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ORIGINAL RESEARCH ARTICLE

The effect of paternal and maternal factors on the prognosis of live birth in couples with recurrent pregnancy loss

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Abstract

Introduction: Currently, recurrent pregnancy loss (RPL) examinations focus on the woman, although paternal factors are also involved. Men in couples with RPL have higher sperm DNA fragmentation levels than fertile men, but the effect of sperm DNA damage on couple's later prognosis is unknown. Advanced maternal age and obesity are associated with RPL, but paternal lifestyle factors are less studied. Therefore, we aimed to study the associations of couples' lifestyle factors, causes of RPL, and sperm DNA fragmentation with their prognosis of future live birth.

Material and methods: This descriptive cohort study comprised 506 couples investigated for RPL at Helsinki University Hospital, Finland, between 2007 and 2016, linked with national health and population registers. The primary outcome was couple's live birth after RPL investigations. Data on couple's background factors, including age, body mass index, smoking, and alcohol use, were collected from medical records. Sperm DNA fragmentation index was analyzed from 211 men using the sperm chromatin dispersion test. The associations between background factors, sperm DNA fragmentation, and cumulative probability of live birth over time were analyzed using cross-tabulations and age-adjusted Cox regression.

Results: In all, 352 of 506 couples (69.6%) achieved live birth. Maternal age, unexplained RPL, prolonged pregnancy attempts before investigations, paternal obesity, and maternal smoking were associated with prognosis: unadjusted hazard ratio for couple's live birth for women aged 35–39 vs younger than 30 years was 0.63 (95% confidence interval [CI] 0.47–0.84), and for 40 years or older was 0.36 (95% CI 0.22–0.58). Age-adjusted hazard ratio for unexplained vs explained RPL was 1.39 (95% CI 1.12–1.72), for couple's pregnancy attempt at least 4 years vs less than 2 years was 0.50 (95% CI 0.33–0.76), for paternal body mass index at least 30 kg/m^2 vs less than

Abbreviations: BMI, body mass index; DFI, sperm DNA fragmentation index; RPL, recurrent pregnancy loss; TSH, thyroid-stimulating hormone; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

25 kg/m^2 was 0.67 (95% CI 0.46–0.98), and for maternal smoking was 0.71 (95% CI 0.51–0.99). Altogether, 96/135 (71.1%) couples with normal (<15%), 38/60 (63.3%) with intermediate (15–30%), and 11/16 (68.8%) with high sperm DNA fragmentation index achieved live birth ($p = 0.56$).

Conclusions: In couples with RPL, prolonged pregnancy attempts, a cause found in RPL examinations, lifestyle factors, and maternal age are negatively associated with their prognosis of future live birth. Sperm DNA fragmentation was not associated, but the number of men with damaged spermatozoa was small. We suggest that clinicians include women and men in RPL counseling because couple's joint lifestyle seems to determine their later prognosis.

KEYWORDS

body mass index, lifestyle factors, male factors, recurrent miscarriage, recurrent pregnancy loss, smoking, sperm DNA fragmentation

1 | INTRODUCTION

Recurrent pregnancy loss (RPL), defined as two or more pregnancy losses,[1] is a shared problem for a couple. Nevertheless, RPL examinations are primarily focused on the woman as opposed to the male partner.[2] Research related to RPL examinations has only recently, albeit only slowly, begun to focus also on the male partner.[3,4] Spermatozoa are responsible for half of the genes of the pregnancy, and therefore it is likely that their quality affects the success of the pregnancy.[5] Although conventional sperm analysis has little value in RPL evaluation, sperm DNA fragmentation tests seem promising.[6] Compared with fertile men, men from couples with RPL have higher sperm DNA fragmentation levels,[7] but knowledge of the effect of sperm DNA damage on couple's subsequent pregnancy outcomes is lacking.

Lifestyle has significant impact on reproductive health. Maternal age and obesity are risk factors for sporadic[8,9] and recurrent[10,11] miscarriage, but less is known about the contribution of paternal age and body mass index (BMI). Maternal[12] and paternal[13] smoking and maternal alcohol consumption[14] increase the risk of sporadic miscarriage, but knowledge of their contribution to RPL is still lacking. A prognostic model found that multiple risk factors, including maternal and paternal age, BMI, and maternal smoking, when combined, predicted the subsequent ongoing pregnancy in RPL couples.[3] Also, a recent study found that maternal obesity and smoking, but not unhealthy paternal lifestyles, were associated with increased time to conception and viable pregnancy in RPL couples.[4] Still, no studies have evaluated the associations of both parents' lifestyles with their future prognoses of live birth.

We aimed to perform a descriptive analysis of underlying causes and risk factors for RPL and study how the background factors, especially both parents' unhealthy lifestyles (obesity, smoking, and alcohol consumption) and sperm DNA fragmentation, associate with couple's prognosis of live birth after RPL examinations.

Key message

Recurrent pregnancy loss examinations focus on women, while men are often ignored. This study shows that the couple's common background factors, such as prolonged pregnancy attempts and unhealthy lifestyles, are negatively associated with their later prognosis of live birth.

2 | MATERIAL AND METHODS

2.1 | Study population and collection of clinical data

The study population included all couples investigated for RPL at Helsinki University Hospital, Helsinki, Finland, and Hyvinkää Hospital, Hyvinkää, Finland, between 2007 and 2016 (Figure 1). Power calculations were not performed. The criteria for investigations were couples with three or more consecutive clinical first-trimester pregnancy losses or two or more losses with at least one in the second trimester and woman's age younger than 42 years. Clinical pregnancy loss was defined as a spontaneous loss of an intrauterine pregnancy confirmed by ultrasonography or histopathology or a positive serum or urine human chorionic gonadotropin at 6 or more gestational weeks. Biochemical pregnancy was defined as a pregnancy loss before 6 gestational weeks, diagnosed by only a positive human chorionic gonadotropin. Live birth was defined as a child born alive and stillbirth as a stillborn child at 22^{+0} or more gestational weeks or with a birthweight of 500 g or more.

