Human Reproduction, Vol.31, No.11 pp. 2428–2434, 2016
Advanced Access publication on September 2, 2016 doi:10.1093/humrep/dew169

human reproduction

ORIGINAL ARTICLE ***Early pregnancy***

Recurrent pregnancy loss: what is the impact of consecutive versus non-consecutive losses?

P. Egerup[1], A.M. Kolte[1], E.C. Larsen[1], M. Krog[1], H.S. Nielsen[1], and O.B. Christiansen[1,2,3,*]

[1]Recurrent Pregnancy Loss Unit, Fertility Clinic 4071, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen, Denmark [2]Department of Obstetrics and Gynaecology, Aalborg University Hospital, Reberbansgade, DK-9000 Aalborg, Denmark [3]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

*Correspondence address. E-mail: olbc@rn.dk

Submitted on April 11, 2016; resubmitted on May 31, 2016; accepted on June 8, 2016

STUDY QUESTION: Is there a different prognostic impact for consecutive and non-consecutive early pregnancy losses in women with secondary recurrent pregnancy loss (RPL)?

SUMMARY ANSWER: Only consecutive early pregnancy losses after the last birth have a statistically significant negative prognostic impact in women with secondary RPL.

WHAT IS KNOWN ALREADY: The risk of a new pregnancy loss increases with the number of previous pregnancy losses in patients with RPL. Second trimester losses seem to exhibit a stronger negative impact than early losses. It is unknown whether the sequence of pregnancy losses plays a role for the prognosis in patients with a prior birth.

STUDY DESIGN, SIZE, DURATION: This retrospective cohort study of pregnancy outcome in patients with unexplained secondary RPL included in three previously published, Danish double-blinded placebo-controlled trials of intravenous immunoglobulin (Ivlg) conducted from 1991 to 2014. No other treatments were given. Patients with documented explained pregnancy losses (ectopic pregnancies and aneuploid miscarriages) were excluded.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Of the 168 patients included in the trials, 127 had secondary RPL and experienced a subsequent live birth or unexplained pregnancy loss in the first pregnancy after giving informed consent to participate in the trials (the index pregnancy). Data analyzed by multivariate analysis included the independent variables age, the number of early pregnancy losses before and after the last birth, respectively and a second trimester pregnancy loss before or after the last birth, respectively. The outcome variable was unexplained loss in the index pregnancy.

MAIN RESULTS AND THE ROLE OF CHANCE: In patients with secondary RPL, both a late and each early loss before the last birth did not significantly influence the risk of a new pregnancy loss in the index pregnancy: incidence rate ratio (IRR) 1.31 (95% CI 0.62–2.77) and IRR 0.88 (95% CI 0.70–1.11), respectively. In contrast, the impact on risk of pregnancy loss conferred by a late and by each early pregnancy loss occurring after the birth was significant: IRR 2.15 (95% CI 1.57–2.94, $P < 0.0001$) and IRR 1.14 (95% CI 1.04–1.24, $P = 0.002$), respectively.

LIMITATIONS, REASONS FOR CAUTION: Of the patients, 48% were treated with Ivlg, which could influence the results. However, allocation to Ivlg was random and prognostic variables were equally distributed in Ivlg and placebo-treated patients.

WIDER IMPLICATIONS OF THE FINDINGS: A birth in women with secondary RPL eradicates the negative prognostic impact of previous pregnancy losses and this finding is important for our understanding of the pathogenesis. It indicates that only consecutive pregnancy losses should count in the definition of RPL.

STUDY FUNDING/COMPETING INTERESTS: There was no particular funding for this study. The authors declare that there is no conflict of interest.

TRIAL REGISTRATION NUMBER: Not applicable for two of the included randomized controlled trials. For the last trial: Clinical.Gov NCT00722475.

© The Author 2016. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

Key words: recurrent pregnancy loss / recurrent miscarriage / secondary recurrent pregnancy loss / cohort study / randomized controlled trial

Introduction

Recurrent pregnancy loss (RPL) is defined by ESHRE as three or more consecutive pregnancy losses and affects 1–3% of women attempting to have a child. Although some risk factors for RPL are known, such as high levels of antiphospholipid antibodies, parental chromosome aberrations and some uterine anatomical abnormalities, the vast majority of cases remain unexplained.

