Semen quality and reproductive hormones in sons of subfertile couples: a cohort study

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Objective: To study the associations between parental subfecundity, assessed by time to pregnancy and use of medically-assisted reproduction, and reproductive health of young men.
Design: Cohort study.
Setting: Denmark.
Patient(s): A total of 1,058 men in the Fetal Programming of Semen quality cohort, a subcohort of the Danish National Birth Cohort.
Intervention(s): From 2017–2019, men were recruited and provided semen and blood samples. Information on parental time to pregnancy and use of medically-assisted reproduction (including type of treatment) as well as demographic, health, and lifestyle factors were available. We estimated the crude and adjusted relative percentage differences with 95% confidence intervals (CIs) in the outcomes according to time to pregnancy and use of medically-assisted reproduction, using multiple adjusted negative binomial regression analysis.
Main Outcome Measure(s): Semen characteristics (semen volume, sperm concentration, total sperm count, sperm motility, and morphology), testicular volume, and reproductive hormone levels (follicle stimulating hormone, luteinizing hormone, testosterone, estradiol, sex hormone-binding globulin, and free androgen index).
Result(s): Overall, semen quality and levels of reproductive hormones were not lower among sons of subfecund parents reporting a time to pregnancy >6 months or use of intrauterine insemination. Sons conceived after in vitro fertilization or intracytoplasmic sperm injection, had a higher semen concentration (29%; 95% CI, −7%–79%) and a higher percentage of sperm with normal morphology (20%; 95% CI, −8%–56%), but with 95% CI overlapping the null. Moreover, these sons had slightly higher estradiol levels (30%; 95% CI, 7%–57%). The absolute differences seen were small, and the clinical significance of these differences are unknown.
Conclusion(s): We found no major difference in semen quality or reproductive hormones in sons conceived by subfertile couples or with the use of medically-assisted reproduction. (Fertil Steril® 2022;118:671-78. ©2022 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key words: Cohort study, fecundity, fertility, infertility, reproduction, reproductive hormones, risk factors, semen quality, subfecundity

Infertility is an increasing global health problem, affecting approximately 15% of couples worldwide (1), and male factor infertility is a major contributing factor. The decline in couple fecundity presumably is caused by biological, social, and behavioral aspects. It has been suggested that family planning and use of contraception during the past century have increased subfecundity. The most fecund couples have fewer children, whereas couples with low fecundity produce more

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children than before due to fertility treatment (2). This hypothesis assumes that intergenerational transmission of subfecundity is substantial, which is poorly documented (3).

During the last decades, an increasing number of children have been conceived by use of medically-assisted reproduction (MAR) (4). Medically-assisted reproduction consists of a wide range of treatment options depending on the underlying cause of infertility; from ovulation induction and intrauterine insemination (IUI) used in couples without male factor infertility, to the more invasive and advanced techniques, such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). It is well known that poor semen quality is a major contributing cause of couple infertility (5, 6), and for years, couples where the male has severely poor semen quality needed donor sperm to achieve a pregnancy by IVF. In the early 1990s, the introduction of the ICSI technique was a major breakthrough in the treatment of severe male factor infertility and, thus, the technique is used increasingly worldwide (7). In Denmark, ICSI is still used primarily in couples with severely low semen quality, but ICSI is used increasingly for other reasons, such as poor fertilization.

Although the short-term health of children conceived by MAR have been studied extensively (8–12), less is known about the potential long-term consequences, including reproductive health. In recent years, the area has received more attention, and parental subfecundity has been associated with the risk of the genital anomalies, cryptorchidism, and hypospadias (13), which may be associated with poor semen quality in adulthood (14).

In 2007, Jensen et al. (15) and Ramlau-Hansen et al. (16) published the first studies on couple subfecundity, time to pregnancy (TTP), and fertility treatment in relation to semen quality in sons. Jensen et al. (15) found that the 47 men whose mothers had received fertility treatment had poorer semen quality across all semen parameters and that 30% of these men had a sperm count <20 million/mL. In line with this, Ramlau-Hansen et al. (16) reported that 30 sons of couples examined or treated for infertility had a lower sperm concentration, total sperm count, motility, and morphology than sons unexposed to infertility treatment. In 2016, Belva et al. (17) studied semen quality in a Belgian cohort of 54 young men conceived by ICSI. They found that those conceived by ICSI had a lower sperm concentration and total sperm count than males conceived naturally.

Male factor infertility remains a major global health problem and natural concerns have been raised on whether poor semen quality is the result of intergenerational transmission to the next male generations. Further, with the increasing use of MAR, in particular IVF and ICSI, there is a need to investigate if any paternally inherited or technically-associated adverse effects may exist on reproductive health. This large cohort study studied the association between parental subfecundity, use of MAR, and markers of reproductive health in young men.

MATERIALS AND METHODS

Data Source and Study Population

This cohort study is based on data from the Fetal Programming of Semen quality (FEPOS) cohort, a subcohort nested within the Danish National Birth Cohort (DNBC) (18).

The DNBC is a national birth cohort, established between 1997 and 2002, including approximately 92,000 pregnant women, recruited at the first antenatal visit in general practice around gestational week 6–12 (18). The pregnant women underwent computer-assisted telephone interview twice during pregnancy (typically in gestational weeks 17 and 32) and twice postpartum.