We collected clinical data from the medical records. Women's and men's ages at the first RPL visit were categorized as less than 30, 30–34, 35–39, and 40 years or older. Lifestyle factors included couple's BMI, smoking, and alcohol use. BMI was categorized to less than 25 (normal), 25–29.9 (overweight), and 30 kg/m^2 or more (obese).

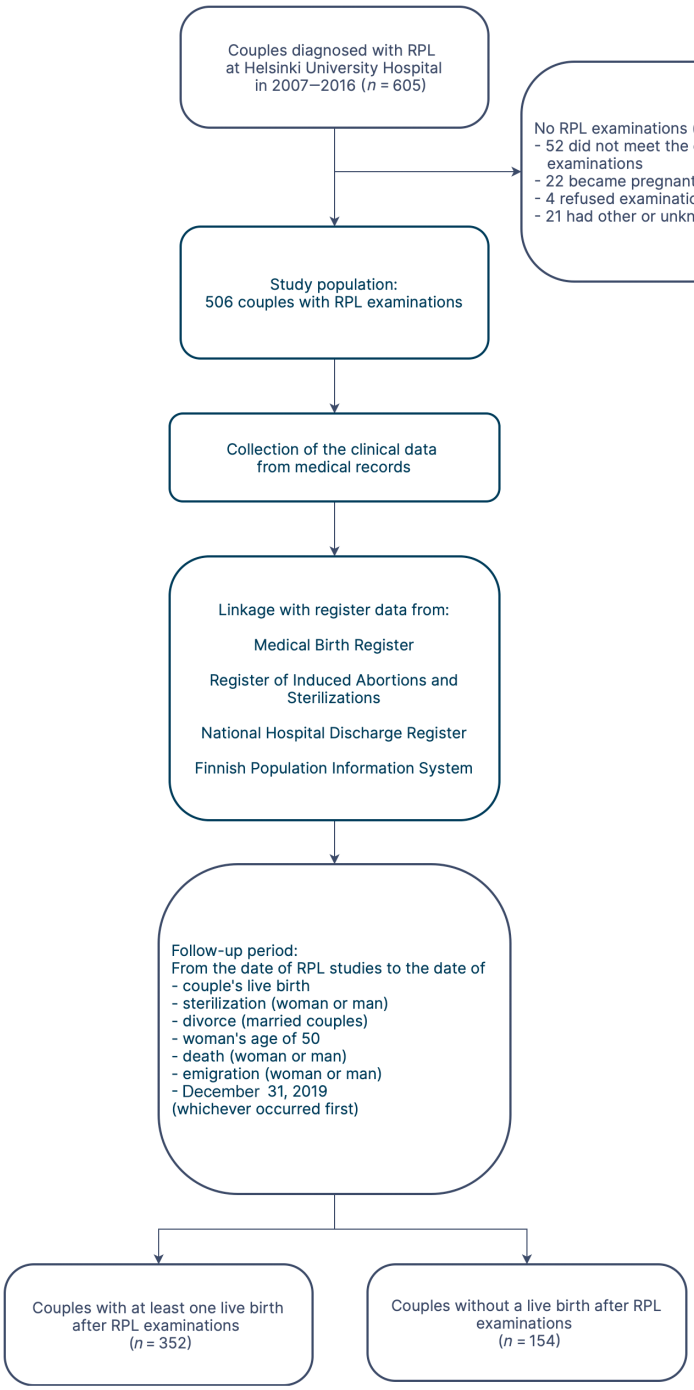

FIGURE 1 Flowchart of the study design. RPL, recurrent pregnancy loss

Current smokers (≥1 cigarettes per day) and snuff users were considered smokers; former, occasional, and non-smokers were considered non-smokers. We used two cut-off values for alcohol consumption: 1 unit or more vs less than 1 unit weekly and, for excessive alcohol consumption, 7 units or more vs less than 7 units weekly for women and 14 or more vs less than 14 for men. We also collected data on couples' pregnancy outcomes, duration of pregnancy attempts before RPL examinations, infertility history, and treatments used to prevent subsequent pregnancy loss.

Investigations for RPL included both partners' karyotypes, vaginal two-dimensional ultrasonography (three-dimensional ultrasonography, sonohysterography, or hysteroscopy when needed), phospholipid antibodies, hereditary thrombophilia tests, complete blood count, fasting glucose, thyroid-stimulating hormone (TSH), and prolactin. Between 2011 and 2016, the laboratory analyzed the thyroid peroxidase antibodies of 225 women and sperm DNA fragmentation index (DFI) with sperm count, concentration, and motility of 211 men. Table S1 presents the criteria for abnormal test results.[15–18]

Sperm DNA fragmentation was analyzed using the sperm chromatin dispersion test (Halosperm®, Halotech DNA). Fresh unfixed sperm cells were incubated subsequently in acid and lysis solutions, which, in a controlled manner, first denatures sperm DNA and then removes DNA packing proteins. Specimens were stained with

Wright's methylene blue and visualized under a conventional microscope. In normal spermatozoa, the non-fragmented DNA forms loops, creating a large halo surrounding the head of the sperm. Spermatozoa with fragmented DNA show intermediate, small, or no halo. The percentage of spermatozoa with fragmented DNA defines DFI. We considered DFI less than 15% as normal, 15–30% as intermediate, and more than 30% as high.