One observation regarding RPL has been consistently made: the risk of future pregnancy loss increases with the number of prior pregnancy losses (Clifford *et al.*, 1997; Brigham *et al.*, 1999; Kolte *et al.*, 2014).

The explanation for this association is unclear. With an increased number of pregnancy losses, the age of the woman increases; however, the number of previous losses is also a strong determinant of subsequent pregnancy outcome after adjustment for maternal age (Lund *et al.*, 2010; Kolte *et al.*, 2014).

Pregnancy loss in the second trimester (≥14 gestational week) is rare. Drakeley *et al.* (1998) reported that 25% of patients with RPL had experienced at least late pregnancy loss, but patients with late losses might have been selectively included. Kolte *et al.* (2014) reported a 3% rate of prior late pregnancy losses in patients admitted with RPL. A mid-trimester pregnancy loss seems associated with a subsequent poor pregnancy prognosis (Strobino *et al.*, 1986; Cowshock *et al.*, 1990).

For many years, the definition of RPL has been two or three consecutive pregnancy losses. However, some research groups and scientific societies now advocate for defining RPL as two or three not necessarily consecutive miscarriages (van den Boogaard *et al.*, 2013; Practice Committee of the ASRM, 2013), a definition used in a recent large randomized placebo-controlled trial (RCT) (Coomarasamy *et al.*, 2015). No study has so far investigated whether the sequence of pregnancy losses plays a role for the prognosis for live birth; that is, whether a specific number of pregnancy losses distributed before and after a birth displays the same prognostic impact as the same number of consecutive pregnancy losses after a birth. Such knowledge is important for adequate allocation of patients to different treatment arms in RCTs and for providing advice in clinical practice.

In the present study, the main objective was to examine the prognostic impact of pregnancy losses happening before or after a birth in patients with RPL, after adjustment for relevant variables. We did this in a cohort of RPL patients included in three previously published RCTs of intravenous immunoglobulin (Ivlg).

Materials and Methods

Included randomized controlled trials

Patients who participated in three Danish RCTs of treatment for RPL with Ivlg were included in this study (Christiansen *et al.*, 1995, 2002, 2015). Information about the RCTs is provided in Supplementary Table SI. Patients were included in the RCTs after written informed consent. The RCTs were approved by the local ethics committee and by the Danish National Board of Health and the most recent one was registered with ClinicalTrials.gov. no. NCT00722475. The trials were conducted according to the regulations of good clinical practice (GCP) for trials on medical products and monitored by external CGP monitors.

Patients were classified into two main groups: (i) primary RPL with losses prior to gestational week 22 only and no live birth or stillbirth and (ii) secondary RPL with at least one pregnancy progressing to ≥22 weeks resulting in a live birth or stillbirth, followed by ≥3 consecutive pregnancy losses prior to Week 22. A few patients with ≥3 pregnancy losses and who could not fit into any of these groups were included as unclassified RPL.

Pregnancy losses were defined as clinical miscarriages confirmed by ultrasound and/or histology until gestational week 22 and biochemical pregnancies and pregnancies of unknown location (PULs). Both biochemical pregnancies and PULs had been confirmed by a positive urine- or plasma-hCG measurement documented in records of the hospitals or practitioners. In concordance with an ESHRE consensus statement (Kolte *et al.*, 2015), we use the joint terminology non-visualized pregnancy losses (NVPL) including both biochemical pregnancies and PULs.

In the first two RCTs, patients were included and randomized to Ivlg or placebo as soon as a urine- or plasma-hCG was positive, whereas in the third RCT (Christiansen *et al.*, 2015) patients were included only when two or more plasma-hCG measurements revealed a minimum 30% increase per 24 h. In six cases, plasma-hCG was not adequately increasing, no treatment was provided and, in each case, the pregnancy ended as an NVPL. These patients were included in the RCT after a subsequent conception. In the present cohort study, the focus was on first pregnancy (called the index pregnancy) after the patient had signed the informed consent document for participation in the RCT. The pregnancies included in the publications of the RCTs were identical to the index pregnancies of the present study for the two first RCTs (Christiansen *et al.*, 1995, 2002) and in the third RCT (Christiansen *et al.*, 2015); they were identical to the index pregnancies except for the six cases described above. The patients could participate only once in an RCT and therefore each patient contributed with only one index pregnancy in the study.

