Seminal significance: the forgotten father in recurrent pregnancy loss

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CHAPTER 2

Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis

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ABSTRACT

Background

Although spontaneous miscarriage is the most common complication of human pregnancy, potential contributing factors are not fully understood. Advanced maternal age has long been recognised as a major risk factor for miscarriage, being strongly related with fetal chromosomal abnormalities. The relation between paternal age and the risk of miscarriage is less evident, yet it is biologically plausible that an increasing number of genetic and epigenetic sperm abnormalities in older males may contribute to miscarriage. Previous meta-analyses showed associations between advanced paternal age and a broad spectrum of perinatal and paediatric outcomes. This is the first systematic review and meta-analysis on paternal age and spontaneous miscarriage.

Objective and rationale

The aim of this systematic review and meta-analysis is to evaluate the effect of paternal age on the risk of spontaneous miscarriage.

Search methods

PubMed, Embase and Cochrane databases were searched to identify relevant studies up to August 2019. The following free text and MeSH terms were used: paternal age, father's age, male age, husband's age, spontaneous abortion, spontaneous miscarriage, abortion, miscarriage, pregnancy loss, fetal loss and fetal death. PRISMA guidelines for systematic reviews and meta-analysis were followed. Original research articles in English language addressing the relation between paternal age and spontaneous miscarriage were included. Exclusion criteria were studies that solely focused on pregnancy outcomes following artificial reproductive technology (ART) and studies that did not adjust their effect estimates for at least maternal age. Risk of bias was qualitatively described for three domains: bias due to confounding, information bias and selection bias.

Outcomes

The search resulted in 975 original articles. Ten studies met the inclusion criteria and were included in the qualitative synthesis. Nine of these studies were included in the quantitative synthesis (meta-analysis). Advanced paternal age was found to be associated with an increased risk of miscarriage. Pooled risk estimates for miscarriage for age categories 30-34, 35-39, 40-44 and ≥45 years of age were 1.04 (95% CI 0.90, 1.21), 1.15 (0.92, 1.43), 1.23 (1.06, 1.43) and 1.43 (1.13, 1.81) respectively (reference category 25-29 years). A second meta-analysis was performed for the subgroup of studies investigating first trimester miscarriage. This showed similar pooled risk estimates for the first three age categories and a slightly higher pooled risk estimate for age category ≥45 years (1.74; 95% CI 1.26, 2.41).

Wider implications

Over the last decades, childbearing at later ages has become more common. It is known that frequencies of adverse reproductive outcomes, including spontaneous miscarriage, are higher in women with advanced age. We show that advanced paternal age is also associated with an increased risk of spontaneous miscarriage. Although the paternal age effect is less pronounced than that observed with advanced maternal age and residual confounding by maternal age cannot be excluded, it may have implications for preconception counselling of couples comprising an older aged male.

INTRODUCTION

Advanced maternal age is an extensively studied risk factor for adverse reproductive outcome.(1-10) The reproductive risks associated with advanced maternal age (usually defined as age ≥ 35 years) form an integral part of preconception counselling and are well known to the general public.(11). Moreover, clinical policy is based on this knowledge, for instance, maternal age-related access criteria for in vitro fertilisation (IVF) treatment.(12) In contrast, less attention has been paid to the potential effect of paternal age. There are, however, studies indicating that this is unjustified. In 2018, Oldereid et al. evaluated the influence of paternal factors on a broad spectrum of perinatal and paediatric outcomes.(13) They found associations between advanced paternal age and adverse outcomes in the offspring, particularly with psychiatric disorders like autism spectrum disorders and schizophrenia but also with stillbirth and several birth defects. The age of the father and the mutation rate in the offspring are found to be strongly related, possibly due to the larger number of germline divisions that have occurred in older males.(14, 15) Next to a higher frequency of point mutations, there is evidence suggesting that increasing paternal age is associated with sperm DNA strand breaks, genetic imprinting errors and chromosomal anomalies, all of which are factors related to miscarriage.(16-18) As such, from a biological point of view, it seems justified to consider paternal age as an independent risk factor for miscarriage.

Spontaneous miscarriage is the most common complication of human pregnancy; it is estimated that at least 30% of all pregnancies and 10–15% of clinically recognised pregnancies end in miscarriage.(4, 19) Miscarriage refers to a spontaneous demise of pregnancy before the fetus reaches viability (before 24 weeks of gestational age); however, in many studies it is defined as a pregnancy loss that occurs before 20 completed weeks of gestational age.(20, 21) The majority of studies on miscarriage and its associated factors are focused on female factors. Cytogenetic and chromosomal microarray analysis studies on miscarriage specimens have shown that genetic abnormalities play a role in 50–70% of cases.(22-24) The prevalence of genetic abnormalities is highest in miscarriage samples from the first trimester, particularly in miscarriage samples of embryonic stage.(23) Advanced maternal age is strongly related with fetal chromosomal abnormalities, mainly aneuploid conceptions.(4, 25, 26) Besides maternal age, other factors such as uterine anomalies, poorly controlled diabetes and thyroid autoimmunity are related to miscarriage.(25, 27-29) In addition, associations have been found with behavioural and environmental factors including maternal obesity, smoking, alcohol and caffeine consumption, the use of non-steroidal anti-inflammatory drugs and acute and chronic stress.(30-35)

Despite our current knowledge, the cause of miscarriage is not always well-understood, especially in couples with recurrent miscarriages.(36, 37) Since the male partner contributes half of the genetic material of the embryo, studying paternal factors will possibly contribute to unravelling the complex aetiology of pregnancy loss. This may help to provide answers to affected couples, of whom many experience a high psychological impact and emotional burden.(38)

This is the first systematic review and meta-analysis evaluating the effect of paternal age on spontaneous miscarriage. We provide an overview of epidemiological studies evaluating the association between paternal age and spontaneous miscarriage and we discuss possible underlying explanatory mechanisms.

METHODS

We have conducted a systematic review and meta-analysis following the PRISMA guidelines.(39) This systematic review was registered and accepted for inclusion in the international prospective register of systematic reviews PROSPERO (ID CRD42019132886).

Systematic search

A systematic search of PubMed, Embase and Cochrane electronic databases was performed to identify relevant studies from inception until 12 August 2019. We used the following free text and MeSH terms: paternal age, father's age, male age, husband's age, spontaneous abortion, spontaneous miscarriage, abortion, miscarriage, pregnancy loss, fetal loss, fetal death. The full electronic search strategy for PubMed is shown in Supplementary Table 1. Additional searches in Google Scholar were conducted, and reference lists of identified articles were manually searched for additional relevant references.

The literature search was performed by two researchers (N.F. and E.L.) and a librarian. The results of the search were exported to a citation manager (EndNote), and duplicates were removed. The screening was performed by two researchers (N.F. and E.L.). There were two stages of screening for study inclusion: in the first stage, titles and abstracts were screened and in the second stage, full manuscripts of the articles identified in the initial screening were retrieved and read in detail. Any discordance on selecting studies and assessing risk of bias (see further) was resolved by consensus. If no agreement was obtained, the opinion of a third observer (M.H.) was sought to gain consensus.