We considered RPL to be unexplained if the woman's age was less than 40 years, her BMI was less than 30 kg/m^2, and no uterine malformation, abnormal karyotype, antiphospholipid syndrome, hereditary thrombophilia, or polycystic ovary syndrome was diagnosed, nor high DFI (>30%), TSH, prolactin, fasting glucose, or thyroid peroxidase antibodies were observed.

2.2 | Collection of the follow-up data from the national registers

Couples were followed from their first visit for RPL to their first live birth, sterilization, divorce (married couples), emigration, death, woman's age of 50 years, or December 31, 2019, whichever came first. For monitoring couples after RPL investigations, we used data from the medical records linked with the national health registers maintained by the Finnish Institute for Health and Welfare and the Finnish Population Information System, maintained by the Digital and Population Data Services Agency. The Finnish Social and Health Data Permit Authority, Findata, gathered and linked register data with clinical data using participants' unique personal identity codes, which are given to all Finnish citizens and permanent residents.

We obtained data on live births, stillbirths, and children's identity codes from the Medical Birth Register and data on induced abortions and sterilizations from the Register of Induced Abortions and Sterilizations. The National Hospital Discharge Register provided data on pregnancy losses treated in public hospitals. Pregnancy losses more than 90 days apart were considered separate losses.

The Finnish Population Information System provided data on women's and men's emigrations and deaths, women's marriage histories, and identity codes of the men's liveborn children (adoptions excluded). By comparing identity codes of children born to a woman with identity codes of the male partner's children, we were able to match couple's live births.

2.3 | Statistical analyses

We analyzed data using SAS 9.4 and IBM SPSS Statistics 27. The primary outcome was the couple's live birth after RPL investigations. We used cross-tabulations with chi-squared test for independence to screen associations between background factors and the outcome. Factors considered were maternal and paternal age; smoking; alcohol consumption; number of couple's clinical, second-trimester, and all pregnancy losses (biochemical pregnancies and stillbirths included); primary vs secondary RPL (primary RPL being defined as no previous childbirth); firstborn child's sex in secondary RPL; infertility treatments; duration of pregnancy attempt before RPL examinations; unexplained vs explained RPL; abnormal karyotype; uterine malformation; hereditary thrombophilia; TSH; thyroid peroxidase antibodies; hemoglobin; glucose; polycystic ovary syndrome; and, in a subgroup of 211 couples with DFI analysis, sperm parameters and DFI.

Factors showing an association in crosstabulation, lifestyle factors, or those associated with RPL in literature were used in the Cox regression analysis to determine unadjusted and age-adjusted hazard ratios with 95% confidence intervals for the couples' live birth over time in all 506 couples, and in a subgroup of 361 couples with three or more clinical pregnancy losses. In 211 couples with DFI analysis, differences in the median DFI between couples who achieved live birth and those who did not, were compared with Mann–Whitney *U* test. Correlations between DFI, male age, and BMI were calculated using Spearman's correlation. Associations between treatments and pregnancy outcomes were analyzed with a chi-squared test. A significance level of *P* value less than 0.05 was used in all analyses. We did not apply corrections for multiple comparisons.

2.4 | Ethics statement

The study received research permissions from Helsinki University Hospital (HUS/138/2017, June 28, 2017) and Findata (THL/4217/14.02.00/2020, October 14, 2020). In Finland, register-based studies do not need approval from ethics committees.

3 | RESULTS

The study population included 506 couples, of whom 361 (71.3%) had experienced three or more clinical pregnancy losses and 211 had sperm DFI results (Figure 2). All met the European Society of Human Reproduction and Embryology's definition for RPL.[1] In 15 cases, the man's identity could not be ascertained. Table 1 shows the basic characteristics of all 506 couples and 361 couples with three or more pregnancy losses. The median number of clinical pregnancy losses in all couples was three (range zero to six). Those 32 couples with zero or one clinical loss had biochemical, ectopic, or pregnancies of unknown locations or losses in another relationship. Women's mean age was 33.7 years (19.6–43.9 years) and men's was 35.7 years (20.7–68.2 years); 50/506 (9.9%) women and 104/492 (21.1%) men were 40 years or older. Sixty of 505 (11.9%) women and 63/373 (16.9%) men were obese; 13/505 (2.6%) women and 8/373 (2.1%) men had BMI of 35 kg/m^2 or more. We observed one or more unfavorable lifestyles, such as obesity, smoking, or excessive alcohol consumption, in 254/506 (50.2%) couples. One or more test results were abnormal in 212/506 (41.9%) couples (Table 2). RPL remained unexplained in 280/506 (55.3%) couples. The background factors of the 361 couples with three or more losses were comparable to the background factors of all couples (Tables 1 and 2). Of

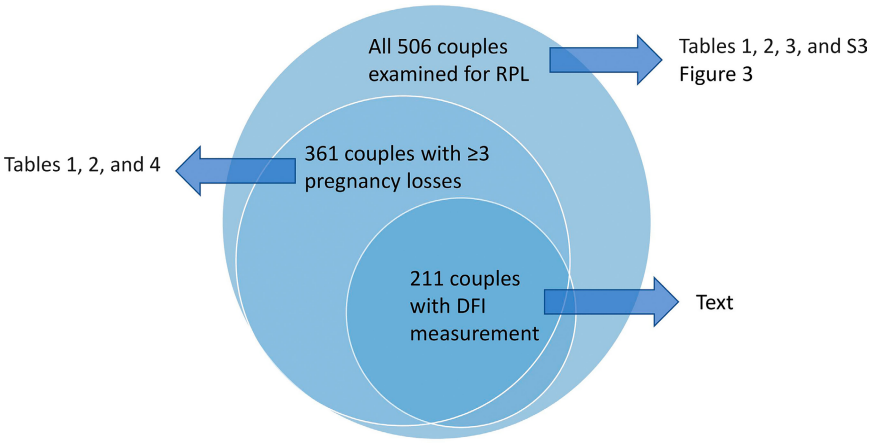

FIGURE 2 The total number of all couples and subpopulations with three or more pregnancy losses and sperm DNA fragmentation index (DFI) measurement. The arrows show where each population's results are presented in the manuscript. RPL, recurrent pregnancy loss

the couples with DFI measurement, 135/211 (64.0%) had normal, 60/211 (28.4%) had intermediate, and 16/211 (7.6%) had high DFI.

