Hum. Reprod. Advance Access published March 6, 2014
Human Reproduction, Vol.0, No.0 pp. 1–7, 2014
doi:10.1093/humrep/deu042

human reproduction ORIGINAL ARTICLE *Early pregnancy*

Non-visualized pregnancy losses are prognostically important for unexplained recurrent miscarriage

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Submitted on December 20, 2013; resubmitted on February 3, 2014; accepted on February 5, 2014

STUDY QUESTION: Are non-visualized pregnancy losses (biochemical pregnancy loss and failed pregnancy of unknown location combined) in the reproductive history of women with unexplained recurrent miscarriage (RM) negatively associated with the chance of live birth in a subsequent pregnancy?

SUMMARY ANSWER: Non-visualized pregnancy losses contribute negatively to the chance for live birth: each non-visualized pregnancy loss confers a relative risk (RR) for live birth of 0.90 (95% CI 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage.

WHAT IS KNOWN ALREADY: The number of clinical miscarriages prior to referral is an important determinant for live birth in women with RM, whereas the significance of non-visualized pregnancy losses is unknown.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort study comprising 587 women with RM seen in a tertiary RM unit 2000–2010. Data on the outcome of the first pregnancy after referral were analysed for 499 women.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study was conducted in the RM Unit at Rigshospitalet, Copenhagen, Denmark. We included all women with unexplained RM, defined as ≥3 consecutive clinical miscarriages or non-visualized pregnancy losses following spontaneous conception or homologous insemination. The category 'non-visualized pregnancy losses' combines biochemical pregnancy loss (positive hCG, no ultrasound performed) and failed PUL (pregnancy of unknown location, positive hCG, but on ultrasound, no pregnancy location established). Demographics were collected, including BMI, age at first pregnancy after referral and outcome of pregnancies prior to referral. Using our own records and records from other Danish hospitals, we verified the outcome of the first pregnancy after referral. For each non-visualized pregnancy loss and miscarriage in the women's reproductive history, the RR for live birth in the first pregnancy after referral was determined by robust Poisson regression analysis, adjusting for risk factors for negative pregnancy outcome.

MAIN RESULTS AND THE ROLE OF CHANCE: Non-visualized pregnancy losses constituted 37% of reported pregnancies prior to referral among women with RM. Each additional non-visualized pregnancy loss conferred an RR for live birth of 0.90 (95% CI 0.83; 0.97), which was not statistically significantly different from the corresponding RR of 0.87 (95% CI 0.80; 0.94) conferred by each clinical miscarriage. Among women with ≥2 clinical miscarriages, a reduced RR for live birth was also shown: 0.82 (95% CI 0.74; 0.92) for each clinical miscarriage and 0.89 (95% CI 0.80; 0.98) for each non-visualized pregnancy loss, respectively. Surgically treated ectopic pregnancies (EPs) were significantly more common for women with primary RM and no confirmed clinical miscarriages, compared with women with primary RM and ≥1 clinical miscarriage (22 versus 6%, difference 16% (95% CI 9.1%; 28.7%); RR for ectopic pregnancy was 4.0 (95% CI 1.92; 8.20).

LIMITATIONS, REASONS FOR CAUTION: RM was defined as ≥3 consecutive pregnancy losses before 12 weeks' gestation, and we included only women with unexplained RM after thorough evaluation. It is uncertain whether the findings apply to other definitions of RM and among women with known causes for their miscarriages.

WIDER IMPLICATIONS OF THE FINDINGS: To our knowledge, this is the first comprehensive investigation of prior non-visualized pregnancy losses and their prognostic significance for live birth in a subsequent pregnancy in women with unexplained RM. We show that a prior non-visualized pregnancy loss has a negative prognostic impact on subsequent live birth and is thus clinically significant.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: N/A.

Key words: recurrent miscarriage / biochemical pregnancy loss / pregnancy of unknown location / non-visualized pregnancy loss / retrospective cohort study

Introduction

The term early pregnancy loss covers three different clinical scenarios: miscarriage, where transvaginal ultrasound (TVS) or histological findings document an intrauterine demise before 12 weeks' gestation; ectopic pregnancy (EP), where TVS or laparoscopy identifies a pregnancy outside the uterine cavity; and biochemical pregnancy loss, where there is a positive pregnancy test but no ultrasound has been performed. In the event where a woman has a positive pregnancy test and TVS is performed, but neither an intrauterine nor an ectopic pregnancy is seen, the pregnancy is classified as a 'pregnancy of unknown location' (PUL) (Barnhart et al., 2011). Following an initial classification of PUL, the possible final diagnoses are: an ongoing intrauterine pregnancy; an ectopic pregnancy; a failed PUL; or an intrauterine miscarriage (Barnhart et al., 2011).

