D-deficient group was significantly lower compared to the non-deficient group (43.9%, 208/474 vs 50.9%, 325/639, OR 0.755, 95% CI 0.595–0.959, P = 0.021, unadjusted). The clinical/ongoing pregnancy rate, live birth rate and miscarriage rate in the fresh cycle did not show significant differences between the vitamin D deficient and non-deficient groups		

THYROID HORMONES

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Unuane, D., Velkeniers, B., Anckaert, E., Schiettecatte, J., Tournaye, H., Haentjens, P. and Poppe, K. Thyroglobulin	CSS	95 patients unexplained among other cause patients	Thyroid function	TSH, Ft4, TAI	86% TAI negative and 14% positive, same as all cause infertility and slightly higher than fertile controls (87% normal)	Thyroid testing important in infertility but no different in unexplained	No extra benefit of testing in unexplained

autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? Thyroid. 2013; 23 (8): 1022-8.							
Duran, ., Ozlü, T., Koç, O., Eşitken, C. and Topçuoğlu, A. Relationship of thyroid hormone levels and thyroid autoantibodies with early pregnancy loss and infertility. J Obstet Gynaecol. 2013; 33 (8): 862-4.	CCS	25 unexplained, 45 controls	Thyroid function	TSH, fT4, fT3, anti-TPO, anti-TG	UI ft4 1.14 vs 0.88 and fT3 3.48 vs 4.7 ($p<0.001$) but in normal range. No difference in TAI	Changes in thyroid in unexplained but not autoimmunity	Thyroid results were in normal range
Rehman, R., Rajpar, H. I., Ashraf, M., Iqbal, N. T., Lalani, S. and Alam, F. Role of oxidative stress and altered thyroid hormones in unexplained infertility. J Pak Med Assoc. 2020; 70 (8): 1345-1349.	CCS	44 unexplained, 44 controls	Thyroid function	Thyroid tests including T4,T3,TSH,oxidative stress markers	TSH slightly higher than controls (1.49 vs1.12 $p=0.027$) T4 was also higher in unexplained $p<0.001$	Unexplained and thyroid related	While there were statistical difference , well within normal range

Orouji Jokar, T., Fourman, L. T., Lee, H., Mentzinger, K. and Fazeli, P. K. Higher TSH Levels Within the Normal Range Are Associated With Unexplained Infertility. J Clin Endocrinol Metab. 2018; 103 (2): 632-639.	CCS	187 unexplained infertility vs 52 male infertility	TSH and prolactin	Absolute levels and correlations	Unexplained TSH higher 1.95 (1.5-2.6) vs male TSH 1.66 (1.25-2.17) p=0.003. More women had level >2.5uU/ml in unexplained 26.9 vs13.5%. TPO higher in male factor and prolactin similar results	TSH higher in unexplained than male infertility couples even after allowing for variables.	Useful but no real controls as partners of male infertility may not be true controls

PROLACTIN

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Subramanian, M. G., Kowalczyk, C. L., Leach, R. E., Lawson, D. M., Blacker, C. M., Ginsburg, K. A., Randolph, J. F., Jr., Diamond, M. P. and Moghissi, K. S. Midcycle increase of prolactin seen in normal women is absent in subjects with unexplained infertility. Fertil Steril. 1997; 67 (4): 644-7.	CS	prospective cohort study. 12 fertile, 12 unexplained women	Prolactin	Midcycle increase in prolactin	Midcycle bioactive prolactin (34.2±8.3 vs. 19.2±3.4 ng/ml) but not immunoactive (26.9±4.3 vs. 22.1±2.6 ng/mL) were reduced in unexplained women compared to controls	May play a subtle role in unexplained	All other times of the cycle were normal so hard to be sure this is important especially since bioactive was normal

Orouji Jokar, T., Fourman, L. T., Lee, H., Mentzinger, K. and Fazeli, P. K. Higher TSH Levels Within the Normal Range Are Associated With Unexplained Infertility. J Clin Endocrinol Metab. 2018; 103 (2): 632-639.	CSS	Cross-sectional study187 unexplained infertility vs 52 male infertility	TSH and prolactin	Absolute levels and correlations	Unexplained TSH higher 1.95 (1.5-2.6) vs male TSH 1.66 (1.25-2.17) p=0.003. More women had level >2.5uU/ml in unexplained 26.9 vs13.5%. TPO higher in male factor and prolactin similar results	TSH higher in unexplained than male infertility couples even after allowing for variables.	Useful but no real controls as partners of male infertility may not be true controls
Qu, T., Yan, M., Shen, W. J., Li, L., Zhu, P., Li, Z., Huang, J., Han, T., Hu, W., Zhou, R., Li, P., Xu, L., Huang, T., Zhong, Y. and Gu, J. Predictive serum markers for unexplained infertility in child-bearing aged women. Am J Reprod Immunol. 2020; 83 (1): e13194.	CS	prospective cohort study. 84 women with unexplained infertility vs44 fertile women	25 hormones and cytokine markers particularly prolactin, MCP-1 and leptin	Absolute levels and predictive model with ROC calculated	Using prolactin, MCP-1 and leptin in a predictive model significant ROC of 0.89. Other contributors included inhibin alpha, G-CSF, IL10, IL4, IL9, follitropin, LIF	Suggest use of predictors may improve detection of unexplained infertility	I was unable to sort out which components were increased or decreased
Veena, B. S., Upadhya, S., Adiga, S. K. and Pratap, K. N. Evaluation of oxidative stress, antioxidants and prolactin in infertile women. Indian J Clin Biochem. 2008; 23 (2): 186-90.	CCS	case-control study. 13 unexplained among many other causes of infertility and 25 controls	Prolactin, MDA, LDH, nitrite and FRAP levels as oxidative stress markers and antioxidants	Absolute levels	Prolactin no different but MDA increased (3.92 vs 2.82) while nitrite less (3.0vs 5.0 umol/l). LDH also increased (83.4 vs 67.9 U/L)	Increased ROS elements while antioxidants not increased. Claims hyperprolactinemia can produce this no backed by data)	Prolactin of no value for prediction

BMI

Reference	Study Type	Patients	Diagnostic test evaluated Reference standard test	Outcome measures	Effect size	Authors conclusion	Comments
Noventa, M., Quaranta, M., Vitagliano, A., Cinthya, V., Valentini, R., Campagnaro, T., Marci, R., Paola, R. D., Alviggi, C., Gangemi, M., Saccardi, C., Nardelli, G. B. and Gizzo, S. May Underdiagnosed Nutrition Imbalances Be Responsible for a Portion of So-Called Unexplained Infertility? From Diagnosis to Potential Treatment Options. Reprod Sci. 2016; 23 (6): 812-22.	CS	epidemiological survey. 198 unexplained and 59 pregnant controls	Dietary tests including energy intake, exercise	Dietary and exercise measurements	UI 33% daily physical exercise vs 69%. Calories for UI 2688 vs control 2115 significant p<0.001. Unexplained had lower intake of carbohydrates, higher lipids. Many vitamins were lower in the intake.	Italian cohort unexplained had inappropriate calorie intake and macronutrient intake. Fatty acid and vitamins also changed.	Useful approach to study
Lintsen, A. M., Pasker-de Jong, P. C., de Boer, E. J., Burger, C. W., Jansen, C. A., Braat, D. D. and van Leeuwen, F. E. Effects of subfertility cause, smoking and body weight on the success rate of IVF. Hum Reprod. 2005; 20 (7): 1867-75.	Rest	1828 first IVF cycles out of 8457 total cycles compared with other causes.	BMI	Live birth rate, miscarriage , implantation rate	There was a significantly higher live birth rate per cycle in women with normal weight (BMI ≥20–25 kg/m2) and slight overweight (BMI 25–27 kg/m2) compared with women with evident overweight with a BMI ≥27 kg/m2. The unfavourable effect of overweight was largest for women with unexplained subfertility. Underweight women had similar LBR compared to women of normal weight.	Smoking and overweight harmful. Patients would benefit from stopping smoking and reducing weight	Observational but difficult to elicit cause

Wang, L. T., Wang, C. X., Sun, H. L., Wang, X., Li, X. F., Wang, Y. L. and Li, Q. C. Effect of BMI on blood value of patients on HCG day with IUI treatment. BMC Womens Health. 2020; 20 (1): 105.	Rest	2319 cycles of IUI in unexplained infertility women.	BMI and hormone levels	Hormone levels	E2 day of hCG lower in overweight/obese on day of HCG (natural and stimulated cycles) where patient <35 years but not in over 35 years. In older women E2, Prog and LH were lower in woman with greater weight.	BMI affects E2, LH, Prog values but not the pregnancy rate.	Observational data

III. Treatment

III.1 Expectant management

PICO Question: What is the value of expectant management compared to active treatment for patients with UI?

