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ORIGINAL ARTICLE *Reproductive epidemiology*

Risk of hospitalization for early onset of cardiovascular disease among infertile women: a register-based cohort study

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STUDY QUESTION: Is female infertility predictive of a woman's future risk of early cardiovascular disease (CVD)?

SUMMARY ANSWER: Female infertility does not seem to be predictive of early CVD during a mean follow-up of 9 years.

WHAT IS KNOWN ALREADY: Associations between infertility and comorbidity have been found in several studies, but data on the association between female infertility and risk of CVD are scarce and inconclusive.

STUDY DESIGN, SIZE, DURATION: In this nationwide cohort study, we included 87 221 women registered in the Danish National IVF register, undergoing medically assisted reproduction (MAR) between 1st of January 1994 and 31st of December 2015. The cohort was followed for incident hospitalization due to CVD in the Danish National Patient Register from enrollment to 31 December 2015. Women with a history of CVD prior to enrollment were excluded. Cox proportional hazard models with age as the underlying time scale were used to estimate hazard ratios (HR) with 95% CI of CVD among women with an infertility diagnosis, compared to women without an infertility diagnosis. All analyses were adjusted for educational attainment.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Female infertility and the reason for infertility was diagnosed and registered in the IVF register by specialists in Danish public and private fertility clinics since 1st of January 1994. In our cohort, 53 806 women (61.7%) were diagnosed with female factor infertility, while 33 415 (38.3%) did not have a female factor infertility diagnosis and made up the reference group.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 686 (1.3%) infertile women were hospitalized for CVD compared to 250 (0.7%) among women without an infertility diagnosis during a mean follow-up time of 9 years. We found no increased risk of early CVD in our analyses (adjusted HR 0.98, 95% CI: 0.85;1.14). Likewise, analyses stratified by specific infertility diagnosis, showed no risk difference.

LIMITATIONS, REASONS FOR CAUTION: We were unable to adjust for confounding parameters such as body mass index, cigarette smoking or alcohol consumption. These results may not be generalizable to infertile women who do not seek out fertility treatment, or infertile women with other lifestyle characteristics than Danish women.

WIDER IMPLICATIONS OF THE FINDINGS: Diagnosing female infertility or the time of MAR does not seem to be a window of opportunity where early screening for cardiovascular disease risk factors can have a prophylactic potential.

STUDY FUNDING/COMPETING INTEREST(S): This study is part of the ReproUnion collaborative study, co-financed by the European Union, Interreg V ÖKS. None of the authors declare any conflict of interest.

Key words: female infertility / comorbidity / cardiovascular disease / infertility / register-based cohort study

Introduction

Cardiovascular diseases (CVDs) are responsible for one in three deaths worldwide (World Health Organization (WHO), 2017). Female infertility is a disease of the reproductive system that affects many women and couples worldwide (Boivin *et al.*, 2007). Female infertility and CVD share some common pathways, through endocrine conditions, such as polycystic ovarian syndrome (PCOS), menstrual irregularities, endometriosis, hypothyroidism and metabolic syndrome (Solomon *et al.*, 2002; Flynn *et al.*, 2006; Wang *et al.*, 2011; Kvaskoff *et al.*, 2015; Awlaqi *et al.*, 2016; Delitala *et al.*, 2017; Glintborg *et al.*, 2018). Psychological stress can be a mediating factor between infertility and CVD, as infertility can induce psychological stress (Rooney and Domar, 2018), which may increase the risk of CVD (Steptoe and Kivimäki, 2012). Also, genetic alterations, epigenetic disturbances and exposure to endocrine disrupters are hypothesized to affect both reproductive health and the risk of comorbidity and mortality (Tarín *et al.*, 2015). As with male infertility (Glazer *et al.*, 2017), associations between female infertility and comorbidities have been reported (Tobias *et al.*, 2015; Hanson *et al.*, 2017), and infertility was suggested as a potential early biomarker of future health risks (Cedars *et al.*, 2017; Senapati, 2018).