The couples' mean follow-up time was 3.2 years (standard deviation 2.7, range 0.2–12.6 years). Altogether, 352/506 (69.6%) of all couples and 254/361 (70.4%) with three or more clinical pregnancy losses had at least one live birth after RPL investigations.

Couples with abnormal test results were treated according to the underlying condition (Table S2). In unexplained RPL, one or more medications, such as aspirin, heparin, progesterone, or prednisolone, were used in 86/548 (15.6%) pregnancies. Infertility treatments were used in unexplained RPL for the treatment of concomitant infertility, leading to 55 pregnancies. The outcomes did not significantly differ between treated and non-treated pregnancies. Men with high sperm DFI received counseling on lifestyle modification, but no treatments. All conceptions in couples with high DFI were spontaneous.

The cross-tabulations showed a significant association between couples' prognoses of live birth and maternal and paternal age, paternal BMI, duration of pregnancy attempts before RPL examinations, unexplained vs explained RPL, TSH level, and uterine malformation (Figure 3; Table S3). Compared with normal values, neither high nor intermediate DFI was associated with a couple's prognosis of live birth, but DFI was studied only from 211 men, of which 16 had high DFI levels. The median DFI did not differ between the couples who achieved a live birth (median DFI 11%, range 0%–53%) and those who did not (median 13%, range 2%–68%). There was a weak positive correlation between DFI and male age (Spearman's correlation coefficient $r_s = 0.20$, $p = 0.003$) but not between DFI and BMI ($r_s = 0.048$, $p = 0.53$). The prognosis was not associated with conventional sperm parameters, such as total sperm count, concentration, or motility.

Table 3 shows hazard ratios with 95% confidence intervals for live birth after RPL investigations in all 506 couples. Maternal age of 35 years or older vs younger than 30 years was associated with reduced chances for live birth. In couples where RPL remained unexplained after examinations, these had a higher likelihood of achieving a live birth. However, those couples where the duration of pregnancy attempts was longer (>4 years) were associated with a decreased prognosis of live birth, as opposed to a shorter duration (<2 years). Maternal obesity was not associated with the likelihood of live birth. However, in obese men or if the man's BMI was unknown, likelihood of live birth was decreased compared with men with normal BMI. Maternal smoking, adjusted by age, and TSH level less than 0.5 mU/L were associated with reduced chances of live birth. The clinical backgrounds of women with low TSH varied: 6/21 had hyperthyroidism, whereas others used thyroxine for hypothyroidism or had normal TSH when controlled. After adjusting by woman's age, men aged 30–34 years had better chances for live birth than men younger than 30 years, but advanced paternal age (35 years or older) was not associated with prognosis.

Couples with three or more clinical pregnancy losses were less likely to achieve live birth if the woman's age was 35 or older vs younger than 30 years or if they had attempted pregnancy over 4 or more years vs less than 2 years before RPL examinations. Couples with unexplained RPL had a better prognosis than those with an explanation for their miscarriages (Table 4).

4 | DISCUSSION

Our data show that 40% of couples had abnormal test results in RPL investigations, and 50% had at least one unhealthy lifestyle. The overall prognosis was good, especially in unexplained RPL, even without treatments. Three years after examination, 70% of couples achieved a live birth. Maternal age of 35 years or older, pregnancy attempts for at least 4 years, paternal obesity, and maternal smoking were associated with decreased chances of having a child after RPL investigations. Sperm DNA fragmentation seems not to impact prognosis.

Couples who had tried for pregnancy for 4 years or more were 50% less likely to have a child than couples with less than 2 years of pregnancy attempts, which is a novel finding. The explanation might be a couple's secondary infertility or a decision not to try for a new pregnancy after many years of unsuccessful pregnancy attempts. Couples with unexplained RPL were 40% more likely to achieve a live birth than couples with an explanation for their miscarriages, which is in line with an earlier study reporting a higher live

TABLE 1 Basic characteristics of all couples investigated for recurrent pregnancy loss (RPL) and couples with three or more pregnancy losses; results are presented as means (standard deviation [range]) or *n* (%)