Clomiphene citrate with timed intercourse (+/- ovulation trigger)

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Bhattacharya, S., Harrild, K., Mollison, J., Wordsworth, S., Tay, C., Harrold, A., McQueen, D., Lyall, H., Johnston, L., Burrage, J., Grossett, S., Walton, H., Lynch, J., Johnstone, A., Kini, S., Raja, A. and Templeton, A. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised	RCT	Inclusion criteria were at least two years of infertility, bilateral tubal patency (demonstrated by laparoscopy or hysterosalpingography), ovulation demonstrated by appropriately timed mid-luteal progesterone, and normal semen variables (according to World Health Organization criteria).We also included couples with minimum sperm motility of 20% or minimal endometriosis (rAFS stage 1). Blinding was not possible	**Expectant managemen**t (n=193): This involved 6 months during which no clinic visits or medical interventions were scheduled. Couples were given general advice regarding the need for regular intercourse, but no specific measures such as basal temperature charts or luteinising hormone kits were	live birth per woman, clinical pregnancy rate per woman, multiple PR, acceptability, adverse events, anxiety, depression	**expectant management:** LBR: 32/193 (17%) vs. **CC**: LBR: 26/192 (14%), 3 women conceived spontaneous (2%). Compared with expectant management, the odds ratio of a live birth was 0.79 (95% CI 0.45 to 1.38) with clomiphene citrate. Compared with expectant management, the adjusted HR for the time to a pregnancy leading to a live birth was 0.83 (99% CI 0.42 to 1.63). CPR: expectant management and clomifene citrate (17% v 15%), NS; multiple PR: 1% vs 1%; miscarriage rate: 30% vs 26%; ectopic pregnancy: 2% vs 0%. women on	CC seems to be no more effective than expectant management in couples with unexplained infertility.	

controlled trial. Bmj. 2008; 337 a716.		because of the nature of the interventions.	recommended. **Clomiphene citrate** (n=192): oral dose of 50 mg between day 2-6 of each treatment cycle. Couples were advised to have intercourse on days 12-18 of the cycle. If three or more ovarian follicles were detected by scan in the first cycle, the cycle was cancelled and the couple advised to avoid intercourse. Duration of intervention: 6 months		active treatments found the process of treatment more acceptable than those randomised to expectant management.		
Fisch, P., Casper, R. F., Brown, S. E., Wrixon, W., Collins, J. A., Reid, R. L. and Simpson, C. Unexplained infertility: evaluation of treatment with clomiphene citrate and human chorionic gonadotropin. Fertil Steril. 1989; 51 (5): 828-33.	RCT	155 couples with UI in a double-blind, prospective study. Inclusion: primary infertility of 2 or more years' duration; normal history and physical examination; proven ovulation by either regular cycles and biphasic basal body temperature charts, serum progesterone (P) > 10 ng/ml in the midluteal phase or an in-phase, secretory endometrial biopsy in the late luteal phase; a normal HSG; a normal laparoscopy	**Group 1:** placebo (two tablets) taken by mouth on cycle days 5 to 9 followed by i.m. saline injections on cycle days 19, 22, 25, and 28. **Group 2:** placebo tablets with i.m. hCG injections 5,000 IU on cycle days 19, 22, 25, and 28. **Group 3:** CC tablets 100 mg on cycle days 5 to 9 with saline	pregnancy rates	**Group 1 vs. 2 vs. 3 vs. 4**. The **pregnancy rates** were 0% (0/36), 11% (4/36), 19% (7/37; p<0.05 vs. group 1), and 7.6% (3/39), respectively.		

		done within the last 2 years confirming bilateral tubal patency and no other pelvic pathology; a normal serum prolactin; and at least two normal semen analyses fitting the following criteria: volume > 1 cc, count ~ 20 X 106 sperm/cc, morphology > 60% normal, and motility> 50%.	injections as in group 1. **Group 4:** CC and hCG injections with dosage and schedule as noted previously. Each patient received the same treatment for all four cycles. Patients were followed for 6 months after the end of the trial.				
Wordsworth, S., Buchanan, J., Mollison, J., Harrild, K., Robertson, L., Tay, C., Harrold, A., McQueen, D., Lyall, H., Johnston, L., Burrage, J., Grossett, S., Walton, H., Lynch, J., Johnstone, A., Kini, S., Raja, A., Templeton, A. and Bhattacharya, S. Clomifene citrate and intrauterine insemination as first-line treatments for unexplained infertility: are they cost-effective? Hum Reprod. 2011; 26 (2): 369-75.	RCT	Inclusion criteria were at least two years of infertility, bilateral tubal patency (demonstrated by laparoscopy or hysterosalpingography), ovulation demonstrated by appropriately timed mid-luteal progesterone, and normal semen variables (according to World Health Organization criteria).We also included couples with minimum sperm motility of 20% or minimal endometriosis (rAFS stage 1). Blinding was not possible because of the nature of the interventions.	**Expectant managemen**t (n=): This involved 6 months during which no clinic visits or medical interventions were scheduled. Couples were given general advice regarding the need for regular intercourse, but no specific measures such as basal temperature charts or luteinising hormone kits were recommended. **Clomiphene citrate** (n=): oral dose of 50 mg between day 2-6 of each treatment cycle. Couples were	cost-effectiveness	average cost for CC: £87.65 (mainly ultrasound scans) vs. £0 for expectant; the bootstrapped 95% CI for the cost difference between EM and CC (IUI) is £303–£370 (£286–£353). EM has the lowest cost per live birth at £72 (£0–£206), whereas CC has the highest at £2611 (£1870–£4166).	CC has a very small chance of being cost-effective, regardless of the value of the ceiling ratio.	

			advised to have intercourse on days 12-18 of the cycle. If three or more ovarian follicles were detected by scan in the first cycle, the cycle was cancelled and the couple advised to avoid intercourse. Duration of intervention: 6 months				

INTRA-UTERINE INSEMINATION (IUI) IN A NATURAL CYCLE VS EXPECTANT MANAGEMENT

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Bhattacharya, S., Harrild, K., Mollison, J., Wordsworth, S., Tay, C., Harrold, A., McQueen, D., Lyall, H., Johnston, L., Burrage, J., Grossett, S., Walton, H., Lynch, J., Johnstone, A., Kini, S., Raja, A. and Templeton, A. Clomifene citrate or unstimulated intrauterine insemination compared with expectant	RCT	Inclusion criteria were at least two years of infertility, bilateral tubal patency (demonstrated by laparoscopy or hysterosalpingography), ovulation demonstrated by appropriately timed mid-luteal progesterone, and normal semen variables (according to World Health Organization criteria).We also included couples with minimum sperm motility of	**Expectant management** (n=193): This involved 6 months during which no clinic visits or medical interventions were scheduled. Couples were given general advice regarding the need for regular intercourse, but no specific measures such as basal	live birth per woman, clinical pregnancy rate per woman, multiple PR, acceptability, adverse events, anxiety, depression	Treatment vs expectant: LBR: 38/165 vs. 26/167;	No indication that treatment with IUI was effective over no treatment after two failed IUI cycles, in couples with unexplained subfertility and a poor prognosis on natural conception. Only when in vitro fertilization (IVF) cycles were performed, treatment	

management for unexplained infertility: pragmatic randomised controlled trial. Bmj. 2008; 337 a716.		20% or minimal endometriosis (rAFS stage 1). Blinding was not possible because of the nature of the interventions.	temperature charts or luteinising hormone kits were recommended. **IUI:** A single insemination was performed 20-30 hours after an endogenous surge was detected.				