However, studies on the association between female infertility and CVD are scarce and the association is only studied in study populations where female infertility is either self-reported or subject to risk of misclassification. A Swedish cohort study with a median follow-up time of 11.9 years found that women who reported at least 5 years of subfertility prior to successful pregnancy had an increased risk of CVD later in life, compared to women with a history of subfertility for less than 5 years (Parikh *et al.*, 2012). Another cohort study with a median follow up time of 9.7 years found no increased risk of coronary heart disease (CHD) or stroke among women who had received medically assisted reproduction (MAR) 2 years prior to delivery (Udell *et al.*, 2013). A third cohort study with a median follow up of 8.6 years found higher risk of hypertension, but not higher risk of diabetes, CHD and stroke, among women who had received successful MAR treatments prior to delivery compared to women who delivered after natural conception (Westerlund *et al.*, 2014). However, it may be difficult to draw strong conclusions from these studies as they use proxies such as couple's infertility for female infertility, which may be solely due to male factor in many cases. In addition, all studies only included women who eventually became mothers which is of concern as women who remain childless and women who receive unsuccessful MAR treatment may have higher risk of CVD (Lawlor *et al.*, 2003; Parikh *et al.*, 2010; Udell *et al.*, 2017). A recent cross-sectional analysis by Gleason et al. (2019) showed that self-reported infertility was associated with metabolic syndrome and CVD. Other cross-sectional studies have investigated CVD risk factors (e.g. hyperlipidemia, diabetes, hypertension) among infertile women or women with self-reported infertility, but these studies have also been conflicting and inconclusive (Verit *et al.*, 2014, 2017; Farland *et al.*, 2015; Kurabayashi *et al.*, 2016; Mahalingaiah *et al.*, 2017), and reverse causation is impossible to rule out due to cross-sectional designs. These few and conflicting results underline the need for large prospective studies on incident CVD among infertile women.

This nationwide cohort study will be the first to explore the association of register-based diagnoses of female factor infertility and incident register-based diagnoses of early CVD, with adjustment for known confounding factors including PCOS and endometriosis.

The aim of this study is to contribute to the investigation of female infertility as a potential early biomarker of future health risks and investigate whether early screening for CVD risk factors at the time of MAR may have a prophylactic effect on early CVD events.

Materials and Methods

Setting and data source

In Denmark, heterosexual and lesbian couples and single women are offered MAR. In public fertility clinics, treatment is tax financed and treatment is offered up to female age of 40 years, to couples not having a common child or to childless single women. The private fertility clinics offer treatment up to female age of 45 years. Prior to 1st of January 2007 medical doctors were permitted only to offer treatment to heterosexual cohabiting or married couples. Around 50% of all treatments are performed at the public fertility clinics. It is mandatory by Danish law for all fertility clinics (public and private) to report data for each treatment cycle to the national IVF register established in 1994. Until 2008, the IVF register include data on ART treatments (i.e. only *in vitro* treatment methods). Since 2008, the IVF register additionally includes data on *in vivo* methods as well (e.g. intrauterine insemination treatment cycles, and ovulation induction).

Study population

We defined a cohort of all women in the Danish national IVF register who received MAR from 1st of January 1994 to 31st of December 2015. The women had to live in Denmark and have a unique personal identification number (social security number) at the time of inclusion in the IVF register. Data regarding emigration and death were obtained from the Danish Central Person Register (Pedersen, 2011).

The study was approved by Danish Data Protection Agency J. no.: BFH-2015-091. According to Danish legislation, register-based studies without direct contact to individuals do not require approval from the scientific ethics committee.