In all three RCTs, the patients had: normal uterine anatomy assessed by hysteroscopy, hysterosalpingography or uterine sonography, regular menstrual cycles with minimum 21 and maximum 35 days intervals, and normal screening for hypo- or hyperthyroidism. The couples all had normal karyotypes. In two of the RCTs, patients with antiphospholipid antibodies were excluded. In one RCT (Christiansen *et al.*, 2002), three patients were positive for lupus anticoagulant and/or anticardiolipin antibodies according to the international consensus criteria.

In each of the RCTs, no other medicine that could potentially interfere with pregnancy outcome such as progesterone, estrogen, prednisone, heparin or low-dose aspirin was allowed.

In the combined cohort of 174 patients, three patients had tubal pregnancies requiring surgery. Karyotyping of miscarried fetuses was successful in 24 cases. One trisomy and two triploidies were detected; the remaining showed a normal fetal karyotype. The patients with documented ectopic pregnancies or karyotypically abnormal embryos were excluded from further analysis.

Information about prior pregnancy outcome in the 168 patients in the combined cohort is provided in Table I. Only late losses of fetuses with normal autopsy and no suspicion of chromosomal abnormalities were included. Of the 127 secondary RPL patients, three had consecutive pregnancy losses after an unexplained stillbirth but no losses before; the remaining 124 secondary RPL patients had consecutive pregnancy losses after a live birth and 45 of them also had losses before the birth.

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

Table I Reproductive outcome in the study cohort of patients with recurrent pregnancy loss (RPL) before the index pregnancy.

	All patients ($n = 168$)		
Patients with one live birth	108		
Patients with two live births	13		
Patients with 3–4 births	3		
Patients with a stillbirth	14		
Patients with late losses	33		
Patients with only first trimester losses	135		
		Secondary RPL ($n = 127$)	
		Before last birth	After last birth
Patients with late loss (range)		3 (1)	15 (1–3)
Patients with first trimester loss (range)		45 (1–3)	127 (1–8)
Type of first trimester pregnancy losses			
Non-visualized losses (%) ($n = 138$)		15 (10.9)	123 (89.1)
Clinical losses (%) ($n = 454$)		52 (11.5)	402 (88.5)
All (%) ($n = 592$)		67 (11.3)	525 (88.7)

Long-term prognosis

The long-term reproductive prognosis in patients who had an unexplained pregnancy loss in the index pregnancy while participating in the first two RCTs was evaluated using data from the Danish National Birth Register in 2010 as part of a previous study (Lund *et al.*, 2012). The patients in the two first RCTs had at least 10 years of follow-up after their participation. Patients participating in the third RCT (Christiansen *et al.*, 2015) were contacted by e-mail or telephone in 2015. Those who had had a pregnancy loss while participating in the RCT were asked whether they subsequently had achieved pregnancy and about the outcome. With regard to the third RCT, we included only patients who were randomized until January 2011 in the follow-up study. Thus, all patients had a follow-up period of at least 5 years.

Statistics

Data were analyzed in the statistical software package Stata II. Univariate analyses were performed by χ^2 tests and multivariate analyses were done by Poisson regression with a robust error variance calculating incidence rate ratios (IRRs). Robust Poisson regression provides more conservative and robust estimates of relative risks than logistic regression when outcomes are binary (Zou, 2004). In the multivariate analysis, in all 168 patients, the following independent variables were included: (i) age at the time of index pregnancy, (ii) number of early pregnancy losses, (iii) presence of a late pregnancy loss, (iv) number of live births and (v) presence of stillbirth. In the multivariate analysis of 127 patients with secondary RPL, the following independent variables were included: (i) age at the time of index pregnancy, (ii) number of early pregnancy losses before and after the last birth, respectively; (iii) presence of a late pregnancy loss before or after the last birth, respectively; (iv) number of live births; (v) presence of stillbirth; (vi) female sex of the last child and (vii) whether the partner fathering the last three pregnancy losses was not the father of the last child. The dependent (outcome) variable was in both groups live birth/unexplained pregnancy loss in the index pregnancy.