OVARIAN STIMULATION WITH IUI VS EXPECTANT MANAGEMENT

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Ayeleke, R. O., Asseler, J. D., Cohlen, B. J. and Veltman-Verhulst, S. M. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev. 2020; 3 (3): Cd001838.	SR	2 RCTs	OS+IUI vs expectant management in a natural cycle	LBR, multiple pregnancy rate, cumulative pregnancy rate, miscarriage rate,	**OS+IUI vs expectant management.** cLBR in couples with poor prognosis: OR 4.48, 95% CI 2.00 to 10.01, 1 RCT, 334 women; cLBR in couples with moderate prognosis: OR 0.82, 95% CI 0.45 to 1.49; 1 RCT, 334 women. Multiple PR: OR 3.01, 95% CI 0.47 to 19.28; 2 RCTs, 454 women. cPR in couples with poor prognosis: OR 4.68, 95% CI 2.22 to 9.86; 1 RCT, 201 women; cPR in couples with moderate prognosis: OR 0.80, 95% CI 0.45 to 1.42; 1 RCT, 253 women. Miscarriage rate: OR 2.87, 95% CI 1.18 to 7.01; 2 RCTs, 454 women.		

IVF VS EXPECTANT MANAGEMENT

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Pandian, Z., Gibreel, A. and Bhattacharya, S. In vitro fertilisation for unexplained subfertility. Cochrane Database Syst Rev. 2015; 2015 (11): Cd003357.	SR	2 RCTs,	OS+IVF vs expectant management	Live birth rate per woman, pregnancy rate per woman (i.e. the number of pregnancies for each randomly assigned woman over a particular period of time), multiple pregnancy rate per woman, OHSS, miscarriage rate	LBR: 1 cycle of IVF vs 3 months of expectant: OR 22.0, 95% CI 2.56-189.38, 51 women, 1RCT); CPR: 1 cycle of IVF vs 3-6 months of expectant: OR 3.24, 95% CI 1.07 to 9.80, two RCTs, 86 women		
Carosso, A. R., van Eekelen, R., Revelli, A., Canosa, S., Mercaldo, N., Stura, I., Cosma, S., Scarafia, C., Benedetto, C. and Gennarelli, G. Expectant Management Before In vitro Fertilization in Women Aged 39 or Above and Unexplained Infertility Does Not Decrease Live Birth Rates Compared to Immediate Treatment. Reprod Sci. 2022; 29 (4): 1232-1240.		retrospective CS. N=635 couples with UI and female age 39 or more	n=359 immediate treatment 276 expectant for one year	live birth	LBR: 70 (19.5%) in immediate group (11 natural, 59 IVF) and 57 (20.7%) in those who waited.(37 natural, 20 IVF). NS cLBR same for expectant treatment for 1 year and immediate IVF treatment in couples with female age of 39 years and above.		

III.2 Active treatment

PICO Question: If active treatment is pursued, which type of active treatment for UI?

Timed intercourse

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Harira, M. Use of Letrozole versus clomiphene-estradiol for treating infertile women with unexplained infertility not responding well to clomiphene alone, comparative study. Middle east fertility society journal. 2018; 23 (4): 384-387.	RCT	172 women. Patients with male factor infertility, hyperprolactinemia, thyroid disorder and/ or with body mass index >30 kg/m2 were excluded. All included women (172) were subjected to Clomiphene citrate (CC) 100 mg/d from 3rd to 7th day of menstruation, despite good follicular response (presence of follicles ≥18 mm in diameter) there was poor endometrial thickness. Groups were comparable at baseline.	**Group A**: n=86 CC 100 mg daily from cycle day 3 to 7 with estradiol valerate 4 mg from cycle day 8–14. **Group B**: letrozole 5 mg daily from cycle day 3 to day 7 using a computer generated randomization list and sequentially numbered opaque sealed envelopes	clinical pregnancy rate (presence of gestational sac in uterine cavity detected by transvaginal ultrasound), ongoing pregnancy rate (pregnancies continued beyond 20 weeks gestation), miscarriage rate (termination of pregnancy before the 20th gestational weeks), ectopic pregnancy rate and high ordered pregnancy rate.	CC+E2 vs. Ltz, no significant differences in clinical PR (11/86 (12.7%) vs. 1/86 (16.2%)); ongoing PR (7/86 (8.1%) vs. 11/86 (12.7%)); miscarriage rate (4/11 (4.6%) vs. 3/11 (3.4%)); multiple PR (2/86 (2.3%) vs. 0/86 (0%)); no cases of OHSS in either group		

Ibrahim, M. I., Moustafa, R. A. and Abdel-Azeem, A. A. Letrozole versus clomiphene citrate for superovulation in Egyptian women with unexplained infertility: a randomized controlled trial. Arch Gynecol Obstet. 2012; 286 (6): 1581-7.	RCT	270 women. Exclusion criteria were women with PCOS, FSH [10m IU/ml, endometriosis, hypo- or hyperthyroidism, Cushing syndrome, hyperprolactinemia, age\19 years or more than 38 years and diabetes mellitus. Groups were comparable at baseline.	**group 1** (letrozole group, n = 136): letrozole 2.5 mg/day from cycle day 3 to 7; **group 2**: n=134 women who received CC 100 mg/day from cycle day 3 to 7. all women were given hCG 10.000 IU i.m. when at least one mature follicle becomes ≥18 mm in diameter.	clinical pregnancy rate, side effects	The clinical PR was significantly greater in letrozole group (23.07 vs 10.68 %, P<0.001). Follow up of the pregnancy till the end of eighth week was observed. The abortion rate and the multiple pregnancy rate were significantly greater in CC group (35.71 vs 6.66 % and 21.42 vs 3.33 %, respectively). No patients had ectopic pregnancy or OHSS in both groups (Table 3). In the letrozole group, 20 women (15.38 %) had gastrointestinal symptoms in the form of nausea, vomiting, stomach pain and constipation while 8 (6.15 %) women experienced hot flashes, flushing and increased sweating. In CC, 22 (16.79 %) women experienced gastrointestinal upset in the form of nausea, occasional vomiting or more frequent bowel motions, and 8 (6.10 %) women experienced generalized malaise.		

TIMED INTERCOURSE VS. IUI IN A NATURAL CYCLE

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Bhattacharya, S., Harrild, K., Mollison, J., Wordsworth, S., Tay, C., Harrold, A., McQueen, D., Lyall, H., Johnston, L., Burrage, J., Grossett, S., Walton, H., Lynch, J., Johnstone, A., Kini, S., Raja, A. and Templeton, A. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. Bmj. 2008; 337 a716.	RCT	Inclusion criteria were at least two years of infertility, bilateral tubal patency (demonstrated by laparoscopy or hysterosalpingography), ovulation demonstrated by appropriately timed mid-luteal progesterone, and normal semen variables (according to World Health Organization criteria).We also included couples with minimum sperm motility of 20% or minimal endometriosis (rAFS stage 1). Blinding was not possible because of the nature of the interventions.	**Clomiphene citrate (n=192):** oral dose of 50 mg between day 2-6 of each treatment cycle. Couples were advised to have intercourse on days 12-18 of the cycle. If three or more ovarian follicles were detected by scan in the first cycle, the cycle was cancelled and the couple advised to avoid intercourse. Duration of intervention: 6 months **IUI:** A single insemination was performed 20-30 hours after an endogenous surge was detected.	live birth per woman, clinical pregnancy rate per woman, multiple PR, acceptability, adverse events, anxiety, depression	**Treatment vs expectant:** LBR 23/173 (13%) vs. 38/165 (23%)		