Eligibility criteria

Inclusion criteria were original research articles in English language addressing the relation between paternal age and spontaneous miscarriage. Exclusion criteria were studies that solely focused on pregnancy outcomes after artificial reproductive technology (ART) and studies that did not adjust their effect estimates for at least maternal age.

Data extraction

Two reviewers (N.F. and E.L.) extracted data from all selected articles on study design, country, publication year, study period, population characteristics, inclusion and exclusion criteria, exposure and outcome definitions, outcome ascertainment, sample size, type of effect measures, adjusted effect estimates with 95% confidence interval (CI) or P value, variables adjusted for in the analyses and statistical methods of adjustment for maternal age.

Risk of bias assessment

There is lack of a single obvious candidate tool for assessing quality of observational epidemiological studies.(40) Moreover, as stated by Dekkers et al. in the COSMOS-E (Conducting Systematic Reviews and Meta-Analyses of Observational Studies of Etiology) guideline(41), a 'one size fits all' approach for assessing quality of these studies is probably misguided, considering the large heterogeneity in observational research. Therefore, it has been recommended to develop a set of criteria for each observational systematic review and meta-analysis and to assess risk of bias in a qualitative manner.(41)

For the research question of this systematic review, we distinguished three relevant domains of risk of bias: bias due to confounding, information bias and selection bias (including bias due to loss of follow-up or missing data). Risk of bias was assessed by two reviewers (N.F. and E.L.). For each individual study, risk of bias within domains and across domains was assessed and described.

Statistical analysis

The selected studies reported outcomes in adjusted odds ratios (AORs), adjusted hazard ratios (AHRs) and adjusted rate ratios (ARRs) with 95% confidence intervals (CI) or P values. These effect measures were treated equally as risk measures. When standard errors were not reported, we calculated them from 95% CIs or P values. To assess the effect of paternal age on first trimester miscarriage separately, we performed a second meta-analysis for the subgroup of studies that focused on miscarriage <13 weeks.

Most studies used the age category of 25–29 years as the reference category. Two studies(42-44) used <25 years as reference; for these studies the reported AORs were rescaled by dividing the AOR by the reported AOR in age category 25–29 years.

Meta-analyses were stratified by the following paternal age categories: 30–34, 35–39, 40–44 and ≥45 years (similar to that in Oldereid et al.(13)). If a study reported more subcategories (i.e. 45–49 years and ≥50 years), the effect sizes of these categories were pooled using a within study fixed effect meta-analysis. One study(45) reported one odds ratio for the age category 29–39 years. We used the same estimate for both 30–34 and 35–39 years, and we adjusted the standard errors, assuming equal sample sizes in both categories.

Two studies analysed different combinations of paternal age and maternal age ('couple age'). To obtain overall AORs and ARRs for paternal age categories adjusted for maternal age, a weighted regression analysis (using fixed effect regression meta-analysis software) was performed with the estimated log AOR as dependent variable and paternal age and maternal age categories as independent variables.

Evidence of publication bias was assessed through qualitative inspection of a funnel plot. Statistical heterogeneity among studies was assessed by inspecting the heterogeneity (I2) statistics. Because of heterogeneity of study populations and study designs, random-effects meta-analysis with DerSimonian and Laird estimation was used for the main analysis (command metan in Stata 14: StataCorp LLC, TX, USA). For sensitivity analysis, fixed-effect estimates were calculated as well. A second sensitivity analysis was conducted to evaluate the influence of the study with the most extreme estimates, by repeating the meta-analysis with exclusion of this study.

RESULTS

Study selection

Details of the study selection process are shown in the PRISMA Flow Diagram (Fig. 1). The systematic search retrieved a total of 1343 articles: 1337 were identified by the search strategy and six additional articles were identified by hand searching other sources. After removing duplicates, 975 articles remained for first-stage screening. After first-stage screening by reviewing titles and abstracts, 954 articles were excluded and 21 articles were identified to assess the full text for eligibility. After this second stage of screening, 11 articles were excluded for reasons that are shown in Fig. 1. Finally, 10 articles met all the inclusion criteria. These were included in this review and were potentially appropriate to be included in meta-analysis. One study was excluded from meta-analysis, because of a different reference category and extremely high risk estimates, which is further explained in the narrative synthesis section.

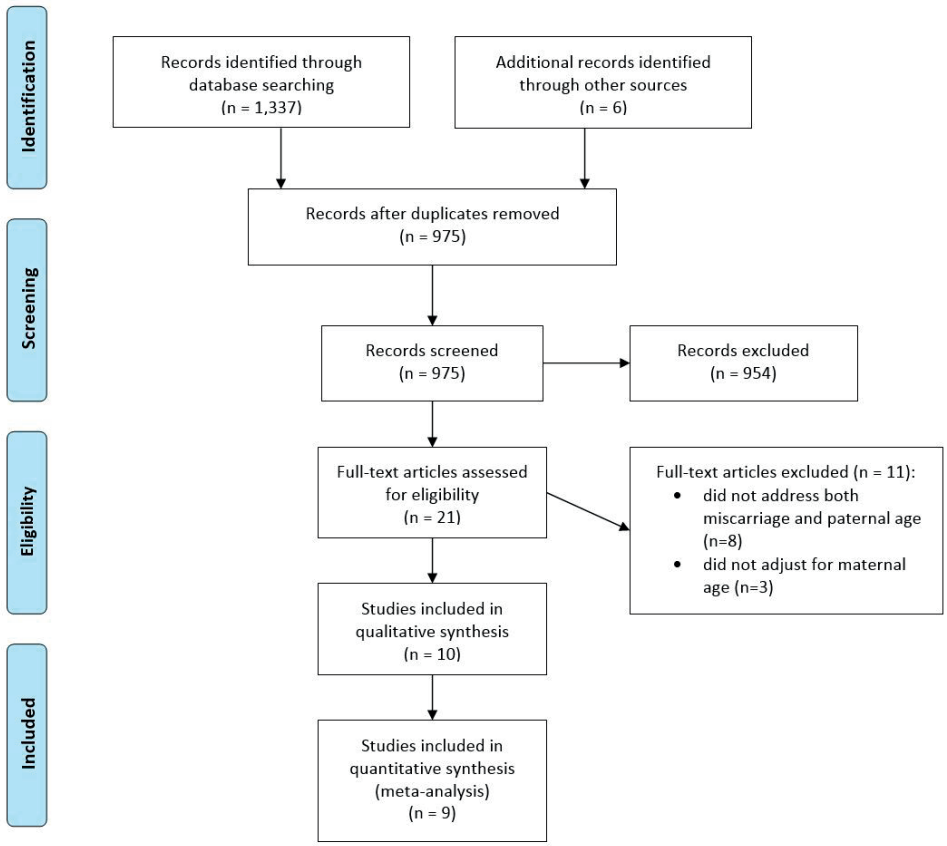

Figure 1. Flow diagram of study selection process

Ten articles met all inclusion criteria and were included in qualitative synthesis. Nine studies were included in the meta-analysis; one study was excluded for reasons explained in the narrative synthesis section.