Results

Overall in the 168 index pregnancies, 80 patients gave birth to live born children, 67 had a clinical pregnancy loss verified by ultrasound and/or histology of tissue obtained by uterine evacuation and 21 had an NVPL verified by repeated plasma-hCG measurements peaking at median 276 ie (range 19–1214 ie) (Table II). Information about treatment is also provided in Table II.

Eight of the clinical miscarriages occurred between gestational week 14 and 22. Two patients experienced stillbirth between Weeks 23 and 27. The clinical picture associated with these late losses was intrauterine fetal death in six cases, rupture of membranes and cervical dilatation in three cases and labor and bleeding in one case. In autopsies of all the cases and in the four karyotyped fetuses, no abnormality was revealed. All 10 late losses occurred in patients with at least one previous second trimester loss. However, among patients with a previous late loss, 20 (83%) had a subsequent first trimester pregnancy loss and only four (17%) had a live birth.

In a univariate analysis, the risk of loss in the index pregnancy increased significantly with the presence of a prior late loss ($P < 0.001$) (Table II).

In the multivariate analysis including all 168 index pregnancies (Table III), each early pregnancy loss and especially the presence of a prior late loss were significant predictors of a new pregnancy loss.

In a univariate analysis of the impact of variables in 127 secondary RPL patients, the strongest predictor of pregnancy loss was again the presence of a late miscarriage in the patient's history (Table IV).

Table Va shows the results of the first multivariate analysis on variables of importance for outcome in patients with secondary RPL. The presence of a late pregnancy loss was the strongest predictor of a poor outcome: IRR for new pregnancy loss 1.65; 95% CI 1.28–2.12. Each early loss also increased the risk of a new pregnancy loss significantly: IRR 1.11; 95% CI 1.02–1.21.

In Table Vb, the analyses were repeated, but now prior early and late pregnancy losses were separated according to whether they happened before or after the last birth. Both late and early losses happening before the last birth seemed not to impact the risk of a subsequent pregnancy loss: IRR 1.31 (95% CI 0.62–2.77) and IRR 0.88 (95% CI 0.70–1.11), whereas the negative prognostic impact of late and early pregnancy losses happening after the birth was statistically significant: IRR 2.15 (95% CI 1.57–2.94) and IRR 1.14 (95% CI 1.04–1.24), respectively. The IRRs of subsequent pregnancy loss of patients with prior late or early losses occurring before a birth were significantly different from the corresponding IRRs for losses happening after a birth ($P < 0.05$).

In a multivariate analysis similar to that given in Table Vb but also including IvIg treatment as an independent variable, the main results did not change: a significant negative impact of both early and late losses after a birth and no impact of losses prior to the birth.

In the long-term follow-up, patients with loss prior to gestational week 14 in the index pregnancy, 22 (38.5%) had had a live birth after at least 5 years of follow-up. In patients with a miscarriage ≥ week 14, four (50.0%)

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

Table II Univariate analysis of the impact of demographic and clinical variables on the risk of a new pregnancy loss in the first pregnancy after accepting participation in one of the three placebo-controlled trials of IvIg in 168 recurrent pregnancy loss patients.

Variable		Subsequent pregnancy outcome		
		Pregnancy loss	Birth	*P*
Age	≤33 years	51 (53.7%)	44 (46.3%)	0.15
	>34 years	37 (50.7%)	36 (49.3%)	
Total number of previous early pregnancy losses	2–4[1]	42 (46.7%)	48 (53.3%)	0.30
	5–6	29 (54.7%)	24 (45.3%)	
	≥7 (7–10)	10 (66.7%)	5 (33.3%)	
Previous late pregnancy loss	0	62 (45.9%)	73 (54.1%)	0.0007
	≥1	26 (78.8%)	7 (21.2%)	
Previous live birth	0	22 (51.2%)	21 (48.8%)	0.58
	1	59 (54.6%)	49 (45.4%)	
	≥2	7 (41.2%)	10 (58.8%)	
Previous stillbirth	0	79 (51.3%)	75 (48.7%)	0.35
	1	9 (64.3%)	5 (35.7%)	
Treatment	Intravenous immunoglobulin	38 (47.5%)	42 (52.5%)	0.043
	Placebo	44 (53.7%)	38 (46.3%)	
	No treatment	6 (100.0%)	0	

[1]Ten patients had no or only one previous first trimester loss.