TIMED INTERCOURSE VS. OVARIAN STIMULATION AND IUI

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Agarwal, S. and Mittal, S. A randomised prospective trial of intrauterine insemination versus timed intercourse in superovulated cycles with clomiphene. Indian J Med Res. 2004; 120 (6): 519-22.	RCT	113 couples with UI. All women had the following test results: biphasic basal body temperature charts, in phase late luteal endometrial biopsy, normal serum levels of thyroid, prolactin, LH and FSH, HSG indicating normal uterine contour and laparoscopy indicating bilateral tubal patency, absence of pelvic adhesions and endometriosis. All men had normal values on at least two standard semen analyses and no antisperm antibodies.	**Group A**: n=69, CC+TI 36-40h after hCG, **Group B**: n=44, CC+IUI 36-40h after hCG. 6 consecutive cycles or until the time of conception. All women received clomiphene citrate 50-150 mg orally from day 3 to 7 of menstrual cycle depending on response. HCG 10,000 IU i.m. was administered when not more than four leading follicles >16 mm were seen.	conception	**TI vs IUI.** conception: 28/69 (41%) vs. 8/44 (18%), NS. Four women undergoing COH/IUI reported signs and symptoms of pelvic infection and uterine cramps. Of the 36 pregnancies delivered, 28 had full term live babies, 2 pairs of twins and 2 premature birth (34-35 wk gestation). Missed abortion occurred in 3 and spontaneous abortion in 1, all requiring vacuum aspiration. There were 2 pregnancies with twin gestation.		
Ayeleke, R. O., Asseler, J. D., Cohlen, B. J. and Veltman-Verhulst, S. M. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev. 2020; 3 (3): Cd001838.	SR	N=2068; Couples with unexplained subfertility, defined as follows. 1. Normal ovulatory status 2. Tubal patency 3. Normal semen sample according to World Health Organization criteria current at the time of the trial. II. Couples who had tried to	OS+IUI vs OS+TI	Primary outcomes Live birth rate per couple: all cycles. Multiple pregnancy rate per couple. Secondary outcomes Pregnancy rate per couple: all cycles. Pregnancy includes clinical pregnancy,	**Live birth rate**: OR 1.59, 95% CI 0.88-2.88, 2 RCT, 208 women. **Multiple PR**: OR 1.46, 95% CI 0.55-3.87, 4 RCT, 316 women. Clinical PR: OR 1.69, 95% 1.14-2.53, 6 RCT, 517 women. **Miscarriage rate:** OR 1.66, 95% CI 0.56-4.88, 2 RCT, 208 women. **OHSS**: OR 2.75, 95% CI 0.11-69.83, 1RCT, 68 women.		

		conceive for at least one year.		and/or ongoing pregnancy, Other adverse events: Moderate or severe ovarian hyperstimulation syndrome (OHSS), rate per woman; Miscarriage rate per couple			

IUI IN A NATURAL CYCLE VS. OVARIAN STIMULATION AND IUI

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Ayeleke, R. O., Asseler, J. D., Cohlen, B. J. and Veltman-Verhulst, S. M. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst Rev. 2020; 3 (3): Cd001838.	SR	N=2068; Couples with unexplained subfertility, defined as follows. 1. Normal ovulatory status 2. Tubal patency 3. Normal semen sample according to World Health Organization criteria current at the time of the trial. II. Couples who had tried to conceive for at least one year.	natural cycle+IUI vs OS+IUI	Primary outcomes Live birth rate per couple: all cycles. Multiple pregnancy rate per couple. Secondary outcomes Pregnancy rate per couple: all cycles. Pregnancy includes clinical pregnancy, and/or ongoing pregnancy, Other adverse events: Miscarriage rate per couple;	**Live birth rate:** OR 2.07, 95% CI 1.22-3.50, 4 RCT, 396 women. **Multiple PR**: OR 3.00 95% CI 0.11-78.27, 1 RCT, 39 women. **PR:** OR 6.43, 95% CI 0.56-73.35, 1 RCT, 26 women. **Miscarriage rate:** OR 5.21, 95% CI 0.19-141.08, 1 RCT, 26 women.		

IVF

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Pandian, Z., Gibreel, A. and Bhattacharya, S. In vitro fertilisation for unexplained subfertility. Cochrane Database Syst Rev. 2015; 2015 (11): Cd003357	SR	IVF vs natural cycle IUI: 2 RCT, 156 women	IVF vs natural cycle IUI	LBR, clinical PR, multiple PR, OHSS, miscarriage rate	**IVF vs natural cycle IUI.** LBR: OR 2.47, 95% CI 1.19-5.12, 2RCT, 156 women; clinical PR: OR 4.83, 95% CI 0.94-24.95, 1 RCT, 44 women; multiple PR: OR 1.03, 95% CI 0.04-27.29, 1 RCT, 44 women.		
Nandi, A., Raja, G., White, D. and Tarek, E. T. Intrauterine insemination + controlled ovarian hyperstimulation versus in vitro fertilisation in unexplained infertility: a systematic review and meta-analysis. Arch Gynecol Obstet. 2022; 305 (4): 805-824.	SR	8 RCT,; 1497 patients	IUI+OS vs IVF	Clinical pregnancy, live birth, multiple pregnancy and OHSS	CPR: 1.66 (1.02-2.70); LBR: 1.53 (1.10-2.32); MPR: 0.83 (0.50-1.38); OHSS: 1.77 (0.49-6.37); LBR in <38: 1.01 (0.88-1.15); LBR in >=38: 2.15 (1.16-4.00)		

PICO Question: What is the value of IVF versus ICSI?

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Foong, S. C., Fleetham, J. A., O'Keane, J. A., Scott, S. G., Tough, S. C. and Greene, C. A. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. J Assist Reprod Genet. 2006; 23 (3): 137-40.		60 with unexplained infertility had IVF or ICSI	Study period 1997 - 2001, participants were followed up end of treatment or to live birth	Fertilisation rate, pregnancy rate, live birth rate	No differences in fertilisation rate (72.2% vs 82.4%), implantation rate (38.2% vs 44.4%), clinical pregnancy rate (50% vs 50%), live birth rate (46.7% vs 50%)	There were no differences in clinical outcomes associated with IVF versus ICSI in the treatment of unexplained infertility.	
Dang, V. Q., Vuong, L. N., Luu, T. M., Pham T. D., Ho, T. M., Ha, A. N., Truong, B. T., Phan, A. K., Nguyen, D. P., Pham, T. N., Pham, Q. T., Wang R., Norman, R. J, Mol, B. W. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised	RCT	Eligible couples were aged at least 18 years and the male partner's sperm count and motility (progressive motility) were normal based on WHO 2010 criteria (total sperm count ≥39 × 10^6 sperm, progressive motility ≥32%).12 Couples had to have undergone two or fewer previous conventional IVF or intracytoplasmic sperm injection attempts, have used an antagonist protocol	Random assignment to IVF (n=199) and ICSI (n=183) group, blinded except for the embryologist and the couple	The primary outcome was changed from ongoing pregnancy resulting in livebirth obtained from all embryos of the started treatment cycle to ongoing pregnancy resulting in livebirth after the first embryo transfer of the started treatment cycle, and the former was	IVF vs ICSI: LBR: 65/183 (35.5%) vs. 73/199 (36.7%), RR 1.03 (95% CI 0.79-1.35), NS		

controlled trial. Lancet 2021; 397: 1554–63.		for ovarian stimulation, and agree to have two or fewer embryos transferred, and not simultaneously be participating in other IVF trials.		changed to a secondary outcome, with a fixed time point at 12 months after randomisation			
Bhattacharya, S., Hamilton, M. P., Shaaban, M., Khalaf, Y., Seddler, M., Ghobara, T., Braude, P., Kennedy, R., Rutherford, A., Hartshorne, G. and Templeton, A. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. Lancet. 2001; 357 (9274): 2075-9.	RCT	N = 100 couples in the UI subgroup analysis of the RCT. 48 had IVF, 52 had ICSI. Female partner <37 years	Participants were followed up to end of scheduled treatment cycle, 10 participants were lost to follow up in the entire study involving 435 cycles, loss to follow up not specified for the UI subgroup	Outcomes provided for UI subgroup = pregnancy rate, fertilisation rate/ oocyte retrieved, fertilisation rate/ oocyte inseminated or injected	Pregnancy rate 1VF 32% vs ICSI 38%, RR 0.83, 95% CI 0.48-1.45; Fertilisation rate/ oocyte retrieved 61% vs 50%, 95% CI for difference 5 to 17, Fertilisation rate per oocyte inseminated or injected 61% vs 70%, 95% CI for difference 2 to 14.	No difference in pregnancy rates between IVF vs ICS, fertilisation rate/ oocyte retrieved significantly higher with IVF than ICSI, fertilisation/ per oocyte inseminated or injected significantly lower with IVF than ICSI	

III.3 Mechanical-surgical procedures

PICO Question: What is the value of mechanical-surgical procedures?