When dealing with an acute early pregnancy complication, the distinction between different types of early pregnancy loss is very important as it has implications for the prognosis, treatment and follow-up of patients (Kirk et al., 2009; Barnhart et al., 2011). In contrast, when considering the past reproductive history of a patient referred to a recurrent miscarriage (RM) unit, the importance of early pregnancy losses, such as failed PULs and/or biochemical pregnancy losses, has not been well studied. We hypothesize that biochemical pregnancy losses and failed PULs share similar prognostic importance. Therefore, in addition to separate analyses for biochemical pregnancy losses and failed PULs, we group these two diagnoses together as 'non-visualized pregnancy losses' defined as a pregnancy loss initially confirmed by a positive hCG, but not visualized by TVS, if performed.

The definition of RM is controversial. A guideline from the European Society of Human Reproduction and Embryology (ESHRE), as well as the Royal College of Obstetricians and Gynaecologists (RCOG), define RM as three or more consecutive pregnancy losses (Jauniaux et al., 2006; Regan et al., 2011). However, the American Society for Reproductive Medicine (ASRM) Practice Committee defines recurrent pregnancy loss as two or more clinical miscarriages confirmed by ultrasound or histology, not necessarily consecutive (ASRM Practice Committee, 2013). Non-visualized pregnancy losses are thus not included in the ASRM Practice Committee definition, nor in other recent publications (Saravelos and Li, 2012).

Non-visualized pregnancy losses in women with RM are increasingly diagnosed because very early pregnancy testing is readily available (Wilcox et al., 1987). Whether non-visualized pregnancy losses should be included in the definition criteria for RM is controversial. If they negatively affect the chance of a subsequent live birth, then non-visualized pregnancy losses are clinically relevant.

To investigate whether prior non-visualized pregnancy losses are clinically relevant, we collected data over 10 years on the outcome of the first pregnancy after referral to the Danish Recurrent Miscarriage Unit.

Materials and Methods

A retrospective cohort study comprising 918 consecutive women seen in the Danish RM Unit at the Fertility Clinic, University Hospital Copenhagen, Rigshospitalet from January 2000 to January 2011 was performed. We included only women who we considered as having unexplained RM, i.e. who fulfilled the following criteria: at least three consecutive pregnancy losses, including both clinical miscarriages and non-visualized pregnancy losses; age <40 years at referral; regular menstrual cycle with length 23–35 days (variation from cycle to cycle was $\leq$2–3 days); normal uterine evaluation by hysteroscopy, hysterosalpingogram or uterine hydrosonography; normal parental karyotypes; and negativity for the lupus anticoagulant. We excluded women who had conceived after IVF/ICSI or donor insemination prior to referral. Figure 1 gives an overview of study flow. In short, 331 women were excluded. Forty women (7%) were lost to follow-up and according to their records, 48 women (9%) did not conceive after referral. Outcome of first pregnancy after referral was registered for 499 women: 290 with primary RM (PRM) (58%) and 209 with secondary RM (SRM) (42%). Of these, 368 (74%) had experienced $\geq$2 clinical miscarriages and thus fulfilled the ASRM criteria for recurrent pregnancy loss as well as the ESHRE/RCOG criteria.

As is standard practice in this RM unit, at first consultation, all women had given a detailed written account of their reproductive history along with documentation on where, when and how their previous pregnancies had been managed. Treatment regimens in a subsequent pregnancy varied according to medical history, and included 'tender loving care' (TLC) with or without intravenous immunoglobulin (IVIg). Twenty-seven women received IVIg or placebo from 2008 to 2013 (NCT00722475). One hundred and ten women received IVIg in a non-randomized fashion, before 2008 or being ineligible for the trial. These women had had at least four early pregnancy losses or at least one unexplained late miscarriage and two early pregnancy losses.

Patients were followed at the RM Unit until 16 weeks' gestation, after which the women were referred for continued monitoring at their local hospital. Information on outcome on first pregnancy after referral was obtained either from patient records or from the women themselves.