Table III Multivariate analysis of the impact (incidence rate ratio = IRR) of demographic and clinical variables on the risk of new pregnancy loss in the first pregnancy after accepting participation in three placebo-controlled trials of IvIg in 168 women with recurrent pregnancy loss.

Variable	IRR	95% CI	*P*
Age	0.99	0.96–1.03	0.99
Each early pregnancy loss	1.11	1.02–1.20	0.013
Late pregnancy loss	1.46	1.23–1.74	<0.001
Previous live birth			
0	1		
1	1.00	0.58–1.73	1.00
≥2	0.77	0.34–1.75	0.53
Stillbirth	1.19	0.74–1.92	0.47

had given birth in the follow-up period. Overall, the live birth rate after a minimum of 5 years was 40.0%

Discussion

This is, to the best of our knowledge, the first study to present the prognostic impact of prior early and late pregnancy losses in women with secondary RPL, distinguishing between losses that occurred before or after the last live birth or stillbirth. In a multivariate analysis, we confirmed previous findings that higher numbers of early losses significantly increased the risk of a new loss, with each prior early loss increasing the risk of new loss by 11% (Tables III and Va). We confirmed the large negative impact of a second trimester loss on subsequent pregnancy outcome in RPL patients and the negative impact was found to be substantially higher than that associated with early pregnancy loss: IRR 1.46–1.65 for late loss compared with IRR 1.11 for each early loss.

A new finding was that the impact of both late and early losses happening prior to the last birth in women with secondary RPL was weak or absent and significantly lower than the negative prognostic impact conferred by a late (IRR 2.15) or early pregnancy loss (IRR 1.14) happening after the last birth (Table Vb). Since almost all secondary RPL patients (98%) had had a prior live birth, the results are actually representative for patients with secondary RPL after a live birth.

In our study, maternal age was not a determinant of new pregnancy loss; this can seem at odds with other studies (Nybo Andersen *et al.*, 2000; Kolte *et al.*, 2014). The most likely explanation is that the age distribution was truncated at 41 years. In RPL patients, Li *et al.* (2002) found completely similar live birth rates after referral in age groups below 40 years and only after 41 years did the subsequent birth rate drop substantially.

Although treatment with IvIg tended to improve outcome in the crude analyses (Tables II and IV), it was not feasible to make adjustment for IvIg treatment in the multivariate analysis. As mentioned previously, in our third RCT there was a protocol-defined linkage between no IvIg treatment in the index pregnancy and a high risk of pregnancy loss; therefore, adjustment for IvIg treatment would substantially overestimate its effect. However, including IvIg treatment as an independent variable in the analysis would generate results very similar to those given in Table Vb.

The multivariate analysis was performed in a cohort of RPL patients derived from three previously published RCTs of IvIg. This limits the number of patients analyzed and it may be argued that they are not representative for the whole RPL population. The strengths of focusing on the patients from the RCTs are numerous: (i) the patients were predefined by the previous participation in RCTs and were thus randomly selected for the present study; (ii) the patients all met criteria, which

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

Table IV Univariate analysis of the impact of demographic and clinical variables on the risk of new pregnancy loss in the first pregnancy after accepting participation in one of three placebo-controlled trials of intravenous immunoglobulin (Ivlg) in 127 patients with secondary recurrent pregnancy loss.

Variable		Pregnancy loss (%)	Birth (%)	P
Age	≤33 years	36 (57.1)	27 (42.9)	0.33
	>34 years	31 (48.4)	33 (51.6)	
Total number of early pregnancy losses	3–4	34 (48.6)	36 (51.4)	0.37
	5–6	24 (54.5)	20 (45.5)	
	≥7 (7–10)	9 (69.2)	4 (30.8)	
No. of early pregnancy losses after birth	2–4	42 (45.7)	50 (54.3)	0.20
	5–6	18 (66.7)	9 (33.2)	
	≥7 (7–10)	7 (87.5)	1 (12.5)	
Late pregnancy loss	0	51 (46.8)	58 (53.2)	0.0009
	≥1	16 (88.9)	2 (11.1)	
Sex of last child	Male	35 (50.0)	35 (50.0)	0.49
	Female	32 (56.1)	25 (43.9)	
Treatment	Ivlg	26 (42.6)	35 (57.4)	0.013
	Placebo	35 (58.3)	25 (41.7)	
	No treatment	6 (100.0)	0 (0.0)	