Resection of polyps or fibroids

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Seyam, E. M., Hassan, M. M., Mohamed Sayed Gad, M. T., Mahmoud, H. S. and Ibrahim, M. G. Pregnancy Outcome after Office Microhysteroscopy in Women with Unexplained Infertility. Int J Fertil Steril. 2015; 9 (2): 168-75.	RCT	200 women with unexplained infertility who were trying to conceive naturally. No suspicion of uterine abnormalities	group 1: office microhysteroscopy (n=100) group 2: no office microhysteroscopy (n=100)	ongoing pregnancy rate	**group 1 vs 2** Ongoing PR: 43/100 vs 10/100; RR 4.30 (95%CI 2.29-8.07) Clinical PR: 57/100 vs. 15/100; RR 3.80 (95%CI 2.31-6.24) Miscarriage rate: 14/100 vs. 5/100; RR 2.8 (95%CI 1.05-7.48)		
Casini, M. L., Rossi, F., Agostini, R. and Unfer, V. Effects of the position of fibroids on fertility. Gynecol Endocrinol. 2006; 22 (2): 106-9.	RCT	94 women with infertility for ≥1 year having fertility-oriented intercourse. Suspicion of uterine abnormalities (polyps, fibroids, septate uterus or intrauterine adhesions)	group 1: removal of fibroids (n=52) group 2: no surgery (n=42)	clinical pregnancy rate miscarriage rate	Uncertain if surgery improved clinical PR compared to expectant management OR 2.44, 95% CI 0.97-6.17. Miscarriage rate: insufficient evidence of a beneficial effect OR 1.54, 95% CI 0.47-5.00.		

TUBAL FLUSHING

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Wang, R., Watson, A., Johnson, N., Cheung, K., Fitzgerald, C., Mol, B. W. J. and Mohiyiddeen, L. Tubal flushing for subfertility. Cochrane Database Syst Rev. 2020; 10 Cd003718.	SR	15 Randomised trials involving 3864 women with infertility.	Intervention: Tubal flushing with different contrast media (oil soluble contrast media(OSCM) or water soluble contrast media (WSCM)), alone or in combination, with each other or no treatment, were compared.	Primary outcome was live birth rate, other outcome measures were clinical pregnancy rate, miscarriage rate, complications such as intravasation and infection.	OSCM vs no treatment: OSCM may increase the odds of live birth (OR 3.27, 95% CI 1.57 to 6.85, 3 RCT's, 204 women). OCSM may increase the odds of clinical pregnancy (OR 3.54, 95% CI 2.08 to 6.02, 4 RCT's, 506 women). WSCM vs no treatment: it is uncertain whether flushing with WSCM increases live birth rate (OR 1.13, 95% CI 0.67 to 1.91, 1 RCT, 334 women). It is uncertain increases clinical pregnancy rate (OR 1.14, 95% CI 0.71 to 1.84, 1 RCT, 334 women). OSCM vs WSCM: live birth rate reported in 3 RCT's. In two a higher live birth rate with OSCM (OR 1.64 95% CI 1.27 to 2.11, 1119 women; OR 3.45, 95% CI 1.97 to 6.03, 398 women). In one no evidence of a difference between groups (OR 0.92, 95% CI 0.60 to 1.40, 533 women) I= 86%, therefore no meta-analysis. Tubal flushing with OSCM vs WSCM probably increases the odds of clinical pregnancy (OR 1.42, 95% CI 1.10 to 1.85, 6 RCT's, 2598 women). Flushing with OSCM	The evidence suggests that compared to no treatment, tubal flushing with oil-soluble contrast media (OSCM) may increase the chance of live birth and clinical pregnancy, while it is uncertain whether tubal flushing with : water soluble contrast media (WSCM) improves those outcomes. Compared to tubal flushing with WBCM, OSCM may improve clinical pregnancy while meta-analysis was not performed due to heterogeneity. Evidence also suggests that OSCM is associated with an increased risk of intravasation. Overall adverse events, especially long-term adverse events, are	

					probably increased the odds in intravasation (OR 5.00, 95% CI 2.25 to 11.12, 4 RCT's, 1912 women). No difference in infection or haemorrhage between OSCM and WSCM and no serious adverse events reported.	poorly reported across the studies.	
van Welie, N., Pham, C. T., van Rijswijk, J. Dreyer, K., Verhoeve, H. R., Hoek, A., de Bruin, J. P., Nap, A. W., van Hooff, M. H. A., Goddijn, M., Hooker, A. B., Gijsen, A. P., Traas, M. A. F., Smeenk, J. M. J., Sluijmer, A. V., Lambers, M. J., van Unnik, G. A., de Koning, C. H., Mozes, A., Timmerman, C. C. M., Lambalk, C. B., Karnon, J. D., Mijatovic, V., Mol, B. W. J. The long-term costs and effects of tubal flushing with oil-based versus water-based contrast during hysterosalpingography. Reprod Biomed Online 2021; 42(1): 150-157	RCT	Couples with male infertility (total motile sperm count after sperm washing of less than 3 million spermatozoa per millilitre), endocrine disorders (e.g. polycystic ovary syndrome, diabetes, hyperthyroidism or hyperprolactinaemia), iodine allergy or a high risk of tubal pathology (a history of pelvic inflammatory disease, previous Chlamydia infection or known endometriosis) were excluded.	1119 women were randomly assigned to HSG with oil-based contrast (n = 557) or water-based contrast (n = 562). The baseline characteristics were similar across the two groups	long-term reproductive outcomes	In the OSCM group, 39.8% of the women needed no other treatment, 34.6 % underwent IUI and 25.6% had IVF/ICSI in the 5 years following HSG. In the WSCM group, 35.0% of the women had no other treatment, 34.2% had IUI and 30.8% had IVF/ICSI in the 5 years following HSG (p=0.113)		

MINIMAL TO MILD ENDOMETRIOSIS

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Bafort, C., Beebeejaun, Y., Tomassetti, C., Bosteels, J., Duffy, J. M. Laparoscopic surgery for endometriosis. Cochrane Database Syst Rev 2020; 10: Cd011031	SR	women with minimal to mild endometriosis	3 RCTs pooled, 528 women.	pregnancy rate	Laparoscopic ablation or excision probably increases pregnancy rate compared to diagnostic laparoscopy only (OR 1.89, 95% CI 1.25 to 2.86, 3 RCTs, 528 participants; moderate quality evidence). Sensitivity analysis excluding poor quality studies (Gad 2012; Moini 2012) did not affect the results of the main analysis for this outcome. No subgroup analysis was possible.		