Information on female infertility

Information on female infertility was retrieved from the Danish National IVF register which was established in 1994 and updated to an electronic version in 2006 (Andersen *et al.*, 1999; Blenstrup and Knudsen, 2011). Important differences between the two versions of the register exist. In the version covering 1994–2005, female infertility was registered as ovulation defect (yes/no), tubal factor (yes/no), and cervico-vaginal uterine factor (yes/no). Couples infertility and male factor infertility was registered as other factors (yes/no), idiopathic (yes/no) and male factor (yes/no). In the version covering 2006 and onwards, infertility diagnoses were obtained through diagnosis codes based on the following International Classification of Diseases −10 (ICD-10) codes: female infertility associated with anovulation (N970), female infertility of tubal origin (N971), female infertility of uterine origin (N972), female infertility of cervical origin (N973), female infertility of other origin (N978), female infertility of ovarian origin (N978B), and female infertility unspecified (N979). To match the category of cervico-vaginal uterine factor (yes/no) from the first version of the register, female infertility of uterine origin (N972) and female infertility of cervical origin (N973) were combined, while

Table I Baseline characteristics of women (*N* = 87 221) with and without female infertility registered in the Danish IVF register 1994–2015.

		Female infertility (*n* = 53 806)	No female infertility (*n* = 33 415)
	n		
Age (years) mean, (SD)	87 221	32.6 (4.8)	31.9 (4.8)
Follow-up time (years) mean, (SD)	87 221	9.7 (6.3)	7.5 (5.3)
Highest level of education	83 320		
Primary school (%)		14	12
High school or equivalent (%)		8	8
Skilled trade (%)		37	36
Bachelor or equivalent (%)		27	28
University degree (%)		14	16
Marital status	70 628		
Male partner (%)		87	100
Single (%)		11	0
Female partner (%)		2	0
Female infertility diagnosis *	87 221		
Idiopathic infertility (%)		38	
Tubal factor † (%)		27	
Ovulation/ovarian factor ‡ (%)		19	
Other reason for infertility (%)		18	
Cervico-vaginal uterine factor § (%)		1	

*The percentages of the adverse female infertility diagnoses adds up to more than 100% because a women could have more than one infertility diagnosis in the first version of the IVF register.
†In 1994–2005 defined as tubal factor yes/no. In 2006–2015 defined as ICD10 N971.
‡In 1994–2005 defined as ovulation defect yes/no and ovarian factor yes/no. In 2006–2015 defined as ICD10 N970 for ovulation defect and N978B for ovarian factor.
§In 1994–2005 defined as cervico-vaginal uterine factor yes/no. In 2006–2015 defined as ICD10 N972 for uterine factor and N973 for cervico-vaginal factor.

female infertility associated with anovulation (N970) and ovarian origin (N978B) were combined to match the category of ovulation defect from the first version of the register. From the second version of the register information on PCOS (DN80) and endometriosis (DE282) was also available. Women in the Danish IVF register without a diagnosis of infertility made up the reference group. For both register periods, the infertility diagnosis of the initial examination date was used.

Diagnosis of cardiovascular disease

Information on hospitalization for cardiovascular disease was obtained from the Danish National Patient Register (NPR), which holds information on diagnoses and treatments for all patients admitted to any public or private hospital (Lynge *et al.*, 2011). Diagnoses related to cerebrovascular disease (ICD 8 codes [1977–1993] 430-434 and ICD10 codes [1994–2015] I60-I65) and ischemic heart diseases (ICD 8 codes 410-412 and ICD 10 codes I21-I25) were extracted.

Covariates

Potential confounders were identified *a priori* using directed acyclic graphs (Greenland *et al.*, 1999). Highest attained education—the most appropriate measure of socioeconomic status for young adults who have yet to establish themselves occupationally (Galobardes *et al.*, 2006), was obtained from Statistics Denmark. Highest attained education can also be a marker of cognitive functioning, receptiveness to health messages and the ability to communicate with and access appropriate health services (Galobardes *et al.*, 2006; Jensen and Rasmussen, 2011). Endometriosis and PCOS diagnoses were available from the version of the IVF register covering 2006–2015. Since female infertility diagnoses was registered differently in the two register periods of the Danish National IVF register (1994–2005 and 2006–2015), an indicator variable of registration period was included in the model.