For this study, the women's information was entered in a Microsoft Office Access 2010 database by two of the authors (A.M.K. and O.B.C.). Double entry was avoided using the unique Danish identification number. Prior to statistical analysis, data quality was checked manually (A.M.K.).

We divided early pregnancy events into the following categories: miscarriage, where ultrasound or histology documented an intrauterine pregnancy loss before 12 weeks' gestation; EP, where a pregnancy loss was visualized outside the uterus by laparoscopy or TVS; failed PUL, where there had been a positive hCG, but no location was established by TVS; biochemical

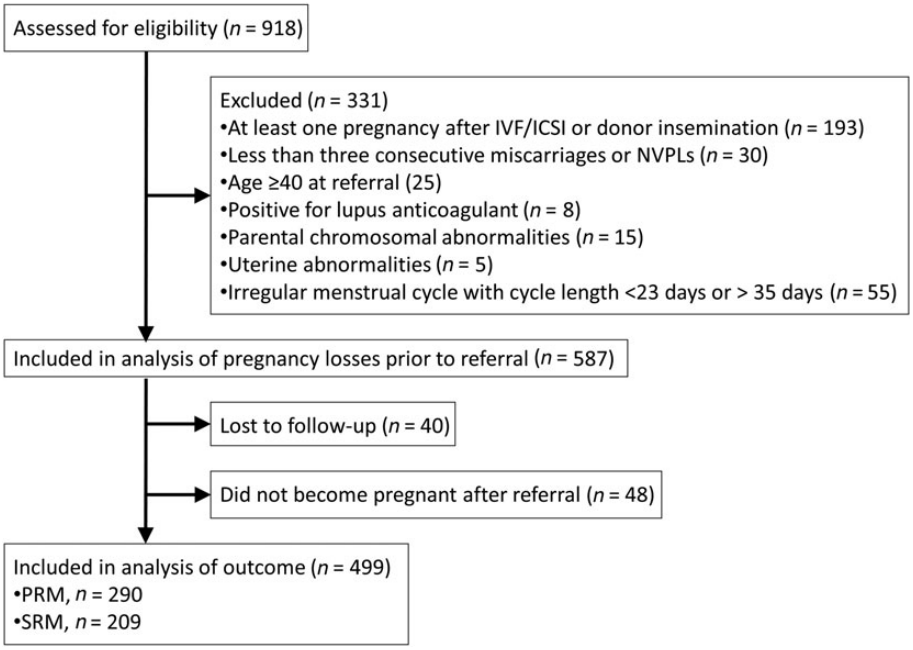

Figure 1 Inclusion of women in the cohort. All women were seen between January 2000 and January 2011. PRM, primary recurrent miscarriage; SRM, secondary recurrent miscarriage; NVPL, non-visualized pregnancy loss.

pregnancy loss, as a positive hCG, but no TVS performed. The two categories 'failed PUL' and 'biochemical pregnancy loss' were combined as 'non-visualized pregnancy losses'. As the study is retrospective, all diagnoses are final.

For 88% of the women in the cohort, we relied on self-reporting and records available at time of referral. However, as a data quality check, we obtained further details on reported non-visualized pregnancy losses for the 61 women born on the first to third of each month.

Statistics

As we have chosen to report our results as relative risk (RR) and the outcome 'live birth' was common (>10%), we used robust Poisson regression instead of standard logistic regression (Deddens and Petersen, 2008). In the Poisson regression analysis we used non-visualized pregnancy losses as the independent variable and corrected for the risk factors PRM versus SRM; age at index pregnancy; the number of prior early and late miscarriages; EPs; and treatment. Equivalent analyses were performed with non-visualized pregnancy losses split into biochemical pregnancy losses and failed PULs. We also used miscarriage as independent variable equivalent to non-visualized pregnancy loss. As standard Poisson regression uses the log-link function, female age in years, early miscarriages, non-visualized pregnancy losses, biochemical pregnancy losses, failed PULs and EPs were modelled as linear variables on the logit scale. Testing for linearity showed no problems for any of the variables. Model control was performed. There were no signs of interaction for any of the variables and thus multiple regression analysis was deemed appropriate. For these analyses the statistical software package STATA 11 was used.