ENDOMETRIAL INJURY/SCRATCH

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Ghuman, N. K., Raikar, S., Singh, P., Gothwal, M., Yadav, G. Improving reproductive outcomes of intrauterine insemination: Does endometrial scratch injury help? A randomised controlled trial. Eur J Obstet Gynecol Reprod Biol 2020. 253: 225-31.	RCT	this study included couples in whom the women were diagnosed with unexplained infertility and had an indication for UI. Inclusion criteria BMI < 30 kg/m2, age 18-35 years, normal US findings and patent tubes. The quality of partners' semen of recruited women was normal. Other exclusion criteria were advanced maternal age and a history of fertility treatment or previous intrauterine procedures in the preceding 3 months.	150 women with UI. Scratch group (n=75) , on day 6-7 of their stimulated cycle. All women received up to 3 cycles IUI with ovarian stimulation.	Clinical PR, ongoing PR, Miscarriage Rate, Pain	**scratch vs. Control** CPR: 8/75 (10.7%) vs. 9/75 (12.0%); RR 0.89, 95% CI 0.36-2.17, p=0.797 ongoing PR: 6/75 (8.0%) vs. 8/75 (10.7%); RR 0.75, 95% CI 0.27-2.06, p=0.575 Multiple PR: 0/75 vs. 1/75		
Jafarabadi, M. N., Bagheri, M., Ebrahimi, Z., Shariat, M. and Haghollahi, F. Endometrial scratching effect on pregnancy rate in intrauterine insemination cycles: a randomized controlled trial. International journal of women's health and reproduction	RCT	The inclusion criteria: women with primary or secondary **infertility with unknown causes**, being within the age range of 21-35 years, having a body mass index (BMI) of 18-30, having a normal hormonal profile (FSH<10) and thyroid test, having no adnexal mass in ultrasound examination, and being in the menstrual cycle of 25-31 days. Cases of	120 women randomized scratch: n=60, scratch on cycle D3 control: n=60 All patients OS with Ltz, hCG 10.000 IU, followed by IUI 36-38h after trigger.	chemical and clinical PR abortion rate	**scratch vs. Control** chemical PR: 12/59 (20.3%) vs. 10/59 (16.9%), NS clinical PR: 11/59 (18.6%) vs. and 10/59 (16.9%), NS abortion rate: 1/59 (1.7%) vs. 3/59 (5.1%), NS		

sciences. 2020; 8 (1): 85-89.		abnormal prolactin, myoma, and systemic disease were excluded from this study. Both groups were matched in terms of age, infertility duration, and history of IUI or IVF.					
Maged, A. M., Al-Inany, H., Salama, K. M., Souidan, II, Abo Ragab, H. M. and Elnassery, N. Endometrial Scratch Injury Induces Higher Pregnancy Rate for Women With Unexplained Infertility Undergoing IUI With Ovarian Stimulation: A Randomized Controlled Trial. Reprod Sci. 2016; 23 (2): 239-43.	RCT	Couples with **unexplained infertility.** Inclusion criteria: semen analysis was normal with volume 2 to 5 mL, concentration >20 million/mL, >50% total motility, and >30% normal forms, at least 1 tube patent, no significant intrauterine or pelvic abnormalities demonstrated USS, hysteroscopy, and/or laparoscopy with serum FSH level of ≤12 mIU/mL. Exclusion criteria: woman's age >40 years, ovarian cyst detected on USS, uterine lesions such as submucosal leiomyoma, and a previous diagnosis of moderate to severe pelvic endometriosis. Also, women with body mass index ≥35 kg/m2, PCOS/anovulatory patients, or signs of hyperandrogenemia.	154 women randomized scratch (group S): n=77, **timing of scratch not specified** *(as with IVF, so on day of IUI?)* control (group C): n=77 All patients OS with CC+hMG, hCG 5000 IU, followed by IUI 24-36h after trigger	clinical PR multiple PR abortion	**scratch vs. Control** clinical PR: 30/77 (39%) vs. 14/77 (18.2%), p<0.05 multiple PR: 2/77 (6.7%) vs. 1/77 (7.1%), NS abortion rate: 5/30 (16.7%) vs. 3/14 (21.4%), NS		

Parsanezhad, M. E., Dadras, N., Maharlouei, N., Neghahban, L., Keramati, P. and Amini, M. Pregnancy rate after endometrial injury in couples with unexplained infertility: A randomized clinical trial. Iran J Reprod Med. 2013; 11 (11): 869-74.	RCT	couples with **unexplained infertility.** Inclusion: normal ovulatory function, normal uterine cavity, and bilateral tubal patency via hystrosalpingography and/or hystrolaprascopy if indicated. between 23 and 35 years of age, had an infertility duration of 2-5 years, BMI of 18-25 kg/m2, AMH of >1 µg/l, FSH <10 mIU/ml on the 3rd day of the cycle, and AFC ≥10-12 follicles. male partners had normal semen analyses parameters: concentration of more than15×106/mL, total count of 39×106, progressive motility more than of 32%, and normal morphology of at least 4%). There were no differences between the two study groups regarding the demographic characteristics, BMI, duration of infertility, basal FSH, AMH, duration and dose of hormone stimulation, endometrial thickness, and number of mature follicles of at least 18 mm.	234 women randomized scratch: n=144, scratch on day of LH surge detection control: n=103, mock scratch by gynaecological examination All women OS with CC+hMG, spontaneous LH surge followed by timed intercourse	pregnancy rate ongoing PR abortion rate	**scratch vs. Controls** OPR: 17/114 (14.9%) vs. 6/103 (5.8%) (OR: 2.83 95% CI 1.07 to 7.49, p=0.03) PR: 20/114 (17.5%) vs. 7/103 (6.7%), p=0.027 abortion rate: 3 (17.64%) vs. 1 (14.28%), p=0.701		

Senocak, G. C., Yapca, O. E. and Borekci, B. Comparison of pregnancy rates between patients with and without local endometrial scratching before intrauterine insemination. J Gynecol Obstet Hum Reprod. 2017; 46 (9): 687-690.	RCT	Inclusion criteria were as follows: women between 19 and 35 years of age; BMI in the normal range; no pathological problems as determined by USS; a basal FSH level of < 10 mU/mL; and normal levels of TSH, LH, prolactin, and estradiol on the third day of the menstrual cycle. All patients included had normal HSG results or a normal tubal passage confirmed by laparoscopy. Patients who had systemic or endocrinological diseases were excluded, as were those with submucous myoma, endometrial polyps, a uterine septum, or a uterine anomaly determined by HSG, hysteroscopy, or laparoscopy. In additional, spermiogram results had to be normal according to WHO. Groups were similar at baseline	80 women randomized scratch: n=40, scratch in the midluteal phase (days 21–25 of the cycle) controls: n=40 All women OS with rFSH, hCG 6500 IU, IUI 36h after trigger	biochemical PR clinical PR	**scratch vs. Controls** biochemical PR: 15/40 (37.5%) vs. 8/40 (20%), NS CPR: 11/40 (27.5%) vs. 5/40 (12.5%), NS		

Wong, T. Y., Lensen, S., Wilkinson, J., Glanville, E. J., Acharya, S., Clarke, F., Das, S., Dawson, J., Hammond, B., Jayaprakasan, K., Kearsley, N., Milner, M., Shankaralingaiah, N., Wood, S., Sadler, L. and Farquhar, C. Effect of endometrial scratching on unassisted conception for unexplained infertility: a randomized controlled trial. Fertil Steril. 2022; 117 (3): 612-619.	RCT	women with **unexplained infertility**. Inclusion criteria: age ≤42 years, BMI ≤35 kg/m², unsuccessfully trying to conceive for at least 12 months; normal ovulation (21–42 day menstrual cycles with variation <8 days); and the male partner had a normal semen analysis according to the WHO criteria	220 women randomized scratch: n=113, scratch between D1-12 of the menstrual cycle; second attempt if the first was unsuccessful control: n=107 Regular unprotected sexual intercourse for 3 cycles	live birth/woman randomized clinical PR ongoing PR multiple PR miscarriage	**scratch vs. Controls** LBR: 10/113 (9%) vs. 7/107 (7%), OR 1.39, 95% CI 0.50-4.03, p=0.53 CPR: 12/113 (11%) vs. 8/107 (7%), OR 1.43, 95% CI 0.56-3.84, p=0.46 OPR: 10/113 (9%) vs. 7/107 (7%), OR 1.39, 95% CI 0.50-4.03, p=0.53 multiple PR: none in either group miscarriage rate: 2/113 (2%) vs. 1/107 (1%), OR 20.01, 95% CI 0.19-43.82, p=0.57		
Yildiz, G., Kurt, D., Mat, E. and Yildiz, P. The effect of local endometrial injury on the success of intrauterine insemination. Journal of Experimental and Clinical Medicine (Turkey). 2021; 38 (4): 521-524.	RCT	Inclusion criteria: Age between 20-40, BMI <30 kg/m2, Primary infertility and at least one year history of infertility, patent bilateral tuba in HSG, FSH value of <10 mIU/ml and LH, estradiol, TSH and prolactin values within normal range, no history of known systemic disease or of regular use of drugs, no history of surgical intervention that can play part in the aetiology of infertility (endometrial polypectomy, myomectomy, endometriosis surgery, congenital uterine anomaly	96 women randomized scratch: n=54, scratch between D21-26 (luteal phase) of menstrual cycle control: n=42 all women OS with rFSH, 250µg rhCG followed by IUI 32-36h after trigger	biochemical PR clinical PR ongoing PR	**scratch vs controls** CPR: 4/54 (10%) vs. 2/42 (4.76%), p=0.18 OPR: 4/54 (10%) vs. 2/42 (4.76%) multiple PR and miscarriage: not observed		

		surgery, ovary cyst surgery, hydrosalpinx surgery etc.), normal pelvic USG, no endometrial biopsy, endometrial curettage and hysterescopic procedure within the last three months, normal spermiogram results according to WHO criteria. There was no statistically significant difference between study and control groups in terms of age of female, age of male, duration of infertility, BMI, serum FSH, LH, levels mean dose of gonadotropin, mean duration of ovulation induction					

III.4 Alternative therapeutic approaches

PICO Question: What is the effectiveness of alternative therapeutic approaches?