Variable	All couples (*N* = 506)	Couples with ≥3 clinical pregnancy losses (*N* = 361)
Age (years)		
Women	33.7 (5.0 [19.6–43.9])	33.8 (5.1 [33.8–43.9])
Men	35.7 (6.1 [20.7–68.2])	35.7 (5.7 [20.7–56.6])
BMI (kg/m^2)		
Women[a]	24.4 (4.4 [17.0–43.4])	24.4 (4.5 [17.0–43.9])
Men[b]	26.5 (3.4 [17.2–42.3])	26.3 (3.6 [17.2–38.6])
Current smoker		
Women	60/506 (11.9)	42/361 (11.6)
Men[c]	110/422 (26.1)	82/309 (26.5)
Alcohol consumption (weekly units)		
Women[d], ≥1	224/500 (44.8)	157/357 (44.0)
Women, ≥7	11/500 (2.2)	8/357 (2.2)
Men[e], ≥1	320/389 (82.3)	229/286 (80.1)
Men, ≥14	21/389 (5.4)	15/286 (5.2)
Type of woman's recurrent pregnancy loss[f]		
Primary	234 (46.2)	163 (45.2)
Secondary	272 (53.8)	198 (54.8)
Couple's previous live births		
0	280 (55.3)	192 (53.2)
1 or more	226 (44.7)	169 (46.8)
Couple's previous stillbirths		
0	493 (97.4)	354 (98.1)
1 or more	13 (2.6)	7 (1.9)
Couple's previous clinical pregnancy losses[g]		
0–2	145 (28.7)	0
3	303 (59.9)	303 (83.9)
4	47 (9.3)	47 (13.0)
5 or more	11 (2.2)	8 (2.2)
Couple's previous second-trimester losses		
0	409 (80.8)	308 (85.3)
1	65 (12.8)	36 (10.0)
2 or more	32 (6.3)	17 (4.7)
Couple's previous biochemical pregnancy losses[h]		
0	376 (74.3)	315 (87.3)
1	84 (16.6)	33 (9.1)
2	32 (6.3)	11 (3.0)
3 or more	14 (2.8)	2 (0.6)
Duration of pregnancy attempt before RPL examinations (years)[i]		
<2	90/306 (29.4)	59/215 (27.4)
2–3	147/306 (48.0)	105/215 (48.8)
4 or more	69/306 (22.5)	51/215 (23.7)

TABLE 1 (Continued)

Variable	All couples (*N* = 506)	Couples with ≥3 clinical pregnancy losses (*N* = 361)
Couple's previous history of infertility		
Infertility examinations	148 (29.2)	97 (26.9)
Infertility treatment[j]	102 (20.2)	71 (19.7)

[a]Data missing from 1.
[b]Data missing from 135.
[c]Data missing from 84.
[d]Data missing from 6.
[e]Data missing from 117.
[f]Primary RPL was defined as RPL without previous childbirth. Secondary RPL was defined as RPL after woman's one or more previous pregnancies ≥22^{+0} gestational weeks or birthweight ≥500 g.
[g]A loss of an intrauterine pregnancy confirmed by ultrasound or histopathology, or positive serum or urine human chorionic gonadotropin at 6 gestational weeks or more.
[h]Pregnancy loss before 6 gestational weeks, diagnosed only by positive serum or urine human chorionic gonadotropin.
[i]Data missing from 200.
[j]Ovulation induction, intrauterine insemination or in vitro fertilization / intracytoplasmic sperm injection.

TABLE 2 Abnormal diagnostic test results identified in couples' recurrent pregnancy loss (RPL) investigations in all couples and a subgroup of couples with three or more clinical pregnancy losses; results are presented as *n* (%)

Diagnostic test result	All couples (*n* = 506)	Couples with ≥3 clinical pregnancy losses (*n* = 361)
Congenital uterine malformation[a]	19/506 (3.8)	15/361 (4.2)
Acquired uterine malformation[b]	8/506 (1.6)	7/361 (1.9)
Chromosomal translocation or inversion in either parent	15/487 (3.1)	9/351 (2.6)
Antiphospholipid syndrome[c]	4/492 (0.8)	4/351 (1.1)
Hereditary thrombophilia[d]	30/492 (6.1)	18/351 (5.1)
TSH <0.5 or >3.6 mU/L	31/465 (6.7)	22/334 (6.6)
Elevated serum TPO antibodies	25/227 (11.0)	22/190 (11.6)
Hemoglobin <117 g/L	37/470 (7.9)	28/332 (8.4)
Fasting glucose >6.0 mmol/L	18/228 (7.9)	13/182 (7.1)
Biologically active prolactin >500 mU/L	0/411 (0)	0/301 (0)
Polycystic ovary syndrome[e]	24/506 (4.7)	19/361 (5.3)
Sperm DNA fragmentation index >30%	16/211 (7.6)	15/191 (7.9)
Total sperm count[f] <39×10^6/mL	24/291 (8.2)	14/228 (6.1)
Sperm concentration[f] <15×10^6/mL	18/297 (6.1)	16/223 (7.2)
Progressive motile sperm[f] <32%	34/295 (11.5)	29/226 (12.8)
Couples with one or more abnormal test results	212/506 (41.9)	158/361 (43.8)

Abbreviations: TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone.
[a]Septate, bicornuate, didelphic, or arcuate uterus.[15]
[b]Submucous fibroids or intrauterine adhesions.
[c]According to criteria of an international consensus statement.[16]
[d]Factor V Leiden or Factor II mutation; or persistent protein C, protein S, or antithrombin deficiency.
[e]According to the Rotterdam criteria.[17]
[f]According to WHO.[18]

birth rate after idiopathic miscarriages (76%) than after miscarriages with an identified cause (65%).[19] Consistent with a review and meta-analysis,[20] pharmacological treatments did not improve pregnancy outcomes in unexplained RPL.

Unhealthy lifestyles were common: in half of the couples, one or both spouses were obese, smoked, or consumed alcohol excessively. These lifestyle factors were selected for the analyses because they should be discussed with every couple as an important part of the