Table V Multivariate analysis of the impact (incidence rate ratio = IRR) of demographic and clinical variables on the risk of new pregnancy loss in the first pregnancy after accepting participation in three placebo-controlled trials of intravenous immunoglobulin in 127 women with secondary recurrent pregnancy loss.

Variable	IRR	95% CI	P
(a) Analysis without separate analysis of pregnancy losses before and after birth			
Age	0.99	0.96–1.03	0.59
Each early pregnancy loss	1.11	1.02–1.21	0.018
Presence of late pregnancy loss	1.65	1.28–2.12	<0.001
Live birth			
0	1		
1	0.79	0.33–1.93	0.61
≥2	0.61	0.20–1.82	0.37
Stillbirth	0.96	0.44–2.08	0.91
Female sex of last child	1.16	0.83–1.63	0.39
New partner	1.03	0.68–1.55	0.90
(b) Analysis with separation of pregnancy losses before and after birth			
Age	0.99	0.96–1.03	0.71
Each early pregnancy loss before birth	0.88	0.70–1.11	0.29
Each early pregnancy loss after birth	1.14	1.04–1.24	0.002
Presence of late pregnancy loss before birth	1.31	0.62–2.77	0.48
Presence of late pregnancy loss after birth	2.15	1.57–2.94	<0.0001
Live birth			
0	1		
1	0.90	0.39–2.06	0.80
≥2	0.76	0.26–2.34	0.62
Stillbirth	0.88	0.45–1.71	0.70
Female sex of last child	1.23	0.89–1.70	0.22
New partner	1.03	0.68–1.55	0.90

were pre-defined in the protocols of the RCTs that were externally monitored according to the GCP rules; (iii) detailed data about each patient's prior and subsequent pregnancy outcomes have been kept in the trial databases; (iv) the participants in the RCTs received only one of two interventions during pregnancy: Ivlg or placebo (albumin) and no other treatments that may influence miscarriage risk were provided; (v) after signing informed consent for participation in the RCTs, all patients were urged to contact the clinic as soon as the pregnancy test was positive and therefore all first subsequent pregnancies were included. The live birth rate in untreated RPL patients included in RCTs is 15–20% lower than in patients not included in RCTs, a reduction, which can probably be attributed to a more complete ascertainment of pregnancies in RCTs (Carp *et al.*, 1997). The quality of data may also be better in randomized than in nonrandomized studies (Ioannidis *et al.*, 2001).

The finding that a previous second trimester pregnancy loss has a negative impact on subsequent pregnancy outcome is not new (Strobino *et al.*, 1986; Cowshock *et al.*, 1990). However, no study has investigated the impact of prior second trimester losses on the risk of subsequent early and late pregnancy loss in RPL patients. Although a prior late loss was strongly associated with the risk of late miscarriage in the index pregnancy, it was also strongly associated with a subsequent early pregnancy loss.

How can we interpret the findings? Women with late pregnancy losses more often than controls carry biomarkers for thrombophilia (Robertson *et al.*, 2006) or increased inflammatory responses (Christiansen *et al.*, 2009; Sundtoft *et al.*, 2016) and have a highly increased risk of later atherosclerotic disease (Ranthe *et al.*, 2013). A late pregnancy loss is an ominous sign indicating that the woman might be in a chronic pro-inflammatory and/or pro-thrombophilic state, which may increase the risk of embryonal/fetal death in all stages of pregnancy.

We have advocated for the opinion that in RPL patients, the chance of subsequent live birth after a defined time range is more relevant than just looking at outcome in the first pregnancy after referral (Lund *et al.*, 2012). This study showed that in the overall RPL population, 67% had achieved a live birth 5 years after referral. The 5-year live birth rate of 40% in patients with a loss in the index pregnancy is much lower and emphasizes that they comprise a subgroup with a poor prognosis where focusing on prognostic factors is clinically relevant.