Antioxidants

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Showell, M. G., Mackenzie-Proctor, R., Jordan, V. and Hart, R. J. Antioxidants for female subfertility. Cochrane Database Syst Rev. 2020; 8 Cd007807.	SR	The SRV Included 63 RCTs involving 7760 women attending a reproductive clinic comparing oral antioxidants (AO) versus placebo, no treatment/standard treatment or another antioxidant. However, this evidence table captures the subgroup analyses performed in women with unexplained infertility comparing oral AO versus placebo or no treatment/standard treatment.	I grp: Oral antioxidant (AO) plus an infertility treatment (IVF/ICSI, IUI, OI+TI or LOD) C grp: Placebo plus same infertility treatment (IVF/ICSI, IUI, OI+TI or LOD) or same infertility treatment (IVF/ICSI, IUI, OI+TI or LOD) alone	Primary: live birth rate per woman randomised (LBR) Secondary: clinical pregnancy rate per woman randomised (CPR)	LBR: Two RCTs enrolled women with unexplained subfertility (OR 1.50 favouring AO, 95% CI 0.60 to 3.72: P = 0.38, I2 = 0%: 2 RCTs, 133 women). CPR: There was no clear evidence of a difference in CPR's when antioxidants were compared with placebo or no treatment in women with unexplained subfertility (OR 0.84 favouring placebo/no treatment , 95% CI 0.61 to 1.16; P = 0.29, I2 = 0%; 4 RCTs, 997 women). The AO's used in the RCT's in the subgroup of women with unexplained infertility include N-acetyl cysteine group (NAC), Vitamin E (VE), & Melatonin (M). These 4 RCTs included Badawy 2006 (AO [NAC] + clomiphene citrate [CC] 50mg days 2-6 V Placebo + clomiphene citrate 50mg days 2-6), Cicek 2021 (AO [VE] + CC 50mg D5-9 + IUI V CC 50mg D5-9 +	Not applicable as the RCT evidence in the subgroup of women with unexplained infertility not mentioned in the conclusion.	

					IUI), Eryilmaz 2011 (AO [M] + IVF V IVF), Espino 2019 (AO [M] + IVF V IVF).		

ACUPUNCTURE

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Guven, P. G., Cayir, Y. and Borekci, B. Effectiveness of acupuncture on pregnancy success rates for women undergoing in vitro fertilization: A randomized controlled trial. Taiwan J Obstet Gynecol. 2020; 59 (2): 282-286.	RCT	Total no. of Ps: 76 women aged 23-45 years with unexplained infertility (not having acupuncture during the last 1 year) undergoing IVF with fresh D3 ET No. of Ps in I grp: 38 No. of Ps in C grp: 38 Relevant baseline characteristics in I grp: Mean age (30.3 years), mean BMI (24.4 kg/m2), mean past IVF number (1.97) Relevant baseline characteristics in C grp: Mean age (31.5 years), mean BMI (23.3 kg/m2), mean past IVF number (1.83) Grps comparable: Yes	I grp: IVF + ET x 1 cycle + acupuncture x 3 sessions (1 week before ET, 30 mins before ET, 30 mins after ET) with IVF C grp: IVF + ET x 1 cycle only	Primary: not stated Secondary: not stated Outcomes were live birth rate (LBR), ongoing PR (OPR), clinical PR (CPR), anxiety levels (STAI-1 state anxiety scale) before 30 min & after 30 min ET with a higher score indicating higher anxiety level.	No effect sizes reported. LBR: I v C grp: 52.8% (19/36) V 27.8% (10/36); p = 0.031 OPR: : I v C grp: 55.6% (20/36) V 30.6% (11/36); p = 0.032 CPR: 63.9% (23/36) V 33.3% (12/36); p = 0.009 Mean (SD) STAI-1 score before ET: I v C grp: 57.3 (9.8) V 57.0 (8.0) ; p = 0.876; p = 0.876 Mean (SD) STAI-1 score after ET: I v C grp: 28.8 (3.3) V 41.1 (6.8); p = 0.000 I calculated the RR on LBR data: RR = 1.9	It was observed that three sessions of acupuncture before and after ET significantly increased the pregnancy rates in women with unexplained infertility. It was also found that acupuncture significantly reduced anxiety levels that occurred before ET.	

NUTRACEUTICALS (INOSITOL)

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Montanino Oliva, M., Buonomo, G., Carra, M. C., Lippa, A. and Lisi, F. Myo-inositol impact on sperm motility in vagina and evaluation of its effects on foetal development. Eur Rev Med Pharmacol Sci. 2020; 24 (5): 2704-2709.	RCT	Total no. of Ps: 86 women with unexplained infertility undergoing 1-3 consecutive cycles of timed intercourse No. of Ps in I grp: 43 No. of Ps in C grp: 43 Relevant baseline characteristics in I grp: not reported Relevant baseline characteristics in C grp: not reported Baseline characteristics in total patient population: Mean age (34.63 years), mean BMI (22.71 kg/m2), Grps comparable: Not known	I grp: MI (myo-inositol) PV suppositories x 3 every 2nd day peri-ovulatory C grp: Placebo PV suppositories x 3 every 2nd day peri-ovulatory Peri-ovulatory was expected day of ovulation (EDO) – 3, EDO – 1 & EDO + 1 where EDO – 3 = day when lead follicle on U/S > 16mm.	Primary: not stated Secondary: not stated Outcomes were pregnancy rate (not defined)	No effect sizes reported. PR: 18.6% (8/43) V 6.97% (3/43); no test of statistical significance performed MCR: 0% (0/43) V 0% (0/43); no test of statistical significance performed I performed a Chi-Square test on PR data: P = 0.106.	MI improves sperm motility and cervical mucus quality, increasing the probability of conception. The absence of adverse events both for the mother and the foetus confirmed the safety of this molecule in pregnancy, supporting even more its use for couples seeking pregnancy.	

TRADITIONAL CHINESE MEDICINE (TCM)

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Choi, S. J., Kim, D. I., Yoon, S. H., Lim, C. Y., Lee, J. M. and Choe, C. M. Effectiveness and safety of Korean medicine for treating women with unexplained infertility: A multi-center observational study. Integr Med Res. 2021; 10 (4): 100751. BACKGROUND: This study was	Case series (uncontrolled before / after study)	Total no. of Ps: 100 women aged 20-44 years with unexplained infertility undergoing treatment for 4 menstrual cycles followed by 3 menstrual cycles of observation No. of Ps in I grp: 100 (90 women completed the study) No. of Ps in C grp: not applicable Relevant baseline characteristics in I grp: Mean age (35.91 years), mean BMI (21.5 kg/m2), Relevant baseline characteristics in C grp: not applicable Groups comparable: not applicable	I grp: Onkyeong-tang (120cc) twice daily between menstrual cycle day (MCD) 3 and 12, and herbal medicine for ovulation and implantation (120cc) twice daily between MCD 13 and 28 for 4 menstrual cycles (They also received acupuncture and moxibustion treatment during 4 menstrual cycles) followed by 3 menstrual cycles of observation	Primary: Clinical PR (CPR Secondary: Ongoing pregnancy rates (OPR); Live birth rates (LBR); Adverse events	LBR: 7.8% (7/90) OPR per pregnancy: 53.85% (7/13 pregnant women) CPR: 14.4% (13/90) Adverse events: 37% (33/90) but none were serious	The findings of this study may provide the possibility of effectiveness and safety of Korea medicine treatment for unexplained infertile women. Further study is required due to lack of control and small sample size in this study.	