Fisher's exact test was used to test the hypothesis of equal proportions of ectopic pregnancies (EPs) between different groups of patients. *T*-test was used for comparison of gestational age between groups of pregnancy loss. For these analyses we used the statistical software package SAS 19.2.

Results

Reproductive history

Of 2781 pregnancies reported at first consultation, 327 were births after Week 22 (12%). Of the 2454 pregnancy losses, there were 1426 miscarriages before Week 12 (58%), 578 biochemical pregnancy losses (23%) and 334 (16%) failed PULs. Thus non-visualized pregnancy losses constituted 37% of all pregnancy losses before referral in this group. Additionally, there were 73 late miscarriages between Week 12 and 22 (3%) and 43 EPs (2%) (see Fig. 2). All EPs had been treated surgically.

Figure 3 shows the distribution of biochemical pregnancy losses, failed PULs and miscarriages by gestational age. The mean gestational age for biochemical pregnancy losses was 6.08 weeks (95% CI for the mean 5.96; 6.19) and for failed PULs 6.59 (95% CI 6.43; 6.75). The difference is 0.51 weeks (95% CI 0.33; 0.70). The mean gestational age for clinical miscarriages was 8.87 (95% CI 8.74; 9.01), significantly higher than for non-visualized pregnancy losses, mean difference 2.60 weeks (95% CI 2.44; 2.76).

As shown in Fig. 4, women with PRM and no clinical miscarriages had a statistically significantly higher frequency of surgically treated EPs than those with at least one clinical miscarriage (22 versus 6%, difference 16% (95% CI 9.1%; 28.7%), corresponding to an RR for having had an EP of 4.0 (95% CI: 1.92; 8.20) in the former group. We did not confirm the finding for women with SRM.

The women for whom we attempted to obtain further details about prior non-visualized pregnancy losses reported a total of 123 non-visualized pregnancy losses, of which 77 (63%) were biochemical pregnancy losses and 46 (37%) were failed PULs. We were able to confirm the self-reported information in all cases except one; the woman reported a biochemical pregnancy loss, which actually was a miscarriage.

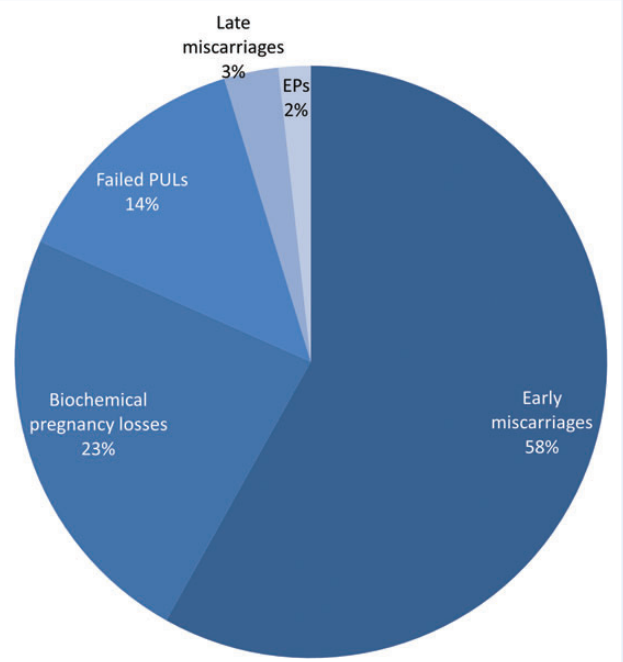

Figure 2 Number and type of pregnancy losses (reproductive history) reported by 587 RM women at first consultation. In addition, 269 births after 22 weeks' gestation were reported. EP: surgically treated ectopic pregnancy; late miscarriage: intrauterine pregnancy loss after 12 weeks' gestation; early miscarriage: histologically or ultrasonically confirmed intrauterine pregnancy loss before 12 weeks' gestation; biochemical pregnancy loss: positive hCG, no ultrasound performed; failed PUL: failed pregnancy of unknown location, positive hCG, but location not established by ultrasound.