Study characteristics

Detailed descriptions of key characteristics for all included studies are summarized in Table 1. With regard to the study designs of the ten included studies; four were cohort studies(42, 46-48) and six were case-control studies(43-45, 49-51). Two of the cohort studies(46, 47) were retrospective studies and two were prospective studies(42, 48). Two of the case-control studies were nested case-control studies(44, 49). As shown in Table 1, three studies took place in the USA(42, 44, 51) (one of these studies used data derived from a historic cohort; the Jerusalem Perinatal Study(44)), two in France(46, 47) (one of these studies was based on the European Study of Infertility and Subfecundity, including data from Denmark, Germany, Italy and Spain(46)), and one each in Denmark(48), the UK(49), Japan(45), China(43) and Pakistan(50). Seven studies were population-based(42, 44, 46-49, 51) and three were hospital-based(43, 45, 50). The sample sizes varied from 600 participants in a case-control study(50) to 23,821 in the Danish study by Nybo Andersen et al.(48). Two studies(44, 51) included only spontaneous pregnancies. In three studies(45, 48, 49) a specified proportion of pregnancies (the highest proportion being 13% in the study of Baba et al.(45)) were conceived after ART, while in one study(46) it was stated that part of the population had fertility problems but not further explained. In four other studies(42, 43, 47, 50) the mode of conception was not stated.

Definition of outcome

Miscarriage is defined as the spontaneous demise of intrauterine pregnancy before 24 weeks of gestational age.(52, 53) In the studies selected for this review, miscarriage was defined by different gestational age ranges. Two studies(42, 47) used a lower threshold for five or six weeks of gestational age, while a common upper threshold was 20 weeks(42, 44, 47, 48, 51). Four studies(43, 45, 49, 50) focused on first trimester miscarriages only (<12 weeks or <13 weeks). Two studies(46, 50) did not specifically define gestational age ranges for miscarriage.

Risk of bias

Risk of bias assessment was carried out for each included study, and the results of this assessment are shown in Supplementary Table II.

Bias due to confounding

When evaluating the effect of paternal age on the risk of miscarriage, maternal age is a major confounding factor, being strongly associated with both the exposure and the outcome. Hence, we decided to include only studies in this review that controlled for maternal age. For other factors, it is less evident whether they are confounding the relation between paternal age and miscarriage or whether they are in the causal pathway. For instance, prior miscarriage is a strong risk factor for a subsequent miscarriage. Six studies(43-46, 48, 49) considered this factor as a potential confounder. However, as

stated by Slama et al.(42, 54), a previous miscarriage might have been caused by an elevated paternal age during the previous pregnancy. From that perspective, it should be thought of as an intermediate variable (or a proxy for an intermediate variable) instead of a confounder. Other factors controlled for in some of the selected studies were maternal smoking(42-48) and alcohol consumption(42, 43, 45, 47, 48). Furthermore, some authors did adjust for potential confounding factors such as education level(44), occupational status(45, 48) and ethnicity(51)

Information bias and selection bias

The studies in this review can be subdivided into two types of designs: population-based studies and hospital-based studies. An advantage of large population-based studies(46-48) is a low risk of selection bias, although as a drawback they often have to rely on self-reports of the women regarding their pregnancy outcomes. This means that miscarriages have not been confirmed. In addition, self-reporting could be subject to recall bias or social desirability bias.(55) In hospital-based case-control studies(43, 45, 50), miscarriages are ascertained by hospital diagnosis. However, conducting a study in a hospital setting may introduce a selection bias, since only a subset of women that miscarried is recruited and this subset may not be representative for all women experiencing a miscarriage. Risk of selection bias due to loss to follow-up or missing data was low for all studies.

Narrative synthesis

We included ten studies in this review and seven studies(42, 44, 46, 47, 49-51) found a significant effect of paternal age on the risk of miscarriage. De la Rochebrochard et al.(46) analysed data of 3,174 couples from four European countries about last planned pregnancies that ended in live birth or miscarriage. They stratified paternal and maternal age in 5-year age classes, with 25–29 years designated as the reference group. Maternal and paternal age were analysed together, defined by the variable 'couple age', consisting of a combination of the age classes of both partners. A significant increased AOR for miscarriage was found if the woman was 30–34 years and the man ≥40 years of age, compared to same-aged women and younger men. When we recalculated the reported AORs to obtain AORs for paternal age effects adjusted for maternal age, we found an increased risk for age category 40–64 years, although this was not significant (AOR 1.31; 95% CI 0.75, 2.28).

In a retrospective study by Slama et al.(47) 1,151 randomly selected French women were interviewed about their pregnancy outcomes between 1985 and 2000. The authors developed a survival model to predict the probability of spontaneous miscarriage as a function of the woman's and man's age. This model showed an increased ARR of 1.95 (95% CI 0.97, 3.92) for spontaneous miscarriage in women aged 25 years with a partner of 35 years or older, compared to women aged 25 years whose partner was younger than 35 years.

Table I. Characteristics of included studies

Author, year, country	Study period	Study design	Study setting	Number of pregnancies or cases and controls	Proportion of ART pregnancies	Definition of miscarriage
De la Rochebrochard et al. (2002), France	1991 - 1993	Retro-spective cohort	Population-based (European Study of Infertility and Subfecundity: Denmark, Germany, Italy, Spain)	3,174 pregnancies	Part of study population had infertility problems, otherwise not stated	Not defined
Slama et al. (2003), France	1985 - 2000	Retro-spective cohort	Population-based	2,414 pregnancies	Not stated	Unplanned termination of pregnancy between 5 and 20 weeks