IV. Quality of Life

PICO Question: Is there a difference in QoL for patients with unexplained versus explained infertility?

Reference	Study Type	Patients	Interventions + comparisons	Outcome measures	Effect size	Authors conclusion	Comments
Santoro, N., Eisenberg, E., Trussell, J. C., Craig, L. B., Gracia, C., Huang, H., Alvero, R., Casson, P., Christman, G., Coutifaris, C., Diamond, M., Jin, S., Legro, R. S., Robinson, R. D., Schlaff, W. D. and Zhang, H. Fertility-related quality of life from two RCT cohorts with infertility: unexplained infertility and polycystic ovary syndrome. Hum Reprod. 2016; 31 (10): 2268-79.	Combination of data from two RCT cohorts	Women with PCOS and their partners (n = 733 and n = 641, respectively), and couples with UI (n = 865 women and 849 men) completed the questionnaires. QoL was determined before the start of treatment in about 45% of the couples; 55% of couples had received prior therapy (same percentages for both cohorts).	The participants completed a validated fertility-specific QOL survey (FertiQOL) at the time of the study screening visit.	The primary outcome for the PPCOSII trial was live birth. The primary outcome in the AMIGOS study was the rate of multiple pregnancies. The outcome measure of the combined study was FertiQOL (= Fertility related Quality of Life)	Women with PCOS had lower total FertiQOL scores (72.3 ±14.8) than those with UI (77.1 ±12.8; P < 0.001); this was true for each domain (except Relational). These differences were largely explained by variation in BMI, hirsutism, household income and age. Women had lower overall FertiQOL scores than their male partners. Males with PCOS partners had higher scores than males with UI (84.9 ±10.2 versus 83.3 ±10.8; P = 0.003). Scores were not consistently associated with conception or pregnancy outcome.	In summary, we used a new instrument, devised to assess specifically the fertility-related QOL (FertiQOL), to test the largest US-based cohort to date and found that QOL is reduced for women with PCOS compared with those with UI. Men have overall less compromise of QOL in association with an infertility diagnosis, but men with UI had lower QOL than men whose partners had PCOS. Finally, QOL did not overall predict	

						conception or live birth in this study.	
Kowalcek, I., Wihstutz, N., Buhrow, G. and Diedrich, K. Subjective well-being in infertile couples. J Psychosom Obstet Gynaecol. 2001; 22 (3): 143-8.	Cohort	**110** infertile couples: 13 with female infertility (group 1), 55 with male infertility (group 2), 31 with infertility in both partners (group 3), 5 with idiopathic infertility (group 4) and 6 unknown. Acording to table 3 **101 women and 98 men** were included (exclusion of 6 'unknwon' couples).	Intervention: von Zerssen symptom checklist (24 items) to establish the degree of subjective wellbeing once during the intake at the fertility clinic (Lübeck).	Mean ratings on the von Zerssen test manual (= subjective well-being). The average values for healthy test persons fall close to 14.3. The mean of somatically ill is 23.7, the mean of psychiatrically ill is 30.	Table 3 (the 6 'unknown' couples are excluded): EXPLAINED INFERTILITY Group 1. Mean women = 17.58 vs. men = 13.17 Group 2. Mean women = 13.07 vs. men = 10.44 Group 3. Mean women = 15.13 vs. men = 11.52 UNEXPLAINED INFERTILITY Group 4. Mean women = 14.8 vs. men = 9.4	With the exception of sterile women of fertile men (group 1), women and men in the overall randomized sample and the diagnostic groups 2, 3, and 4 report fewer general symptoms than the overall population of patients with somatic and psychiatric diseases (abstract).	
Warchol-Biedermann, K. The Etiology of Infertility Affects Fertility Quality of Life of Males Undergoing Fertility Workup and Treatment. Am J Mens Health. 2021; 15 (2): 1557988320982167.	Cohort	Of the 255 baseline respondents, 253 respondents completed the testing twice, 215 respondents completed the testing 3 times, while 185 respondents completed the testing 4 times. One respondent returned an unfinished questionnaire, 4 respondents withdrew from the study, while 65 of them discontinued treatment. Unclear how many participants were included in the end! The baseline sample consisted of 255 married males, who	Respondents completed Emotional, Mind–Body, Relational, and Social subscales of the Polish version of FertiQoL and a baseline demographic survey. The timing of psychological testing was strictly related to andrological visits and to medical procedures, that is, respondents completed the tests	The Core module of FertiQoL consisting of 4 domains (emotional, mind-body, relational, and social).	**The Core FertiQoL score** The mean score in the UFI subgroup, which amounted to 83.97 ± 4.95, at T1 has not significantly changed after the diagnostic disclosure and in the follow-up (at T3 and T4) (p values = .19, = .11, and = .73, respectively) (see Figure 2a for details). **The emotional subscale** The average score in the subgroup with the UFI reached 89.88 ± 8.49 at T1. The analysis could not indicate any significant changes in respondents' scores at T2, T3, and T4 (p values = .27, = .33 and = .61, respectively) (see Figure 2b for details).	The research demonstrated that the FertiQoL scores across the Emotional, Mind–Body, and Relational subscales markedly decreased after the diagnostic disclosure, particularly in the subgroups with male and concurrent male and female factor. Social subscale scores in all subgroups peaked at T1 and remained stable after the diagnostic disclosure (at T2) but	The results of this paper are partially discordant with the results of the study by Santoro et al. (2016). Santoro and co-workers indicated differences in FertiQoL associated with the perceived diagnosis but male UFI participants of Santoro's study were

		were 22–51 years old with a mean age of 30.24 ± 4.29 years. The subjects had a marriage length of between 1 and 11 years (M = 2.16 ± 1.02) and, with the exception of one subject with a child from a previous relationship, were coping with primary infertility (i.e., 99.6% of them had no previous children). The subjects' spouses were 21-42 years old with a mean age of 28.42 ± 3.7 years. Respondents reported having been trying to conceive for 8–24 months (M = 14.53 ± 3.17; Me = 14). Detailed sociodemographics are presented in Table 2.	(1) before their first fertility testing (T1) at the baseline, before a diagnostic disclosure; (2) before the second andrological visit, 2–3 months after the diagnostic disclosure when their emotional response to the diagnosis stabilized (T2); and (3) before the third and the fourth treatment-related or check-up testing appointments (T3, T4). T2, T3, and T4 were 2–3 months apart.		**The Mind-body subscale** The baseline score in the UFI subgroup, which averaged at 93.65 ± 7.97, remained stable after the diagnostic disclosure (T2) (p value = .27). The score significantly increased at T3 (p value = .03) and then plateaued at T4 (p value = .66) (see Figure 2c for details). **The relational subscale** The average score in the UFI respondents, which reached 74.80 ± 6.65 at T1, remained stable after the diagnostic disclosure (T2) (p value = .86). Subsequently, no significant changes could be found at T3 and T4 (p values = .62 and = .92, respectively) (see Figure 2d for details). **Social subscale** The average Social subscale score in the UFI subgroup reached 77.57 ± 5.66 at the baseline (T1). The score remained stable after the diagnostic disclosure (T2) (p value = .63) and in the follow-up (at T3 and T4) (p values = .57 and = .17, respectively) (see Figure 2e for details).	significantly decreased in the follow-up (at T3 and T4). The investigation of the results at the baseline and in the follow-up also demonstrated respondents with UFI were characterized by significantly higher scores in the Emotional, Mind–Body, and Relational domains than those with other diagnoses. Significant differences in FertiQoL scores associated with respondents' infertility factor could be demonstrated at each time point. The study identifies the FertiQoL in unintentionally childless males is significantly affected by their factor of infertility and evolves across the pathway of treatment-related/follow-up appointments.	characterized by lower FertiQoL scores compared with FFI respondents whose partners had polycystic ovary syndrome.