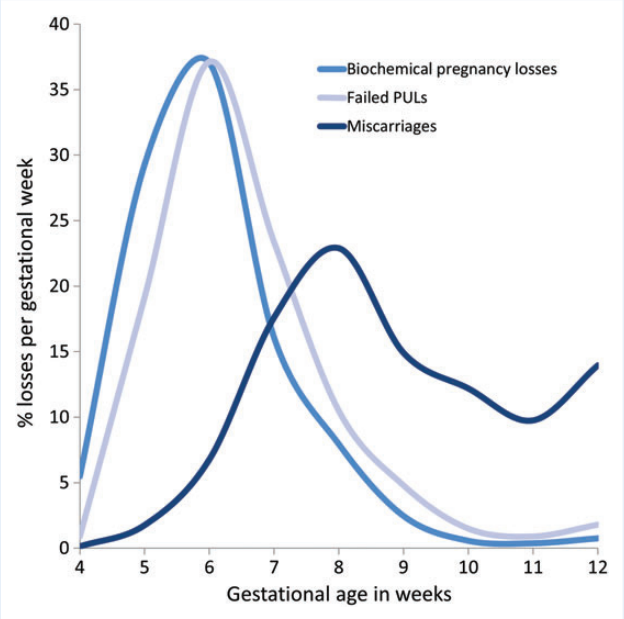

Figure 3 Percentages of biochemical pregnancy losses, failed PULs and miscarriages according to gestational age. PULs, pregnancies of unknown location.

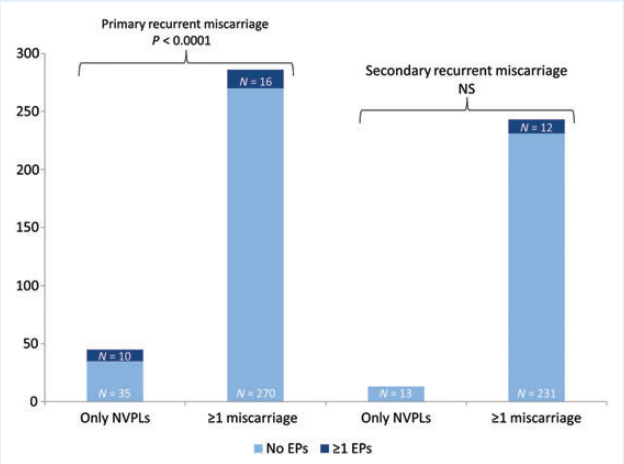

Figure 4 Frequency of a history of surgically treated ectopic pregnancies (EPs) according to presence or absence of confirmed miscarriages in the history, among 587 women; 331 with primary and 256 with secondary recurrent miscarriage. EP: surgically treated ectopic pregnancy: miscarriage, histologically or ultrasonically confirmed intrauterine pregnancy loss before 12 weeks' gestation; NVPLs: non-visualized pregnancy losses; biochemical pregnancy losses and failed pregnancies of unknown location combined.

Thus in 99% of cases, the self-reported information of non-visualized pregnancy losses was confirmed.

Importance of reproductive history on live birth

When analysing all 499 women in the cohort, the RR for live birth for each non-visualized pregnancy loss was 0.90 (95% CI 0.83; 0.97) and for each clinical miscarriage 0.87 (95% CI 0.8; 0.94). For biochemical pregnancy losses the RR for live birth was 0.89 (95% CI 0.82; 0.97) and for failed PULs 0.91 (95% CI 0.82; 1.02). We found no statistically significant difference between the RRs for live birth conferred by each non-visualized pregnancy loss and each miscarriage in any of the analyses.

For women with ≥2 clinical miscarriages, the RR for live birth was 0.89 (95% CI 0.80; 0.98) for non-visualized pregnancy loss and 0.82 (95% CI 0.74; 0.92) for clinical miscarriage and for biochemical pregnancy loss and failed PUL, the RR was 0.88 (95% CI 0.79; 0.98) and 0.89 (0.77; 1.04), respectively.

From Table I we noted that increasing age at first pregnancy after referral was a small, but consistently significant negative prognostic factor in almost all subgroups with RR for live birth ranging from 0.97 to 0.99 for each additional year.

When limiting the Poisson regression analysis to the 312 women (63%) for whom we had registered BMI, there was no significant change in RR for live birth and BMI in itself had no significant effect on outcome, neither as a continuous variable nor as a grouped variable (BMI<20; 20–24; 25–29; ≥30; with BMI 20–24 as reference) (see Table I).

Table I Relative risk (95% CI) of live birth in the index pregnancy, unadjusted for treatment.