Miscarriage ascertainment			Adjusted risk estimates	Risk factors adjusted for	Methods of adjustment for maternal age
Self-reports	Paternal age	Maternal age	AOR (95% CI)	Country, number of the pregnancy, time to pregnancy, maternal and paternal smoking, history of miscarriage, history of ectopic pregnancy, history of induced abortion	Logistic regression Definition of new variable 'couple age', consisting of classes of maternal and paternal age combinations
	20-29	20-29	1.0 (reference)		
	30-34	20-29	1.06 (0.61-1.86)		
	35-39	20-29	1.31 (0.56-3.07)		
	40-64	20-29	1.80 (0.52-6.24)		
	20-29	30-34	1.72 (0.62-4.74)		
	30-34	30-34	1.62 (0.93-2.82)		
	35-39	30-34	1.06 (0.52-2.17)		
	40-64	30-34	2.90 (1.26-6.67)		
	20-29	35-44	9.18 (1.80-46.66)		
	30-34	34-44	3.87 (1.24-12.02)		
	35-39	35-44	3.38 (1.76–6.47)		
	40-64	35-44	6.73 (3.50-12.95)		
	20-29		*1.0 (reference)*		
	30-34		*0.93 (0.60-1.4)*[a]		
	35-39		*0.68 (0.42-1.12)*[a]		
	40-64		*1.31 (0.75-2.28)*[a]		
Self-reports	Paternal age	Maternal age	ARR (p-value or 95% CI)	Area of recruitment	Discrete time survival model Adjusted for maternal age as continuous variable; used age, age squared and age cubed as covariates in the model
	<25	<20	0.8 (0.64)		
	25-29	<20	0.7 (0.71)		
	30-34	<20	2.6 (0.39)		
	<25	20-24	1.2 (0.52)		
	25-29	20-24	1.0 (reference)		
	30-34	20-24	0.5 (0.23)		
	35-39	20-24	5.3 (0.01)		
	<25	25-29	1.3 (0.61)		
	25-29	25-29	1.1 (0.81)		
	30-34	25-29	0.90 (0.70)		
	35-39	25-29	1.1 (0.91)		
	>40	25-29	0.70 (0.77)		
	25-29	30-34	1.5 (0.27)		
	30-34	30-34	1.2 (0.51)		
	35-39	30-34	1.5 (0.28)		
	>40	30-34	1.5 (0.62)		
	25-29	35-39	7.0 (0.03)		
	30-34	35-39	3.3. (0.01)		
	35-39	35-39	2.2 (0.03)		
	>40	35-39	1.1 (0.91)		
	35-39	>40	1.6 (0.68)		
	>40	>40	11.2 (0.00)		
	20	<35	1.36 (0.98-1.90)	Area of recruitment, maternal smoking, maternal alcohol consumption in first trimester, previous history of urogenital disorder	
	25	<35	1.0 (reference)		
	25	≥35	1.95 (0.97-3.92)		
	30	<35	1.12 (0.93-1.35)		
	30	≥35	1.32 (0.84-2.07)		
	35	<35	2.31 (1.42-3.75)		
	35	≥35	1.40 (0.89-2.20)		
	40	≥35	2.76 (1.51-5.04)		
	42	≥35	4.46 (1.90-10.49)		
	25-29		*1.0 (reference)*	Area of recruitment, maternal age	
	30-34		*0.92 (0.57-1.52)*[a]		
	35-39		*1.21 (0.66-2.22)*[a]		
	>40		*1.01 (0.35-2.92)*[a]		

Table I. Continued

Author, year, country	Study period	Study design	Study setting	Number of pregnancies or cases and controls	Proportion of ART pregnancies	Definition of miscarriage
Nybo Andersen et al. (2004), Denmark	1997 - 1999	Prospective cohort	Population-based (Danish National Birth Cohort Recruitment)	23,821 pregnancies	6% of total study population	Early fetal death <20 weeks
Slama et al. (2005), France	1990 - 1991	Prospective cohort	Population-based (Pregnancy Outcome Study: California)	5,121 pregnancies	Not stated	Spontaneous abortion between 6 and 20 weeks
Kleinhaus et al. (2006), USA	1964 - 1976	Nested case-control	Population-based (Jerusalem Perinatal Study)	Cases: n=1,506 Controls: n=12,359 (live births)	Only fertile women, otherwise not stated	Spontaneous abortion <20 weeks
Maconochie et al. (2007), UK	2001	Nested case-control	Population-based (National Women's Health Study)	Cases: n=603 Controls: n=6,116 (ongoing pregnancy >12 weeks)	Cases: 7% Controls: 3%	Early miscarriage <13 weeks
Baba et al. (2011), Japan	2001 - 2005	Matched case-control	Hospital-based	Cases: n=430 Controls: n=830 (term delivery)	Cases: 13% Controls: 12%	Early miscarriage <12 weeks

Miscarriage ascertainment		Adjusted risk estimates	Risk factors adjusted for	Methods of adjustment for maternal age
Hospital diagnosis	Paternal age	AHR (95% CI)	Maternal age, parity, number of previous abortions, maternal alcohol and coffee consumption during pregnancy, maternal and paternal smoking, maternal and paternal occupational status	Cox regression model Maternal age entered in model in three different ways; using age continuously with restricted cubic splines instead of 5-year or 1-year groups yielded similar estimates for paternal age effects
	≤24	1.17 (0.84-1.63)		
	25-29	1 (reference)		
	30-34	0.86 (0.72-1.03)		
	35-39	0.99 (0.79-1.25)		
	40-44	0.77 (0.55-1.09)		
	45-49	0.97 (0.56-1.69)		
	≥50	1.38 (0.66-2.88)		
Hospital diagnosis	Paternal age	AHR (95% CI)	Maternal age, maternal smoking, maternal alcohol consumption, maternal caffeine consumption, paternal smoking in first trimester	Cox regression model Adjusted for maternal age as continuous variable, using a fractional polynomial approach
	<25	1 (reference)		
	25-29	1.47 (1.04-2.08)		
	30-34	1.25 (0.84-1.88)		
	35-39	1.74 (1.12-1.72)		
	40-44	1.45 (0.85-2.46)		
	≥45	1.87 (1.01-3.44)		
	25-29	*1 (reference)*		
	30-34	*0.85 (0.57-1.28)*[b]		
	35-39	*1.18 (0.76-1.85)*[b]		
	40-44	*0.99 (0.58-1.67)*[b]		
	≥45	*1.27 (0.69-2.34)*[b]		
Self-reports	Paternal age	AOR (95% CI)	Maternal age, maternal diabetes, maternal smoking, history of spontaneous abortions, parity, interval from interview to previous pregnancy, maternal and paternal education, history of induced abortions	Unconditional logistic regression Adjusted for maternal age as a continuous variable; used orthogonal coding of parental ages
	<25	0.59 (0.45-0.76)		
	25-29	1 (reference)		
	30-34	1.4 (1.2-1.6)		
	35-39	1.9 (1.6-2.3)		
	≥40	1.6 (1.2-2.0)		
Self-reports	Paternal age	AOR (95% CI)	Maternal age, year of conception, pregnancy order, history of miscarriage, history of live births	Logistic regression Coding of maternal age not stated
	<25	1.18 (0.80-1.73)		
	25	1 (reference)		
	30	1.05 (0.83-1.33)		
	35	1.22 (0.94-1.59)		
	40	1.04 (0.71-1.53)		
	≥45	1.63 (1.08-2.47)		
Hospital diagnosis	Paternal age	AOR (95% CI)	Maternal age[c], year of the event, history of spontaneous abortion, history of induced abortion, treatment of infertility, maternal BMI, maternal smoking, maternal alcohol consumption, maternal employment, paternal smoking	Conditional logistic regression Matched for maternal age ± 3 years
	<29	1 (reference)		
	29-39	1.14 (0.75-1.74)		
	≥40	1.65 (0.94-2.88)		

Table I. Continued

Author, year, country	Study period	Study design	Study setting	Number of pregnancies or cases and controls	Proportion of ART pregnancies	Definition of miscarriage
Jaleel et al. (2013), Pakistan	2007 - 2010	Case-control	Hospital-based	Cases: n=200 Controls: n=400 (ongoing pregnancy >24 weeks)	Not stated	Early miscarriage (otherwise not defined)
Xu et al. (2014), China	2009 - 2012	Matched case-control	Hospital-based	Cases: n=620 Controls: n=1,240 (ongoing pregnancy >12 weeks)	Not stated	Early miscarriage <13 weeks
Nguyen et al. (2019), USA	2011 - 2015	Case-control	Population-based (National Survey of Family Growth)	Cases: 2,300 pregnancies Controls: 10,410 pregnancies (live birth ≥37 weeks)	Only spontaneous pregnancies	Loss of clinically recognized pregnancy ≤12 weeks and <20 weeks