Statistical analyses

Cox proportional hazard models with age as underlying time scale were used to estimate hazard ratios (HR) with 95% CI of incident CVD among women with an infertility diagnosis compared to the reference group of women without an infertility diagnosis. Women with a history of CVD prior to enrollment were excluded. The women in the cohort were considered at risk from enrollment date and censored at the time of hospitalization due to CVD, death, emigration, disappearance or end of follow-up on 31st of December 2015. We

Table II Hazard ratios (HR) with 95% CI for cardiovascular disease in infertile and fertile women registered in the Danish IVF register 1994–2015.

All cardiovascular disease *			
		Crude	**Adjusted §**
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility	250 (0.7%)	1 (ref)	1 (ref)
Female infertility	686 (1.3%)	1.04 [0.90;1.20]	0.98 [0.85;1.14]
Ischemic heart disease †			
		Crude	**Adjusted §**
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility	111 (0.3%)	1 (ref)	1 (ref)
Female infertility	321 (0.6%)	1.02 [0.82;1.26]	0.95 [0.76;1.19]
Cerebrovascular disease ‡			
		Crude	**Adjusted §**
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility	146 (0.4%)	1 (ref)	1 (ref)
Female infertility	384 (0.7%)	1.04 [0.86;1.27]	0.99 [0.82;1.21]

ref = reference
*Defined as ischemic heart diseases and/or cerebrovascular diseases. Ischemic heart diseases were defined as International Classification of Diseases - 10 (ICD-10) I21, I22, I23, I24 and/or I25, and/or as ICD 8410, 411 and/or 412. Cerebrovascular diseases were defined as ICD 10 I60, I61, I62, I63, I64 and/or I65, and/or ICD 8430, 431, 432, 433 and/or 434.
†Defined as ICD 10 I21, I22, I23, I24 and/or I25, and/or as ICD 8410, 411 and/or 412 for ischemic heart diseases.
‡Defined as ICD 10 I60, I61, I62, I63, I64 and/or I65, and/or ICD 8430, 431, 432, 433 and/or 434 for cerebrovascular disease.
§Adjusted for highest level of education ($n = 83\,320$) and registration period (1994–2005 ($n = 32\,423$) or 2006–2015 ($n = 54\,798$)).

estimated both crude and adjusted HRs adjusting for highest attained education and registration period (1994–2005 and 2006–2015). In secondary analyses, we further adjusted for PCOS and endometriosis, available from 2006–2015, tested for interaction between female infertility and PCOS or between female infertility and endometriosis, and we performed analyses stratifying by type of infertility diagnosis.

Kaplan–Meier plots on each type of female infertility allowed for visual evaluation of proportional hazards assumption. All statistical tests were performed using SAS software (version 9.4 SAS Institute, Cary, NC).

Results

In our cohort of 87 221 women, 62% were diagnosed with female factor infertility and 38% had no infertility diagnosis and made up the reference group. The mean age at baseline was similar between the infertile women and the reference group (32.6 vs. 31.9 years, respectively). The mean follow-up time was 9.7 years among infertile women and 7.5 years among the reference group, and the overall mean follow-up time was 8.9 years. Highest level of education was similar between the groups, with skilled trade representing the largest group. Idiopathic infertility was most the commonly reported female infertility diagnosis (38%), followed by tubal factor (27%) (Table I).

A total of 686 (1.3%) infertile women had new cases of hospitalization due to CVD compared to 250 (0.7%) cases among fertile women. Risk of early CVD was similar in women with and without a diagnosis of infertility (adjusted HR: 0.98 (95% CI: 0.85;1.14)) (Table II). Also, when estimating risk of ischemic heart disease and cerebrovascular disease separately, there was no difference in risk (Table II). We found no interaction between female infertility and PCOS or between female infertility and endometriosis. Adjustment for PCOS and endometriosis showed no increased risk with an adjusted HR 1.04 (95% CI: 0.80;1.37) (Table III). Likewise, no increased risk was found when stratifying by type of infertility diagnosis (Table IV).

Discussion

Main findings

In this nationwide cohort study of 87 221 women identified in the Danish IVF register in 1994–2015, and subsequently followed for hospitalization due to early cardiovascular events during a mean follow-up time of 9 years, we found no association between female infertility and risk of early ischemic heart disease or cerebrovascular disease.