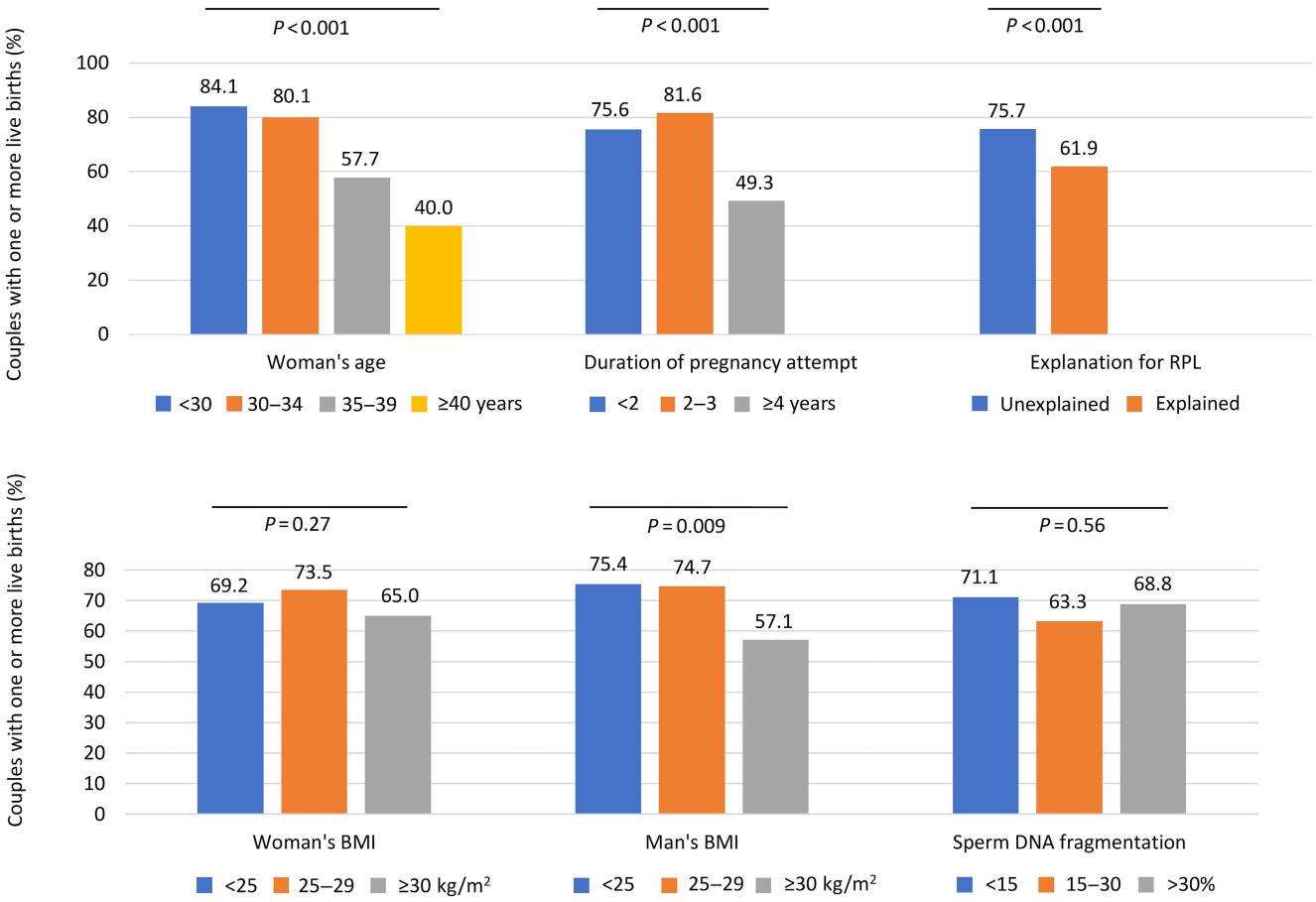

FIGURE 3 Unadjusted percentages of couples who achieved one or more live births after recurrent pregnancy loss (RPL) investigations in all couples ($n = 506$) according to woman's age, duration of the pregnancy attempt, unexplained vs explained RPL, woman's and man's body mass index (BMI), and sperm DNA fragmentation index (DFI). RPL was considered unexplained, when the woman's age was less than 40 years, BMI was less than 30 kg/m^2, and the couple's diagnostic tests were normal

evaluation and because knowledge of their impact on the prognosis of RPL is scarce. Male obesity has previously been associated with reduced fertility[21] and decreased live birth rates in couples undergoing in vitro fertilization,[22] whereas we showed an association between paternal obesity and reduced likelihood of future live birth in RPL. Maternal obesity is a risk factor for sporadic miscarriage[9] and RPL,[11] but we found no association between female obesity and future live birth. An explanation for the conflicting results might be the small number of obese women in our study population, especially those with BMI of 35 kg/m^2 or more. Also, some women might have lost weight before getting pregnant again, possibly increasing their likelihood of live birth.

Maternal smoking was negatively associated with prognosis, which is in line with the results of a meta-analysis,[12] which found that smokers are 1.2-fold more likely to miscarry than non-smokers, and risk increased with the number of cigarettes smoked per day. Although a similar dose-dependent association between paternal smoking and sporadic pregnancy loss was recently reported,[13] we did not find an association. Our study may lack power, or some men might have stopped smoking before the couple's subsequent pregnancy. Maternal alcohol consumption increases the risk of sporadic miscarriage,[14] but we could not identify any studies evaluating the effect of paternal alcohol consumption. We found no association between a couple's alcohol use and future prognosis, but participants, especially heavy drinkers, may have underestimated their alcohol consumption, possibly leading to underestimation of the association.

As expected,[8,10,19] the prognosis of live birth was strongly related to maternal age. Men younger than 30 years of age appeared to have worse prognoses than the older men, which contradicts earlier literature showing a similar risk of sporadic miscarriage between men aged 25–29 and 30–39 years.[23] We believe that other than biological reasons are behind our results, because the fertility of men less than 30 years of age should be normal. They are still relatively young and possibly stopped trying to conceive, or their relationships ended more often than those of older men.