[a] *Recalculated from the risk estimates reported for the combinations of paternal and maternal age, as described in Statistical analysis;* [b] *Rescaled to reference category 25-29, as described in Statistical analysis;*
[c] *Matched for maternal age (±3 years)*
ART, artificial reproductive technology; AOR, adjusted odds ratio; AHR, adjusted hazard ratio; ARR, adjusted rate ratio; CI, confidence interval

Miscarriage ascertainment		Adjusted risk estimates	Risk factors adjusted for	Methods of adjustment for maternal age
Hospital diagnosis	Paternal age	AOR (95% CI)	Maternal age, paternal genital tract infection	Logistic regression Coding of maternal age not stated
	≤35	1 (reference)		
	36-40	16.44 (6.612-40.896)		
	41-45	13.738 (4.376-43.127)		
	>45	7.042 (1.269-39.090)		
Hospital diagnosis	Paternal age	AOR (95% CI)	Maternal age[c], history of early miscarriage, history of induced abortion, vitamin supplementation, maternal smoking and alcohol consumption, maternal night shift work, frequent staying up late, physical exercise	Conditional logistic regression Matched for maternal age ± 3 years
	<25	1 (reference)		
	25-29	0.94 (0.81-1.28)		
	30-34	1.04 (0.85-1.32)		
	35-39	0.97 (0.79-1.37)		
	≥40	1.16 (0.86-1.42)		
	25-29	*1 (reference)*		
	30-34	*1.11 (0.90-1.40)[b]*		
	35-39	*1.03 (0.84-1.46)[b]*		
	≥40	*1.23 (0.91-1.51)[b]*		
Self-reports	Paternal age	AOR (95% CI)	Maternal age, ethnicity, income, marital status, pregnancy intention	Generalized estimating equations logistic regression Maternal age entered in model in four age categories
	<20 weeks			
	<25	1.03 (0.85-1.25)		
	25-29	1 (reference)		
	30-34	1.04 (0.83-1.29)		
	35-39	1.11 (0.81-1.52)		
	40-44	1.10 (0.70-1.74)		
	45-49	1.49 (0.71-3.13)		
	≥50	2.05 (1.06-3.93)		
	≤12 weeks			
	<25	1.07 (0.86-1.32)		
	25-29	1 (reference)		
	30-34	1.10 (0.86-1.39)		
	35-39	1.08 (0.76-1.52)		
	40-44	1.10 (0.67-1.82)		
	45-49	1.49 (0.65-3.40)		
	≥50	2.30 (1.17-4.52)		

Nybo Andersen et al.(48) used data of 23,281 pregnancies from a Danish prospective cohort study to assess the association between paternal age and fetal death. They stratified for early (<20 weeks of gestation) and late (≥20 weeks of gestation) fetal death. Paternal age was categorised in 5-year age groups with the last group covering ≥50 years. The authors found an increased hazard ratio for early fetal death for fathers ≥50 years (AHR 1.38; 95% CI 0.66, 2.88), using 25–29 years as the reference group. They entered maternal age in three different ways in the model. Treating maternal age continuously with restricted cubic splines instead of 5- or 1-year age groups yielded similar estimates for paternal age effects, implying that there was no strong residual confounding by maternal age. To ensure that the effect of paternal age was not due to confounding by subfertility or infertility, they performed a second analysis restricted to couples who conceived without fertility treatment and they found comparable AHRs.

A second study of Slama et al.(42) with a prospective design assessed the risk of spontaneous miscarriage between 6 and 20 weeks of pregnancy in a Cox model. The risk of spontaneous miscarriage was 1.27 times increased for fathers with a paternal age of 35 years and more, compared to fathers younger than 35 years old (AHR 1.27; 95% CI 1.00, 1.60). When they coded paternal age in smaller age groups (and maternal age continuously, using a fractional polynomial approach), they found the highest risk of spontaneous miscarriage for men aged >45 years (AHR 1.87; 95% CI 1.01, 3.44, reference group men aged 18–24 years). We rescaled the AHRs using 25–29 years as the reference category, and this yielded lower AHRs of 0.99 (95% CI 0.58, 1.67) in category 40–44 and 1.27 (95% CI 0.69, 2.34) in the ≥45-year age group.

In a nested case-control study derived from the Jerusalem Perinatal Study, Kleinhaus et al.(44) compared 1506 couples with previous pregnancy ending in spontaneous miscarriage with a control group comprising 12359 couples with prior live birth. They used paternal age categories of 5 years, with 25–29 years being the reference group. The AORs for miscarriage <20 weeks of gestation for the age groups 30–34 (AOR 1.4; 95% CI 1.2, 1.6), 35–39 (AOR 1.9; 95% 1.6–2.3) and ≥40 years (AOR 1.6; 95% CI 1.2–2.0) were all significantly increased.

Maconochie et al.(49) studied various socio-demographic and behavioural factors in relation to last pregnancy outcomes. Cases consisted of 603 women whose most recent pregnancy was a first trimester (<13 weeks) miscarriage. Controls were 6116 women whose most recent pregnancy had progressed beyond 12 weeks. In fathers ≥45 years of age the AOR for first trimester miscarriage was significantly increased (AOR 1.63; 95% CI 1.08, 2.47; reference group 25–29 years).

Baba et al.(45) and Xu et al.(43) conducted similarly designed studies to identify risk

factors for first trimester miscarriage. These hospital-based case-control studies were matched for maternal age, with total sample sizes of 1290 and 1860, respectively. For fathers aged ≥40, Baba et al. found an AOR for miscarriage of 1.65 (95% CI 0.94, 2.88) and Xu et al. an AOR of 1.16 (95% CI 0.86, 1.42). In both studies, only women who miscarried and were hospitalised for a medical procedure were selected as cases; women with spontaneous miscarriages without additional treatment were not included. Baba et al. used women who underwent term deliveries in the same hospital as controls. The control group of Xu et al. consisted of women who attended the outpatient clinic for prenatal care and were past 13 weeks of gestation.

In a case-control study conducted in a hospital in Karachi, Pakistan, pregnant women aged 20-35 years were included.(50) Cases were women with first trimester miscarriage and controls were those admitted for delivery beyond 24 weeks of gestation. Studied factors were maternal age, paternal age, parental tobacco use and male genital tract infection. The final logistic regression model yielded extremely large effects of paternal age on the risk of first trimester miscarriage compared to all other studies, with AORs of 16.44 (95% CI 6.61, 40.90) in age category 36–40 years, 13.74 (95% CI 4.38, 43.13) in age category 41–45 years and 7.04 (95% CI 1.27, 39.09) in age category >45 years. In contrast to the other studies, paternal age≤35 years and maternal age≤31 years were used as reference categories. The reported data was insufficient to rescale the AORs to reference category 25–29 years, as we did for other studies. Part of the explanation for the deviating risk estimates could be that in this study population, there was less correlation between maternal and paternal ages, meaning there were relatively many couples consisting of older fathers and young mothers. We did not include this study in our meta-analyses, as this study might involve a selected population, reflected by the extreme and potentially unrealistic effects of paternal age that could not be compared to other studies because of the different reference category that was used.