Variable	All women	Women with ≥2 clinical miscarriages	Women receiving 'tender loving care'
Pregnancy losses	*N* = 499	*N* = 368	*N* = 344
Age at first pregnancy after referral	0.98 (0.96; 0.99)	0.98 (0.96; 1.00)	0.97 (0.96; 0.99)
Prior miscarriage	0.87 (0.80; 0.94)	0.82 (0.74; 0.92)	0.86 (0.78; 0.96)
Prior non-visualized pregnancy loss[a]	0.90 (0.83; 0.97)	0.89 (0.80; 0.98)	0.90 (0.82; 1.00)
Prior biochemical pregnancy loss	0.89 (0.82; 0.97)	0.88 (0.79; 0.98)	0.92 (0.83; 1.02)
Prior failed pregnancy of unknown location	0.91 (0.82; 1.02)	0.89 (0.77; 0.04)	0.84 (0.71; 0.99)
BMI	*N* = 312	*N* = 228	*N* = 221
BMI ≤20	1.13 (0.90; 1.41)	1.28 (1.00; 1.63)	1.00 (0.77; 1.31)
BMI 21–25	1.00 (reference)	1.00 (reference)	1.00 (reference)
BMI 26–29	1.02 (0.77; 1.35)	1.00 (0.70; 1.43)	0.85 (0.58; 1.24)
BMI ≥30	1.04 (0.81; 1.35)	1.06 (0.79; 1.43)	0.97 (0.70; 1.33)

[a]Non-visualized pregnancy loss: combines biochemical pregnancy losses and failed pregnancies of unknown location.

We analysed the 290 PRM and 209 SRM women separately and the RR did not change significantly, but as expected, the confidence intervals widened due to smaller numbers in each subgroup. Data on PRM versus SRM women are available online as Supplementary data, Table SI.

Treatment

In an analysis of RR for live birth by non-visualized pregnancy loss, clinical miscarriage and biochemical pregnancy loss and failed PULs among the 344 women who received only TLC we found comparable results as for the total group of 499 patients, as can be seen in Table I.

Ninety-eight women received IVIg in addition to TLC and had an RR for live birth of 1.27 (95% CI 1.07; 1.52) compared with TLC alone, and the RR of IVIg for live birth for patients with PRM was 1.39 (1.10; 1.76). Adjustment for treatment did not significantly alter the effect of non-visualized pregnancy losses, miscarriages, biochemical pregnancy losses and failed PULs on the RR for live birth (see Table II).

Discussion

Non-visualized pregnancy losses represent a significant proportion of the pregnancy losses experienced by women referred to the Danish RM clinic. We have demonstrated that non-visualized pregnancy losses and miscarriages both have a negative prognostic influence on the chance for live birth in the first pregnancy after referral among women with unexplained RM. The number of clinical miscarriages before referral has been reported to be an important determinant for RM women's prognosis for live birth (Brigham et al., 1999; Lund et al., 2012). To our knowledge, this is the first investigation of the prevalence and prognostic significance of non-visualized pregnancy losses in women with RM.

Presently, non-visualized pregnancy losses in the history of women with RM are largely ignored by gynaecologists and general practitioners. The finding that non-visualized pregnancy losses and early miscarriages have a similar negative effect on RR for live birth is thus very important. At least for women with RM, our findings support the assumption that the majority of failed PULs are early intrauterine miscarriages (Kirk et al., 2009).

When interpreting our results it is important to note that the definition of RM applied in the study was three or more consecutive early pregnancy losses. Even though our calculations were based on a linear model on the logit scale, we are unable to project the results to sporadic pregnancy losses or other definitions of RM, e.g. two consecutive or non-consecutive early pregnancy losses. We did show a statistically significant reduction in relative risk for live birth also for women who fulfil the ASRM definition of recurrent pregnancy loss, i.e. ≥2 clinical miscarriages. The findings in this study should prompt further inquiry into an evidence-based definition of RM.

As the cohort only included women with unexplained RM, our results may not apply to other groups of patients, such as patients with chromosomal abnormalities, irregular menstrual cycles and patients with RM after IVF/ICSI.

To our knowledge, there are no data regarding the cost-effectiveness of RM evaluation or treatment if non-visualized pregnancy losses are included in the definition, although this is already clinical practice in several European countries such as Great Britain and Denmark. As resources in clinical care are limited, this would be a logical next step by health care economists and relevant policy makers.