Increased formation of reactive oxygen species in semen may damage sperm DNA.[24] The quality of the embryo fertilized by spermatozoa with fragmented DNA may decline,[25] contributing to implantation failure or pregnancy loss.[26] Although men in couples with RPL have higher DFI than fertile men,[7] and high DFI is associated with increased miscarriage rate after in vitro fertilization,[27] sperm DNA damage seems not, according to our data, to be associated with

TABLE 3 Unadjusted and age-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for couples' prognosis of live birth after recurrent pregnancy loss (RPL) investigations according to risk factors in all couples ($n = 506$). p values less than 0.05 (bold) are considered statistically significant

	Prognosis of live birth							
Risk factor	No of couples	No of couples with live birth	Unadjusted HR	95% CI	p	Adjusted HR[a]	95% CI	p
Woman's age (years)								
<30	107	90	1.00			1.00		
30–34	181	145	1.03	0.90–1.61	0.85	0.99	0.75–1.33	0.99
35–39	168	97	0.63	0.47–0.84	**0.002**	0.62	0.44–0.88	**0.007**
≥40	50	20	0.36	0.22–0.58	**<0.001**	0.35	0.20–0.61	**<0.001**
Man's age (years)								
<30	70	59	1.00			1.00		
30–34	163	126	1.18	0.87–1.58	0.29	1.41	1.01–1.96	**0.04**
35–39	155	104	0.92	0.67–1.25	0.58	1.30	0.90–1.89	0.17
≥40	104	56	0.66	0.47–0.95	**0.02**	1.07	0.69–1.66	0.97
Number of pregnancy losses[b]								
2	65	49	1.00			1.00		
3	319	221	0.92	0.65–1.31	0.65	0.91	0.65–1.29	0.61
4	85	59	1.06	0.71–1.58	0.77	1.01	0.68–1.51	0.97
5	28	18	0.68	0.38–1.22	0.19	0.74	0.41–1.33	0.31
≥6	9	5	0.88	0.43–1.83	0.74	0.95	0.46–1.96	0.88
Unexplained[c] vs explained RPL								
Explained RPL	280	212	1.00			1.00		
Unexplained RPL	226	140	1.47	1.19–1.82	**<0.001**	1.39	1.12–1.72	**0.003**
Duration of the pregnancy attempt before RPL examinations (years)								
<2	90	68	1.00			1.00		
2–3	147	120	1.13	0.84–1.52	0.42	1.08	0.80–1.46	0.61
≥4	69	34	0.49	0.32–0.74	**<0.001**	0.50	0.33–0.76	**0.001**
Unknown	200	130	0.81	0.60–1.08	0.15	0.81	0.61–1.10	0.17
Woman's BMI (kg/m^2)								
<25	328	227	1.00			1.00		
25–29	117	86	1.08	0.84–1.39	0.54	1.09	0.86–1.39	0.52
≥30	60	39	0.82	0.59–1.16	0.26	0.84	0.60–1.18	0.30
Man's BMI (kg/m^2)								
<25	142	107	1.00			1.00		
25–29	166	124	0.93	0.73–1.22	0.64	0.98	0.76–1.27	0.87
≥30	63	27	0.65	0.44–0.95	**0.03**	0.67	0.46–0.98	**0.04**
Unknown	135	94	0.67	0.50–0.89	**0.006**	0.68	0.51–0.90	**0.008**
Woman's smoking								
No	446	315	1.00			1.00		
Yes	60	37	0.83	0.60–1.15	0.25	0.71	0.51–0.99	**0.04**
Man's smoking								
No	312	215	1.00			1.00		
Yes	110	77	1.05	0.81–1.35	0.73	0.97	0.75–1.26	0.81
Unknown	84	60	1.03	0.78–1.37	0.81	1.01	0.76–1.33	0.96
Woman's plasma TSH level								
0.5–3.6 mU/L	434	308	1.00			1.00		
>3.6 mU/L	10	7	0.95	0.45–2.00	0.88	1.01	0.48–2.13	0.98
<0.5 mU/L	21	9	0.47	0.24–0.91	**0.03**	0.47	0.24–0.91	**0.02**

Abbreviations: BMI, body mass index; TSH, thyroid stimulating hormone.

[a]HRs for women's age are adjusted by man's age, and HRs for all other variables are adjusted by woman's age.

[b]Couple's clinical and biochemical pregnancy losses and stillbirths before RPL examinations.

[c]Woman's age <40 years, BMI <30 kg/m^2, and normal diagnostic test results.

TABLE 4 Unadjusted and age-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) for couples' prognosis of live birth after recurrent pregnancy loss (RPL) investigations according to risk factors in couples with three or more pregnancy losses ($n = 361$). p values less than 0.05 (bold) are considered statistically significant