Table III Hazard ratios (HR) with 95% CI for cardiovascular disease adjusted for PCOS and endometriosis in infertile women in the Danish IVF register 2006–2015.

All cardiovascular disease *		Crude	Adjusted †
	n cases	HR [95% CI]	HR [95% CI]
No female infertility (*n* = 24 118)	89 (0.4%)	1 (ref)	1 (ref)
Female infertility (*n* = 30 680)	145 (0.5%)	1.08 [0.83;1.41]	1.04 [0.80;1.37]

*Defined as ischemic heart diseases and/or cerebrovascular diseases. Ischemic heart diseases were defined as ICD 10 I21, I22, I23, I24 and I25, and/or as ICD 8410, 411 and/or 412. Cerebrovascular diseases were defined as ICD 10 I60, I61, I62, I63, I64 and/or I65, and/or ICD 8430, 431, 432, 433 and/or 434.
†Adjusted for highest level of education, PCOS and endometriosis (*n* = 51 630).

Prior literature

While several studies have examined the association between proxies of female infertility and subsequent CVD or CVD-related risk factors (Lawlor *et al.*, 2003; Parikh *et al.*, 2010, 2012, Udell *et al.*, 2013, 2017; Westerlund *et al.*, 2014; Gleason *et al.*, 2019), this study is the first to relate register-based diagnosed female infertility to incident register-based CVD diagnoses.

Our findings are consistent with Swedish (*n* = 140 458) and Canadian (*n* = 1 186 753) cohort studies finding no increased risk of CVD events, with a follow-up time of 8.6 and 9.7 years, respectively (Udell *et al.*, 2013; Westerlund *et al.*, 2014). Westerlund et al. (2014) did however, report a higher incidence of hypertension indicating a risk profile for CVD. However, these studies were based only on women who had delivered after MAR, a proxy for female infertility, which can have biased results as the reason for IVF treatment of these women may have been male factor infertility. Further, only women with successful IVF treatment resulting in live birth were included, and Udell *et al.* (2017) (*n* = 28 442) reported that women who had unsuccessful fertility therapy had an increased risk for cerebrovascular events, compared to those who had successful therapy during a mean follow up time of 8.4 years (Udell *et al.*, 2017). Moreover, Udell *et al.* (2013) and Westerlund *et al.* (2014) used external reference groups, which may cause bias as Vassard et al. (2018) recently reported lower mortality risks among IVF treated women, compared to age-matched women in the background population. Vassard *et al.* (2018) attributed this difference to a healthy patient effect, as good general health is a prerequisite for fertility treatment. In our study, we included all women in the IVF register, regardless of the outcome of treatment, and we used an internal reference group, limiting a possible healthy patient effect.

Our findings are in contrast with a Swedish cohort study (*n* = 863 324), relating self-reported infertility for more than 5 years prior to childbirth to an increased risk of CVD after adjusting for smoking, BMI and other well-known potential confounders (Parikh *et al.*, 2012). A recent cross-sectional study (*n* = 744) conducted among 20–59-year-old US women with self-reported infertility, reported higher risks of metabolic syndrome and cardiovascular events. However, due to the cross-sectional design of this study, it is difficult to rule out reverse causation (Gleason *et al.*, 2019). Also, nulliparous women have been suggested to have an increased risk of CVD when compared to women with two children (Lawlor *et al.*, 2003; Parikh *et al.*, 2010; Oliver-Williams *et al.*, 2018), and parity may well reflect reproductive health (Kok *et al.*, 2003). However, when using self-reported infertility, which can be a reliable measure of a couple's fertility, and nulliparity, which may be due to reasons other than female infertility, as proxies of female infertility, considerable exposure misclassification can be the result. In our study, we conducted analyses on women who had gone through a clinical fertility examination that limits the risk of exposure misclassification (i.e. limiting the chance of male infertility as the reason for infertility or nulliparity) as female infertility was diagnosed by fertility specialists.