Gestational age

The exclusion of non-visualized pregnancy losses in RM definitions is probably based on reports that a transiently positive pregnancy test at the time of menstrual period is a common finding in normal women (Wilcox et al., 1987). It is therefore noteworthy that the mean gestational age at time of diagnosis of both biochemical pregnancy losses and failed PULs in this study was ~6 weeks.

The gestational age for non-visualized pregnancy losses is based on last menstrual period and may therefore be unreliable. However, as all women in the cohort had regular menstrual cycles with a variation of no more than 2–3 days for each individual woman, we assume that the estimate of gestational age in prior pregnancies is reasonably

Table II Relative risk (95% CI) of live birth in the index pregnancy, adjusted for treatment.

Variable	*N*	All women	*N*	Women with ≥2 clinical miscarriages
All women				
Age at first pregnancy after referral	499	0.98 (0.96; 0.99)	368	0.98 (0.96; 1.00)
Prior miscarriage		0.86 (0.79; 0.93)		0.81 (0.72; 0.91)
Prior non-visualized pregnancy loss[a]		0.89 (0.82; 0.96)		0.88 (0.79; 0.97)
Prior biochemical pregnancy loss		0.88 (0.81; 0.97)		0.88 (0.79; 0.98)
Prior failed pregnancy of unknown location		0.90 (0.80; 1.00)		0.87 (0.74; 1.02)
'Tender loving care'	344	1 (reference)	243	1.00 (reference)
'Tender loving care' and IVIg alone	98	1.28 (1.07; 1.52)	77	1.36 (1.11; 1.67)
'Tender loving care', IVIg and other	12[b]	1.17 (0.73; 1.87)	10[c]	1.43 (0.99; 2.07)
Other	45[d]	0.87 (0.62; 1.21)	38[e]	0.90 (0.60; 1.33)
Primary recurrent miscarriage				
Age at first pregnancy after referral	290	0.98 (0.96; 1.00)	208	0.99 (0.97; 1.01)
Prior miscarriage		0.88 (0.78; 0.99)		0.79 (0.64; 0.97)
Prior non-visualized pregnancy loss		0.91 (0.82; 1.02)		0.88 (0.75; 1.04)
Prior biochemical pregnancy loss		0.90 (0.79; 1.02)		0.84 (0.70; 1.02)
Prior failed pregnancy of unknown location		0.96 (0.86; 1.08)		0.95 (0.78; 1.17)
'Tender loving care'	224	1 (reference)	159	1.00 (reference)
IVIg alone	45	1.39 (1.10; 1.76)	34	1.50 (1.15; 1.96)
IVIg and other	10[f]	1.40 (0.93; 2.12)	8[g]	1.76 (1.35; 2.31)
Other	11[h]	0.83 (0.43; 1.60)	7[i]	0.89 (0.39; 2.03)
Secondary recurrent miscarriage				
Age at first pregnancy after referral	209	0.97 (0.95; 1.00)	160	0.97 (0.94; 1.01)
Prior miscarriage		0.84 (0.75; 0.94)		0.82 (0.72; 0.94)
Prior non-visualized pregnancy loss		0.86 (0.76; 0.97)		0.86 (0.75; 0.99)
Prior biochemical pregnancy loss		0.89 (0.79; 1.01)		0.91 (0.79; 1.04)
Prior failed pregnancy of unknown location		0.79 (0.65; 0.97)		0.77 (0.57; 1.04)
'Tender loving care'	120	1 (reference)	84	1.00 (reference)
IVIg alone	53	1.15 (0.89; 1.48)	43	1.18 (0.87; 1.60)
IVIg and other	2[j]	0 (0.00; 0.00)	2[k]	0.00 (0.00; 0.00)
Other	34[l]	0.85 (0.57; 1.28)	31[m]	0.84 (0.53; 1.33)