A new finding was that among patients with secondary RPL only the losses after the last birth displayed a negative prognostic impact. A (live) birth thus seems to eradicate the negative prognostic influence of pregnancy losses prior to the birth. There can be several explanations for this observation, but it can in our view best be explained in immunological terms. So-called regulatory T lymphocytes (Tregs) in periuterine lymph nodes suppressing T lymphocyte reactions against antigens on the fetus are of crucial importance for the survival of murine pregnancy (Chen *et al.*, 2013) and may also play an important role in human RPL (Kwiatek *et al.*, 2015). It is possible that some RPL patients lack these

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

tolerance-inducing Tregs in uterine lymph nodes, but a successful pregnancy can re-establish the Treg population and induce tolerance to subsequent pregnancies.

In conclusion, a birth seems to eradicate the negative prognostic impact of previous pregnancy losses in unexplained RPL. This finding has two main implications: (i) it strengthens the hypothesis that anti-fetal immunity mediated by T lymphocytes plays an important role in RPL since memory of previous pregnancy outcomes seems to be involved in the pathogenic mechanisms; and (ii) it questions the motion for including non-consecutive pregnancy losses in the diagnosis of RPL. If non-consecutive losses are included in the diagnostic criteria for RPL, results will be 'diluted' in prospective treatment trials because many patients with a favorable pregnancy prognosis will be included. In such studies, it is important to ensure that the number of late pregnancy losses and consecutive early losses after a prior live birth are equal in the treated and non-treated/placebo groups.

Supplementary data

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

Authors' roles

P.E. collected data, participated in the execution of analyses and critically revised the manuscript. E.C.L. and H.S.N enrolled patients in the last trial, participated in the execution of analyses and critically revised the manuscript. A.M.K and M.K. participated in the execution of analyses and critically revised the manuscript. O.B.C. conceived the study, enrolled patients in the trials, participated in the execution of the analyses and drafted the manuscript.

Funding

There was no specific funding of the present study. The first randomized controlled trial was funded by Novo-Nordisk AS, (Bagsvaerd, Denmark) and the second was funded by HemaSure AS (Gentofte, Denmark). The third randomized controlled trial was funded by the Danish Council for Independent Research, grant no. 271-07-0445.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod* 1999; **14**:2868–2871.

Carp HJ, Toder V, Torchinsky A, Portuguese S, Lipitz S, Gazit E, Mashiach S; the Recurrent Miscarriage Immunotherapy Trialists Group. Allogenic lymphocyte immunization after five or more miscarriages. *Hum Reprod* 1997;**12**:250–255.

Chen T, Darrasse-Jeze G, Bergot AS, Courau G, Valdivia K, Strominger JL, Ruocco MG, Chaouat G, Klatzmann D. Self-specific memory regulatory T cells protect embryos at implantation in mice. *J Immunol* 2013; **191**:2273–2281.

Christiansen OB, Mathiesen O, Husth M, Rasmussen KL, Ingerslev HJ, Lauritsen JG, Grunnet N. Placebo-controlled trial of treatment of unexplained secondary recurrent spontaneous abortions and recurrent late spontaneous abortions with i.v. immunoglobulin. *Hum Reprod* 1995; **10**:2690–2695.

Christiansen OB, Pedersen B, Rosgaard A, Husth M. A randomized, double-blind, placebo-controlled trial of intravenous immunoglobulin in the prevention of recurrent miscarriage: evidence for a therapeutic effect in women with secondary recurrent miscarriage. *Hum Reprod* 2002; **17**:809–816.

Christiansen OB, Nielsen HS, Lund M, Steffensen R, Varming K. Mannose-binding lectin-2 genotypes and recurrent late pregnancy losses. *Hum Reprod* 2009;**24**:291–299.

Christiansen OB, Larsen EC, Egerup P, Lunoee L, Egestad L, Nielsen HS. Intravenous immunoglobulin treatment for secondary recurrent miscarriage: a randomized, double-blind, placebo-controlled trial. *BJOG* 2015;**122**:500–508.

Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod* 1997;**12**:387–389.