	Couples' prognosis of live birth							
Risk factor	No of couples	No couples with live birth	Unadjusted HR	95% CI	*p*	Adjusted HR[a]	95% CI	*p*
Woman's age (years)								
<30	75	65	1.00			1.00		
30–34	126	106	1.09	1.08–1.47	0.78	1.09	0.77–1.55	0.72
35–39	121	67	0.64	0.41–0.82	**0.002**	0.61	0.40–0.94	**0.03**
≥40	39	16	0.41	0.35–0.60	**<0.001**	0.35	0.19–0.66	**<0.001**
Man's age (years)								
<30	47	41	1.00			1.00		
30–34	117	94	1.16	0.81–1.64	0.42	1.75	1.12–2.75	**0.02**
35–39	115	78	0.87	0.60–1.25	0.44	1.55	0.92–2.62	0.10
≥40	72	36	0.56	0.36–0.87	**0.009**	1.14	0.61–2.12	0.68
Number of pregnancy losses[b]								
3	262	185	1.00			1.00		
4	67	47	1.17	0.86–1.58	0.32	1.12	0.83–1.52	0.47
5	24	17	0.86	0.51–1.43	0.56	0.95	0.57–1.59	0.84
≥6	8	5	1.04	0.51–2.12	0.91	1.10	0.54–2.23	0.80
Unexplained[c] vs explained RPL								
Explained RPL	203	157	1.00					
Unxplained RPL	158	97	1.60	1.25–2.06	**<0.001**	1.50	1.16–1.93	**<0.001**
Duration of the pregnancy attempt before RPL examinations (years)								
<2	59	45	1.00			1.00		
2–3	105	90	1.29	0.90–1.85	0.16	1.21	0.84–1.73	0.31
≥4	51	26	0.51	0.31–0.83	**0.006**	0.53	0.32–0.85	**0.009**
Unknown	146	93	0.85	0.60–1.22	0.38	0.86	0.60–1.23	0.41
Woman's BMI (kg/m^2)								
<25	233	169	1.00			1.00		
25–29	83	55	0.86	0.63–1.17	0.34	0.90	0.66–1.23	0.51
≥30	45	30	0.77	0.52–1.14	0.20	0.79	0.53–1.17	0.24
Man's BMI (kg/m^2)								
<25	111	85	1.00			1.00		
25–29	125	92	0.82	0.61–1.11	0.20	0.85	0.64–1.15	0.30
≥30	38	23	0.69	0.44–1.10	0.12	0.74	0.46–1.17	0.20
Unknown	87	54	0.61	0.43–0.86	**0.005**	0.59	0.42–0.84	**0.003**
Woman's smoking								
No	319	228	1.00			1.00		
Yes	42	26	0.85	0.58–1.25	0.41	0.69	0.46–1.02	0.07
Man's smoking								
No	227	158	1.00			1.00		
Yes	82	60	1.03	0.77–1.39	0.84	0.96	0.71–1-29	0.76
Unknown	52	36	0.96	0.67–1.36	0.80	0.89	0.62–1.27	0.52
Woman's plasma TSH level								
0.5–3.6 mU/L	312	224	1.00			1.00		
>3.6 mU/L	7	4	0.64	0.24–1.72	0.38	0.67	0.25–1.80	0.43
<0.5 mU/L	15	7	0.53	0.25–1.12	0.10	0.51	0.24–1.08	0.08

Abbreviations: BMI, body mass index; TSH, thyroid stimulating hormone.

[a]HRs for women's age are adjusted by man's age, and HRs for all other variables are adjusted by woman's age.

[b]Couple's clinical and biochemical pregnancy losses and stillbirths before RPL examinations.

[c]Woman's age <40 years, BMI <30 kg/m^2, and normal diagnostic test results.

the future prognosis of live birth in RPL couples. The conventional sperm parameters also lacked an association. Differences in study designs, populations, assays, and cut-off values may explain the conflict between our results and the previous DFI studies. However, infertility treatments are unlikely to compensate for the sperm damage because all pregnancies in couples with high DFI began after natural conception in our population. Men with high DFI received only lifestyle counseling as treatment, but we are unaware if some used antioxidants on their own initiative. We have no follow-up data on DFI, which may have improved or fluctuated[28] over time. In addition, we did not investigate DFI in all men, and the number of men with high DFI was small. Despite these limitations, this study is, to our knowledge, the first to describe the association between sperm DNA damage and the prognosis of RPL. However, larger studies with different assays should confirm our findings.

We used the sperm chromatin dispersion assay to measure DNA fragmentation. Three other major assays are the sperm chromatin structure assay, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL), and the comet assay.[29] The sperm chromatin structure assay is based on denaturation and TUNEL on enzymatic labeling of the fragmented DNA; both use flow cytometry to measure DFI. The comet assay quantifies the shape of the sperm cell nuclei after gel electrophoresis. Interpreting DNA fragmentation results is challenging as all assays use a relatively small proportion of the total sperm sample, which might not be representative. As a result of differences in analysis techniques and threshold values between assays,[29] the present data may not be generalized to all assays. The results of different assays may also vary according to the clinical condition.[29] In the meta-analysis by McQueen et al,[7] men from couples with RPL had significantly higher DFI than control men when sperm chromatin structure assay, TUNEL, or comet assays were used, but not when sperm chromatin dispersion assay was used. Therefore, the other assays may be more useful in RPL.

Our study has several strengths. First, by combining data from different registers, we were able to determine the couple's live births and consider the male partner's contribution to the prognosis, emphasizing our study's novelty. Second, our primary outcome was live birth because the prognosis of having a child is what matters most to the couples. Lastly, treatments for unexplained RPL were rarely used.

Our study also has some limitations. First, our real-world setting did not allow us to control for all the factors affecting prognosis. We had no follow-up data on health behavior, and we do not know if some couples decided not to attempt pregnancy. Also, we were not able to take into consideration the psychological stress experienced by the couple, with its possible adverse effects on the outcome of the pregnancy.[30] Second, although data on women's lifestyle factors were almost complete, men's data were missing more often, which can significantly affect the conclusions drawn from the data. Third, we made multiple comparisons without corrections, meaning that when using a 0.05 significance level, one result out of 20 may be significant by chance. Because corrections will increase the probability of ignoring an association when it exists,[31] we chose not to make them. Lastly, our population represents mainly Finnish RPL couples, so the results may not be generalized to other ethnicities.

5 | CONCLUSION

Our results show that the prognosis of RPL couples' future live birth is good, especially in younger women and unexplained RPL, even without any treatments. A couple's prolonged pregnancy attempts and unhealthy lifestyles are negatively associated with the prognosis. We did not find an association between sperm DNA fragmentation and later prognosis, but larger studies using different assays are needed. We suggest that clinicians consider the couple's perspective in RPL evaluation because their common background factors seem to determine their prognosis of later live birth.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and planning of the study. PLP and HH collected the clinical data. MG was responsible for the registry data, and PP for the DFI analyses. MG and PLP performed the statistical analyses. PLP prepared the manuscript. HH was accountable for the overall study and obtained funding. All authors made critical reviews and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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