IVIg, intravenous immunoglobulin.
[a]Non-visualized pregnancy loss: combines biochemical pregnancy losses and failed pregnancy of unkown location (PUL).
[b]Includes: IVIg+progesterone ($n = 3$); IVIg+heparin ($n = 1$); IVIg+donor lymphocytes ($n = 1$); IVIg+prednisone ($n = 6$); and IVIg+prednisone+progesterone ($n = 1$).
[c]Includes: IVIg+lymphocytes ($n = 1$); IVIg+prednisone ($n = 5$); IVIg+progesterone ($n = 3$); and IVIg+progesterone+prednisone ($n = 1$).
[d]Includes: heparin ($n = 5$), donor lymphocytes ($n = 4$), prednisone ($n = 2$), progesterone ($n = 7$) and participants in a double blind randomized controlled trial of IVIg versus placebo ($n = 27$).
[e]Includes: donor lymphocyte ($n = 4$); progesterone ($n = 5$), heparin ($n = 4$) and participants in a double blinded randomized trial of IVIg versus placebo ($n = 25$).
[f]Includes: IVIg+donor lymphocytes ($n = 1$); IVIg+heparin ($n = 1$); IVIg+prednisone ($n = 6$); IVIg+prednisone+progesterone ($n = 1$); and IVIg+progesterone ($n = 1$).
[g]Includes: IVIg+donor lymphocytes ($n = 1$); IVIg+prednisone ($n = 5$); IVIg+progesterone ($n = 1$); and IVIg+progesterone+prednisone ($n = 1$).
[h]Includes: donor lymphocytes ($n = 4$); prednisone ($n = 2$); heparin ($n = 3$) and progesterone ($n = 2$);
[i]Includes: donor lymphocytes ($n = 4$); progesterone ($n = 1$); and heparin ($n = 2$).
[j]Includes: IVIg+progesterone ($n = 2$).
[k]Includes IVIg+progesterone ($n = 2$)
[l]Includes: heparin ($n = 2$), progesterone ($n = 5$) and participants in double blind randomized controlled trial of IVIg versus placebo ($n = 27$).
[m]Includes progesterone ($n = 4$), heparin ($n = 2$) and participants in a double blinded randomized trial of IVIg versus placebo ($n = 25$).

accurate. This is substantiated by our data validation where the consistency between patient files and information given at first consultation was 99%.

Life style factors

High BMI has been reported to be prognostically negative in RM (Lashen et al., 2004; Lo et al., 2012). In these studies the authors do not distinguish between women with regular and irregular menstrual cycles. In our study, we did not demonstrate a negative impact of high BMI on the chance of live birth.

Increased maternal age decreases the RR for live birth in the first pregnancy after referral. The RR described in this study is for each additional year, and as such aligns well with previously published studies (Brigham et al., 1999; Lund et al., 2012).

Aetiology of non-visualized pregnancy losses

It is probable that some of the non-visualized pregnancy losses are due to chromosome anomalies, as documented by several studies (Wilcox et al., 1987; Zinaman et al., 1996; Wang et al., 2003) and reviewed by Macklon et al. (2002). On the other hand, more of the non-visualized pregnancy losses would have been classified as miscarriages if the women had been monitored as extensively in their first pregnancies as they are in the Danish RM Unit, where all patients are followed with ultrasound from 6 weeks' gestation.

We found that the frequency of surgically treated EPs in PRM women's reproductive history was significantly higher if they had no clinical miscarriages in their reproductive history compared with those with at least one confirmed intrauterine pregnancy loss. Therefore, we propose that at least some of their non-visualized pregnancy losses may be spontaneously resolved EPs.

Conclusions

To our knowledge, this is the first study to report on the occurrence and impact of non-visualized pregnancy losses in women with unexplained RM after spontaneous conception and ≥ 3 consecutive pregnancy losses.

We demonstrate that non-visualized pregnancy losses are frequent. Each additional non-visualized pregnancy loss decreases the RR for live birth with ~10%, which is the same impact conferred by a clinical miscarriage. For women with at least two clinical miscarriages the RR for live birth is decreased with almost 10% by each non-visualized pregnancy loss and by almost 20% for each clinical miscarriage. The data and results support the inclusion of non-visualized pregnancy losses in definitions of RM. Further studies are needed to confirm or refute our findings.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Acknowledgements

We thank biostatistician Helle Højmark Eriksen, Department of Hospital Affairs, Aalborg University Hospital, Aalborg, Denmark for statistical analysis in STATA.

Authors' roles

A.M.K. collected data, entered data in a database, did quality control, performed statistical analyses in SAS 9.2 and wrote the paper. R.H.v.O., S.Q., R.G.F., M.S. and M.G. contributed to interpretation of data and critically revised the manuscript. O.B.C. initiated the study, participated in data collection and contributed to interpretation of data and critically revised the manuscript. All co-authors approved the final manuscript before submission.

Funding

No specific funding was sought for the study.

Conflict of interest

The authors declare that they have no conflicts of interest.

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