Two previous cohort studies found menstrual cycle irregularities, a known cause of infertility (Kok *et al.*, 2003), to be associated with an increased risk of CHD (Solomon *et al.*, 2002; Wang *et al.*, 2011). Both studies argue that the association could be due to an excess of women with PCOS in the studied population, which is a known cause of menstrual cycle irregularities and predisposes to an elevated CVD risk (Solomon *et al.*, 2002; Glintborg *et al.*, 2018). Another known cause of infertility is endometriosis—a condition that has been linked to increased risk of CHD, hypercholesterolemia and hypertension possibly through shared genes and higher levels of systemic chronic inflammation (Mu *et al.*, 2016, 2017). In our study, however, adjusting for endometriosis and PCOS did not change the risk estimate for CVD among women with a generalized female infertility diagnosis (Table III).

In view of our results and others who have published within this field, there is limited data to support an association between female infertility and CVD. This is interesting to note as reports on the male side (Eisenberg *et al.*, 2016), have linked male factor infertility to an increased risk of cardiovascular disease. These differences in risk profiles between men and women may imply different underlying mechanism, which warrants further investigation.

The hypothesis that infertile women may constitute a CVD risk population was not supported by our results. However, as CVDs often are conditions where the risk builds up over several years through atherosclerosis, a longer time of follow-up and the chance to follow these women into and after menopause will provide a more complete answer to the research question. Studies with a long follow-up time relating female infertility to CVD should also be conducted in populations with other lifestyle characteristics than Danish women seeking MAR. Nevertheless, if female infertility is indeed a biomarker of future health risk, despite our results and previous inconclusive reports, it does not seem to be at the time of initiating MAR treatment. However,

Table IV Hazard ratios (HR) with 95% CI for cardiovascular disease in groups with different female infertility diagnoses registered in the Danish IVF register 1994–2015.

All cardiovascular diseases *			
		Crude	**Adjusted** †
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility (*n* = 33,415)	250 (0.8%)	1 (ref)	1 (ref)
Female infertility due to ovulation/ovarian defect (*n* = 10,118)	89 (0.9%)	1.11 [0.87;1.41]	1.04 [0.81;1.33]
		Crude	**Adjusted** ‡
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility (*n* = 33,415)	250 (0.8%)	1 (ref)	1 (ref)
Female infertility due to tubal factor (*n* = 14 639)	358 (2.5%)	1.12 [0.95;1.33]	1.02 [0.85;1.23]
		Crude	**Adjusted** §
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility (*n* = 33,415)	250 (0.8%)	1 (ref)	1 (ref)
Female infertility due to other reasons (*n* = 9414)	84 (0.9%)	0.97 [0.75;1.23]	0.94 [0.73;1.21]
		Crude	**Adjusted** **
	***n* cases**	**HR [95% CI]**	**HR [95% CI]**
No female infertility (*n* = 33,415)	250 (0.8%)	1 (ref)	1 (ref)
Idiopathic female infertility (*n* = 20,484)	184 (0.9%)	0.92 [0.76;1.11]	0.91 [0.75;1.11]

*Defined as ischemic heart diseases and/or cerebrovascular diseases. Ischemic heart diseases were defined as ICD 10 I21, I22, I23, I24 and I25, and/or as ICD 8410, 411 and/or 412. Cerebrovascular diseases were defined as ICD 10 I60, I61, I62, I63, I64 and/or I65, and/or ICD 8430, 431, 432, 433 and/or 434.
†Adjusted for highest level of education (*n* = 41 354) and registration period (1994–2005 (*n* = 12 891) or 2006–2015 (*n* = 30 642)).
‡Adjusted for highest level of education (*n* = 45 700) and registration period (1994–2005 (*n* = 20 895) or 2006–2015 (*n* = 27 159)).
§Adjusted for highest level of education (*n* = 40 465) and registration period (1994–2005 (*n* = 12 087) or 2006–2015 (*n* = 30 742)).
**Adjusted for highest level of education (*n* = 51 270) and registration period (1994–2005 (*n* = 15 591) or 2006–2015 (*n* = 38 308)).
***There was not sufficient power to do the sensitivity analysis among women with female infertility due to cervico-vaginal uterine factor.

we cannot rule out that such an association may appear later in life which warrants further investigation.