Coomarasamy A, Williams H, Truchanowicz E, Seed PT, Small R, Quenby S, Gupta P, Dawood F, Koot YEM, Bender-Atik R *et al*. A randomized trial of progesterone in women with recurrent miscarriage. *N Engl J Med* 2015; **373**:2141–2148.

Cowshock FS, Smith JB, David S, Scher J, Batzer F, Corson S. Paternal mononuclear cell immunization therapy for repeated miscarriage: predictive variables for pregnancy success. *Am J Reprod Immunol* 1990;**22**:12–17.

Drakeley AJ, Quenby S, Farquharson RG. Mid-trimester loss, appraisal of a screening protocol. *Hum Reprod* 1998;**13**:1471–1479.

Ioannidis JPA, Haidich A-B, Pappa M, Pantazis N, Kokori SI, Tektonidou MG, Coutopoulos-Ioannidis DG, Lou J. Comparison of evidence of treatment effects in randomized and non-randomized studies. *JAMA* 2001;**286**: 821–830.

Kolte AM, van Oppenraaij RH, Quenby S, Farquharson RG, Stephenson M, Goddijn M, Christiansen OB; on behalf of the ESHRE Special Interest Group Early Pregnancy. Non-visualized pregnancy losses are prognostically important for unexplained recurrent miscarriage. *Hum Reprod* 2014; **29**:931–937.

Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, Goddijn M, Stephenson MD. Terminology for pregnancy loss prior to viability; a consensus statement from the ESHRE early pregnancy special interest group. *Hum Reprod* 2015;**30**:495–498.

Kwiatek M, Geca T, Krzyanowski A, Malec A, Kwasniewska A. Peripheral dendritic cells and CD4+ CD25+Foxp3+ regulatory T cells in the first trimester of normal pregnancy and in women with recurrent miscarriage. *PLoS One* 2015;**6**:e0124747.

Li TC, Iqbal T, Anstie B, Gillham J, Amer S, Wood K, Laird S. An analysis of the pattern of pregnancy loss in women with recurrent miscarriage. *Fertil Steril* 2002;**78**:1100–1106.

Lund M, Nielsen HS, Hviid TV, Steffensen R, Nyboe Andersen A, Christiansen OB. Hereditary thrombophilia and recurrent pregnancy loss: a retrospective cohort study of pregnancy outcome and obstetric complications. *Hum Reprod* 2010;**25**:2978–2984.

Lund M, Kamper-Jorgensen M, Nielsen HS, Lidegaard O, Andersen AMN, Christiansen OB. Prognosis for live birth in women with recurrent miscarriage: what is the best measure of success? *Obstet Gynecol* 2012;**119**:37–43.

Nybo Andersen AM, Wohlfart J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register study. *BMJ* 2000; **320**:1708–1712.

Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril* 2013;**99**:63.

Ranthe MF, Andersen MF, Wohlfarth J, Bundgaard H, Melbye M, Boyd HA. Pregnancy loss and later risk of atherosclerotic disease. *Circulation* 2013; **127**:1775–1782.

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016

Robertson L, Wu O, Langhome P, Twaddle S, Clark P, Lowe GC, Walker ID, Greaves M, Brenkel I, Regan L *et al*. Thrombophilia in pregnancy: a systematic review. *Br J Haematol* 2006;**132**:171–196.

Strobino B, Fox HE, Kline J, Stein Z, Susser M, Warburton D. Characteristics of women with recurrent spontaneous abortions and women with favorable reproductive histories. *Am J Public Health* 1986;**76**:986–991.

Sundtoft I, Uldbjerg N, Steffensen R, Sommer S, Christiansen OB. Polymorphisms in genes coding for cytokines, mannose-binding lectin, collagen metabolism and thrombophilia in women with cervical insufficiency. *Gynecol Obstet Invest* 2016;**81**:15–22.

Van den Boogaard E, Cohn DM, Korevaar JC, Dawood F, Vissenberg R, Middeldorp S, Goddijn M, Farquharson RG *et al*. Number and sequence of preceding miscarriages and maternal age for the prediction of antiphospholipid syndrome in women with recurrent miscarriage. *Fertil Steril* 2013;**99**:188–192.

Zou G. A modified Poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;**159**:702–706.

Downloaded from http://humrep.oxfordjournals.org/ at Canakkale Onsekiz Mart Universitesi on October 25, 2016