The most recent study of Nguyen et al.(51) used data of 12 710 pregnancies from the US National Survey of Family Growth and assessed the risk of miscarriage <20 and ≤12 weeks separately. They used pregnancies ending in a live birth ≥37 weeks as controls. Pregnancies resulting in spontaneous miscarriage had 2.05 (95% CI 1.06–3.93) times the odds of being from a father aged ≥50 years. For first trimester miscarriage, the AOR for this age category was 2.30 (95% CI 1.17–4.52).

Quantitative synthesis of paternal age effects

The overall meta-analysis (Fig. 2), including nine studies, showed an increasing risk of miscarriage with advancing paternal age. Significant effects in age categories 40–44 years (pooled estimate 1.23; 95% CI 1.06, 1.43) and ≥45 years (1.43; 95% CI 1.13, 1.81) were found. The reference group was 25–29 years for all studies, except for Baba et

al.(45) (<29 years) and de la Rochebrochard et al.(46) (20–29 years).

A second meta-analysis (Fig. 3) was performed including the four studies that were restricted to first trimester miscarriage. A similar pattern of the paternal age effect was found, with a pooled estimate of 1.74 (95% CI 1.26, 2.41) in the highest age category.

In both meta-analyses, there was substantial heterogeneity in the two lower age categories, while in the more advanced age categories the effects across studies were more similar, as indicated by I^2. In Supplementary Fig. S1, funnel plots are displayed for each age category separately, including all nine studies. No clear evidence of small study effects or publication bias was found.

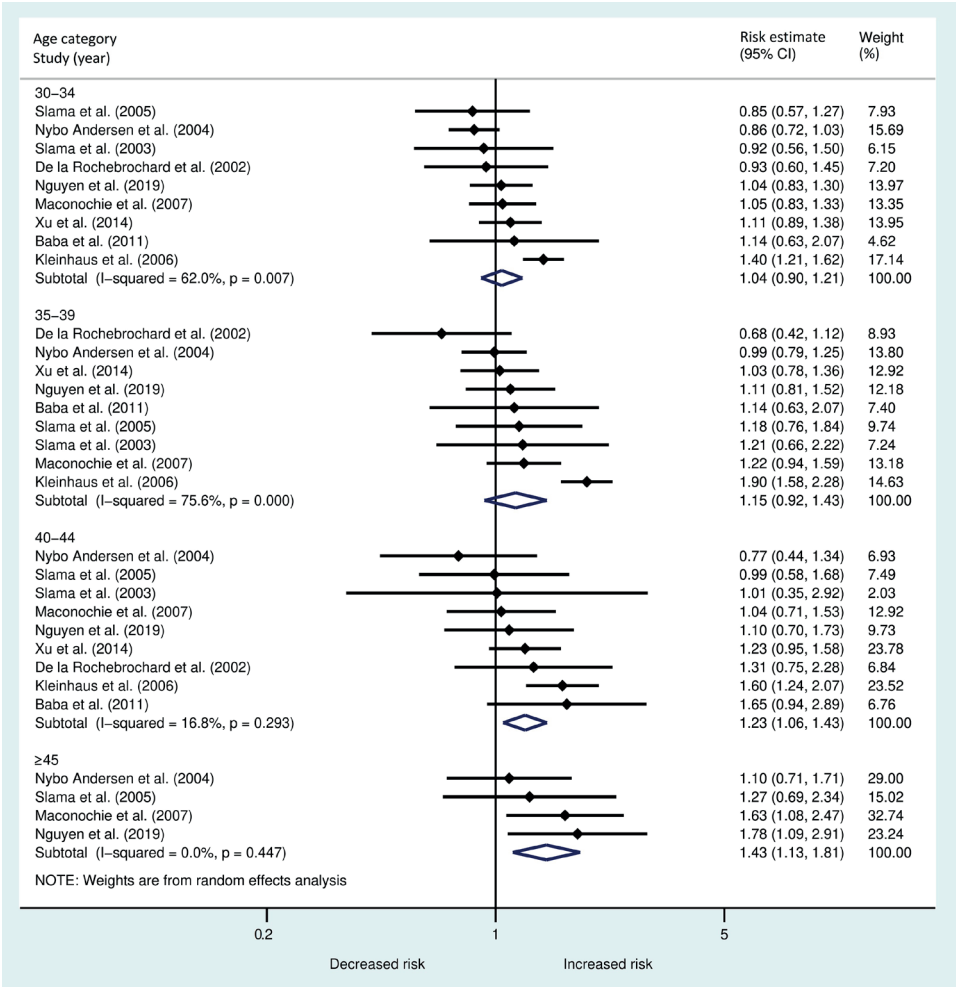

Figure 2. Forest plot describing the association between paternal age in different age categories and the risk of miscarriage <20 weeks

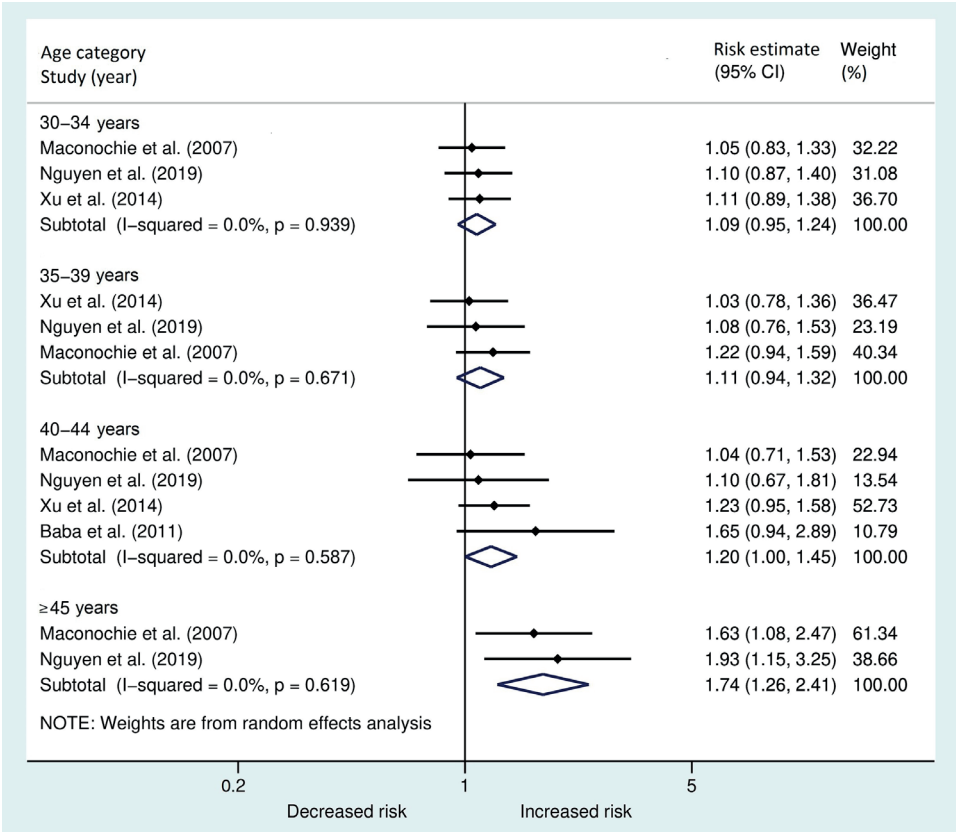

Figure 3. Forest plot describing the association between paternal age in different age categories and the risk of early miscarriage <13 weeks