Strengths and limitations

The major strength of this study is the size, the ability to identify women with diagnosed infertility and the follow-up for CVD events, using national health registers. A recent study on the NPR has shown the validity of CVD diagnoses in the register to be high and the positive predictive values of diagnoses relating to ischemic heart diseases were >90% (Sundbøll *et al.*, 2016). We chose to investigate the incidence of ischemic heart disease and cerebrovascular disease, which we in any case assume to be diagnoses with high specificity and sensitivity. Also, we used an internal reference group which we believe to be a strength. If an external reference group was used, a lower risk of CVD among infertile women would likely be observed, as Vassard et al. (2018) observed lower mortality among this group. However, this means we may have overestimated the risk, despite the null finding of our study, but we believe using an internal reference group limits the source of confounding and limits the possible isolated effect of MAR, as all women in our cohort likely have received some hormonal therapy.

The limitations of this study are firstly that we were unable to adjust for lifestyle factors such as metabolic syndrome, which may affect both fertility and the risk of CVD, or even act as an intermediate step between infertility and CVD. Also, we did not adjust for BMI and cigarette smoking, as these variables only were available from 2006–2015, and in this subpopulation 21% and 22% (respectively) had missing values. We did, however, adjust for educational level, which is a marker of socioeconomic status and closely related to lifestyle, and this may have limited this source of confounding. Secondly, the accuracy of specific infertility diagnoses in the IVF register may be limited, as single women and homosexual women appearing in the IVF register might be subject to exposure misclassification as almost all these women were diagnosed with either 'infertility due to other reasons' or 'idiopathic infertility'—yet some of these women's appearance in the IVF register is related to their desire for access to the use of screened donor semen and treatment at a fertility clinic. This exposure misclassification might also be the explanation for the slightly reduced risk of CVD seen among women diagnosed with either 'infertility due to other reasons' or 'idiopathic infertility' (Table IV). However, when we limited sensitivity analysis to heterosexual couples only and stratified by female infertility diagnosis, results did not change significantly. Thirdly, we did account for parity nor differentiate between women with successful and unsuc-

cessful MAR. This is important to consider in view of results from Udell et al. (2017) indicating that women with unsuccessful treatment have a higher risk of CVD compared to women with successful treatment. However, a recent prognosis of live birth rates for couples undergoing MAR in Denmark showed that up to 80% succeed during a period of 5 years, but prognosis was strongly influenced by female age (Malchau *et al.*, 2017). With a mean follow up of 9 years, we believe that most of the women in the cohort eventually became mothers. Lastly, our results may not be applicable for all infertile women for several reasons. Before initiating MAR in Denmark, women are advised to fulfill certain health parameters such as BMI below 35 and refrainment from smoking—these health parameters themselves could result in lower CVD rate among women participating in MAR. Also, it is plausible that women who are made aware of their reproductive health are more health cautious, which could reduce the risk of future comorbidities. Nonetheless, our results do indeed apply to the numerous women that undergo MAR in countries and clinics where certain health restrictions are required prior to treatment.

Conclusion

In this study of 87 221 women registered in the Danish IVF register 1994–2015 and subsequently followed in the NPR, we did not find an increased risk of incident early CVD among infertile women. Taken together with previous inconclusive studies, our study gives no reason to assume that a female infertility diagnosis or the time of MAR is 'a window of opportunity' where early screening for cardiovascular disease risk factors can have a prophylactic potential. However, these findings should be validated in studies with a longer follow-up time given that cardiovascular disease tends to debut later in life.

Authors' roles

J.P.B. acquired funding for the study. A.B.B., C.H.G., S.S.T, and J.P.B. designed the study. A.B.B. analyzed data, and wrote the manuscript. All authors contributed to data analysis/interpretation, critical revision of the paper, and final approval of the manuscript.

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Conflict of interest

None of the authors declare any conflict of interest.

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