Maternal age effects

Besides analysis of the paternal age effect, four of the included studies(42, 44, 49, 51) evaluated the effect of maternal age on the risk of miscarriage. They reported risk estimates for the maternal age effect, analysed on the same data as used for the paternal age effect. One study(42) provided risk estimates for maternal age, adjusted for paternal age. The other studies did not adjust the maternal age effects for paternal age. For two studies(46, 47) that analysed combinations of paternal and maternal age (couple-age), it was possible to obtain risk estimates for maternal age categories, adjusted for paternal age (in the same way as performed for the paternal age effect, described in the statistical analysis). Maternal risk estimates with a reference category other than 25–29 years were rescaled to reference category 25–29 years when possible. An overview of maternal age effects on the risk of spontaneous miscarriage is shown in Table II.

Table II. Maternal age effects

Author, year, country		Adjusted risk estimates	Risk factors adjusted for
De la Rochebrochard et al. (2002), France	Maternal age	AOR (95% CI)	Paternal age, country, number of the pregnancy, time to pregnancy, maternal and paternal smoking, history of miscarriage, history of ectopic pregnancy, history of induced abortion
	20-29	*1.0 (reference)*	
	30-34	*1.76 (1.10-2.82)*[a]	
	35-44	*6.49 (4.43-9.51)*[a]	
Slama et al. (2003), France	Maternal age	ARR (95% CI)	Paternal age, area of recruitment
	25-29	*1 (reference)*	
	30-34	*1.34 (0.81-2.20)*[a,b]	
	35-39	*2.39 (1.21-4.69)*[a,b]	
	≥40	*6.23 (1.48-26.17)*[a,b]	
Slama et al. (2005),	Maternal age	AHR (95% CI)	Paternal age, maternal smoking, maternal alcohol consumption, maternal caffeine consumption, paternal smoking in first trimester
	<22.5	1.27 (1.04-1.55)	
	22.5-27.4	1	
	27.5-32.4	0.98 (0.84-1.13)	
	32.5-37.4	1.30 (1.03-1.66)	
	37.5-42.4	2.63 (1.86-3.71)	
	≥42.5	8.80 (4.73-16.73)	
Kleinhaus et al. (2006), USA	Maternal age	AOR (95% CI)	Parity, time interval from index pregnancy to interview, history of miscarriage
	25-29	*1 (reference)*	
	30-34	*2 (1.68-2.36)*[b]	
	≥35	*3.77 (3.05-4.68)*[b]	
Maconochie et al. (2007), UK	Maternal age	AOR (95% CI)	Year of conception, history of miscarriage, history of live birth
	<25	1.09 (0.81-1.45)	
	25-29	1 (reference)	
	30-34	1.06 (0.85-1.31)	
	35-39	1.75 (1.37-2.22)	
	≥40	5.16 (3.54-7.52)	
Nguyen et al. (2019), USA	Maternal age	AOR (95% CI)	Ethnicity, income, marital status, pregnancy intention
	<20 weeks		
	<25	0.89 (0.72-1.10)	
	25-29	1 (reference)	
	30-34	0.98 (0.72-1.33)	
	≥35	1.52 (1.04-2.20)	
	≤12 weeks		
	<25	0.86 (0.69-1.09)	
	25-29	1 (reference)	
	30-34	0.92 (0.68-1.24)	
	≥35	1.66 (1.12-2.44)	

[a] Recalculated from the risk estimates reported for the combinations of paternal and maternal age, as described in Statistical analysis; [b] Rescaled to reference category 25-29, as described in Statistical analysis

Significant effects of maternal age ≥35 years were found in all of the above studies, varying from AOR 1.52 (95% CI 1.04-2.20, age category ≥35 years)(51) to AHR 8.80 (95% CI 4.73-16.73, age category ≥42.5 years)(42).

Because of the small number of studies and substantial differences in adjustments of the estimates and used age categories, a meta-analysis of the risk estimates of the maternal age effect was not performed.

Additional analyses

There were no major differences between the pooled estimates of the paternal age effect provided by models with random and fixed effects (Supplementary Fig. S2).

In the sensitivity analysis excluding the study(44) that consequently yielded relatively extreme estimates, the pooled estimates for the paternal age effect in age categories 35-39 and 40-44 years were slightly decreased (-8%). The pattern of the association between paternal age and risk of miscarriage was similar as observed in the main analysis (Supplementary material 3, Fig. S3).

DISCUSSION

In this systematic review and meta-analysis of 10 population-based cohort and case-control studies, advanced paternal age beyond 40 years was found to be significantly associated with an increased risk of spontaneous miscarriage, adjusted for maternal age. This paternal age effect was also observed in a subgroup of studies focusing on first trimester miscarriage.

A major strength of this systematic review and meta-analysis is that we could increase statistical power by combining data of the extreme paternal age categories of different studies. In the individual studies, the analyses were limited by small patient numbers in the more advanced age groups. Often increased risk estimates were found within these categories, although they were not statistically significant. By pooling the effect measures of different studies, we were able to find significant paternal age effects for both the 40–44 and ≥45 age classes.

It is important to mention that investigating a paternal age effect on the risk of miscarriage is challenging, due to the high level of collinearity between paternal and maternal age. To prevent confounding by maternal age, we only selected studies that did control for this variable. However, residual confounding by maternal age may still be present, especially when maternal age is treated as a discrete variable in broad age classes.(46, 56) We evaluated the methods used for adjustment of maternal age in the included studies. The majority of studies carefully adjusted for maternal age, either by matching cases and controls according to maternal age(43, 45), or treating maternal age as a continuous variable, using orthogonal coding of parental ages(44), a fractional polynomial approach(42, 47) or restricted cubic splines(48). Two studies(46, 51) entered maternal age in their model as a categorical variable and two other studies(49, 50) did not state how they treated maternal age in their models.

Other factors taken into account by several authors in the statistical adjustments were maternal smoking and alcohol consumption. The association of these maternal behaviours with spontaneous miscarriage is well-established(31, 32, 57-59). It is debatable to what extent maternal smoking and alcohol consumption are correlated with paternal age, which is another criterion for considering these factors as confounding factors. When such correlations do indeed exist in a study population, as suggested in some of the articles included in this review(42, 44, 48), these factors could potentially bias the estimated association between paternal age and miscarriage. However, it is conceivable that some of the included studies controlled for too many variables. If a study adjusts for a variable that is, instead of being a confounder, in the causal pathway between paternal age and miscarriage, the total causal effect cannot be consistently

estimated (i.e. the effect will be underestimated).(54, 60-62)

In contrast to the risk of overadjustment bias for maternal factors, there might exist residual confounding by paternal factors. Six of the included studies have taken into account at least one paternal factor other than age(42, 44-46, 48, 50). It is, however, possible that the encountered relation between paternal age and miscarriage is biased by other, unmeasured, paternal characteristics.(63-65).

Apart from the risk of confounding, conducting studies that aim to identify the risk of paternal age on spontaneous miscarriage comes with more challenges. Each study design has its own opportunities and obstacles. Population-based studies typically provide more generalisable results. At the same time, they are prone to information bias since they depend on the women's declaration of miscarriage; especially early miscarriage is hard to establish. Furthermore, as previously suggested by other authors, some of the reported miscarriages may actually have been induced abortions.(44, 46, 47) Hospital-based studies have less of a problem with case ascertainment. Nevertheless, these studies are more susceptible to selection bias since they exclusively recruit women who have received medical service for their miscarriage. From the studies included in this review, the cohort studies appear to have more conservative estimates compared to the case-control studies. This finding does not seem to be clearly related to differences in study setting or patient selection. Some of the case-control studies are population-based and others are hospital-based, while the cohort studies are all population-based. Also, the number of variables adjusted for does not substantially differ between the two clusters of studies. Because of the limited number of studies, especially when stratified per age group, sensitivity analysis on study design or meta-regression was not performed.

Supporting the observed epidemiological associations, it is plausible from a biological perspective that advanced paternal age increases the risk of adverse reproductive outcome. In women, the age-related decline in reproductive capacity is explained by a gradual decrease in ovarian reserve and oocyte integrity(66). More frequent chromosome segregation errors result in oocyte aneuploidy, and this is thought to be primarily responsible for maternal age-related miscarriage. In contrast to the process of oogenesis, where germ cell replication is completed at birth, male germ cells divide continuously throughout a man's reproductive lifespan. From entering puberty on, spermatogenic stem cells divide approximately 23 times per year and by the age of 50 years, more than 800 replications have occurred.(14) Therefore, advancing paternal age most likely increases the probability of replication errors in the germ line, resulting in an accumulation of de novo mutations.(15) This process is exacerbated when DNA repair mechanisms are also deteriorating with age.(67) Kong et al. performed whole genome

sequencing on 78 trios of parents and their children and demonstrated a clear association between advanced paternal age and increased number of de novo genetic mutations in the offspring, probably contributing to autosomal dominant disorders and complex disorders such as autism spectrum disorders.(13, 15) Advanced paternal age may also be linked to increased sperm aneuploidy; however, inconsistent findings have been reported in the literature.(68-70) It is suggested that due to continual spermatogenesis, the male gamete is less vulnerable to age-related non-disjunction aneuploidies than its female counterpart.(71)

The influence of paternal age on miscarriage is perhaps acting mostly at the level of sperm DNA integrity. Multiple studies have shown elevated levels of sperm DNA fragmentation in older men, with a more than doubling DNA fragmentation index (DFI) between 20 and 60 years old.(72-74) This is probably due to a combination of age-related mechanisms and inherent characteristics of spermatozoa, such as accumulation of reactive oxygen species, absence of antioxidant capacity and paucity of DNA repair mechanisms.(75) Although conventional sperm parameters such as volume, motility and morphology are declining with rising paternal age(76), those are relatively poor predictors of male fertility potential and miscarriage.(77, 78) In contrast, sperm DNA fragmentation seems directly associated with reproductive outcome. There is solid evidence that an increased level of sperm DNA fragmentation is associated with (recurrent) pregnancy loss.(79-82) In the case of fertilisation, sperm DNA fragmentation can to some extent be repaired by the oocyte. However, with advancing age the oocyte quality is deteriorating, together with its repair capacity.(83) This supports the hypothesis that the impact of paternal age on miscarriage, mediated by increased DFI, is more present in interaction with higher maternal age. This is in line with epidemiological studies that demonstrated such an interaction between advanced paternal and maternal age for the risk of miscarriage.(46) Furthermore, a recent study in IVF/ICSI couples observed a higher miscarriage rate in women beyond 35 years and partners with high sperm DFI, compared to couples with similarly high sperm DFI and younger women.(84) It is noteworthy that quality of sperm, measured either by conventional parameters or DNA integrity, has not been taken into account by any of the studies included in this review. An ongoing prospective study is currently investigating the predictive role of sperm DNA damage in RPL, as well as the relation with paternal age and lifestyle factors.(85)

In this review, we excluded studies that were restricted to couples who conceived after ART, since we were interested in the association between paternal age and miscarriage in the general population. The relationship between advanced parental age, infertility and miscarriage is complex. In some studies, miscarriage rates appear to be higher among ART pregnancies compared to natural pregnancies(86), however, this is not easily interpreted. Assisted pregnancies are usually closely monitored and, as a consequence,

pregnancy losses, especially from early stages, will probably be detected more often than in the general population. In addition, ART-treated couples are generally of more advanced age, which predisposes them to an increased risk of miscarriage. For these reasons, it is difficult to distinguish whether an increased risk of miscarriage in couples receiving fertility treatment is a consequence of the treatment itself, or due to underlying patient characteristics. Studies investigating the effect of paternal age on miscarriage after different forms of ART reported inconclusive results.(87-93) These contradictory data may be explained by the heterogeneity of these studies, the small proportions of older men they included and by the exclusion of women with advanced age or using young oocyte donors in some studies.(71) Furthermore, studies that did not observe an effect of paternal aging on the risk of pregnancy loss were mainly in IVF/ICSI pregnancies from a very heterogeneous population of men with extensive variations in sperm parameters and cause and severity of infertility, which may have diluted an age effect.(94)

While advanced maternal age is generally agreed upon as age≥35, there is currently no consensus for the definition of advanced paternal age. However, ageing is a complex process and it is hard to determine a clear cutoff point, the more because age effects are likely to occur gradually and thresholds are not necessarily the same for all different outcomes that are affected by paternal age. Most studies suggest that infertility and reproductive risks start to increase after the paternal age of 40.(95) This is in accordance with the results of our meta-analyses. Based on our findings, it should be considered to counsel couples with older males about the increased risk of miscarriage at preconception visits. Furthermore, our results are of value for patients with recurrent miscarriages. This condition remains unexplained in the majority of cases(36, 37), and for a proportion of the idiopathic cases, advanced paternal age could be responsible. Currently, there are no studies that did specifically focus on the relation between paternal age and recurrent miscarriages and this should certainly be addressed in future research. Although it is challenging to distinguish paternal age effects from maternal age effects, most studies included in this review made relevant efforts and collectively they suggest the existence of an, albeit small, independent effect of paternal age on the risk of spontaneous miscarriage. Since there are strong biological hypotheses for this paternal effect, it is likely that future studies will establish it even more. Both large population-based registry studies and hospital-based case-control studies may help to validate the paternal age effect on pregnancy loss, provided that they carefully control for maternal age in their statistical analyses. There is a trend toward delayed childbearing in western societies and it has become more common to father children at older age.(96). Hence, we consider it important to not merely focus on the effects of maternal aging on reproductive outcome, but to be aware of risks associated with advanced paternal age as well.

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