The children born to the women included in DNBC have been followed throughout childhood and adolescence until adulthood. Between March 2017 and December 2019, adult sons born between 1998 and 2000 to women who participated in the DNBC were recruited to the FEPOS cohort (19). If the mother had participated in pregnancy interviews and provided blood specimens to the biobank, the sons were eligible to take part in the FEPOS cohort. Further, the young men had to live in the vicinity of the 2 largest cities in Denmark, Copenhagen or Aarhus, and be at least 18 years and 9 months old at recruitment. Among the eligible sons, a random subset was invited to participate through an invitation letter and an electronic informed consent form sent to their personal secure digital mailbox. The young men were encouraged to decline participation if they had undergone sterilization or chemotherapy or if they had only 1 or no testicles in the scrotum, regardless of the underlying reason. Those accepting the invitation were asked to complete a comprehensive online questionnaire addressing health behaviors and reproductive health. Men without both testicles were excluded ($n < 5$) from the analyses of testes volume. Further, they were scheduled for a clinical examination, where the young men provided a semen sample and had blood samples taken.

Exposure Assessment: Parental Time to Pregnancy and Use of Medically-Assisted Reproduction

From the first DNBC interview between 1997 and 2002, conducted around gestational week 17, the women included were asked whether the pregnancy was planned or unplanned. Women with planned pregnancies were asked to provide their TTP in prespecified categories as well as use of MAR before the index pregnancy. The women further provided information on type of fertility treatment used to achieve a pregnancy. Based on this information, we defined the following 6 exposure categories: Couples conceiving spontaneously with TTP $\leq$ 5 months (reference); couples conceiving spontaneously with TTP of 6–12 months; couples conceiving spontaneously with TTP > 12 months; couples conceiving following ovulation induction or IUI; couples conceiving following IVF or ICSI; and unplanned pregnancies.

Outcome Assessment

Recruitment and data collection to the FEPOS cohort took place between March 2017 and December 2019. The main outcomes of interest was semen quality and reproductive hormone levels. The young men delivered a semen sample either at the clinic or at home. They were encouraged to abstain from ejaculation for 48–72 hours before semen sample collection. If collected at home, the semen sample was kept at body temperature during transportation. The abstinence time, spillage,

if any, and time from delivery of the semen sample to the semen analysis were recorded.

The semen samples were analyzed by 1 of 2 trained biomedical laboratory technicians, blinded to the exposure status of the participants. All semen analyses (semen volume, total sperm count, concentration, motility, and morphology) were analyzed according to the most recent edition of the World Health Organization (WHO) manual for examination and processing of human sperm. Semen volume was measured by weight of the sample in the preweighed container. Then the sample was placed in a 37°C incubator for liquefaction. After liquefaction, the samples were analyzed manually for sperm concentration. The percentages of progressive motile (PR), nonprogressive motile (NP), and immotile (IM) sperm cells were counted. In the analyses, motility was assessed as NP+IM to ensure optimal model fit. Total sperm count was calculated by multiplication of semen volume and concentration. Morphology was analyzed at the Reproductive Medicine Centre, Skåne University Hospital, in Malmö, Sweden. Morphologically normal sperm was determined by estimating the percentage of normal sperm cells. Azoospermia was defined as no sperm cells in the semen sample.

Testicular volume was self-measured during the clinical examination using a Prader Orchidometer (Bayer AG, Leverkusen, Germany), a method previously found valid when compared with measurement performed by an expert (20).

Nonfasting venous blood samples were drawn and reproductive hormone levels were measured. The blood samples were collected using a Vacuette Safety Blood Collection Set + Holder (Greiner Bio-One GmbH, Kremsmünster, Austria) and stored in CryoPure Tubes (Sarstedt, Nümbrecht, Germany) at −80°C until analysis. The following reproductive hormones were analyzed; follicle stimulating hormone (FSH), luteinizing hormone, testosterone, estradiol, and sex hormone-binding globulin. If the hormone concentration was below the limit of detection (LOD), the value was replaced with the square-root of the LOD: LOD for FSH was 0.1 IU/L ($n < 5$), LOD for luteinizing hormone was 0.1 IU/L ($n < 5$), LOD for estradiol was 15 pmol/L ($n = 87$), whereas the concentration was above the LOD for Sex hormone-binding globulin and testosterone in all participants. Finally, the free androgen index was calculated using the Vermeulen formula (21).

Covariates

The first DNBC interview conducted in the first trimester of pregnancy between 1997 and 2002, provided information on several maternal health and lifestyle factors. Based on a literature review and directed acyclic graph, a priori decisions were made on which variables were considered potential confounding factors to be included in all models. These included maternal age, maternal cigarette smoking in first trimester, prepregnancy body mass index, and socioeconomic status. Further, to improve precision of estimates, variables expected to correlate strongly with the outcome measures also were included in the final models. For semen characteristics, these variables included place of semen sample collection (at home or at the clinic), abstinence time (days), and spillage of semen sample during collection (yes, no). For the analysis of motility, we also included time from ejaculation to semen sample analyses (minutes) as a precision variable. For the reproductive hormones, we included timing of blood sampling as a precision variable.

Statistical Analyses

Baseline characteristics for mothers and sons were presented as proportions and percentages or median with percentiles according to parental fertility status. Further, the median with percentiles was calculated for all semen parameters and reproductive hormone levels according to parental subfertility status. According to the Danish legislation and General Data Protection regulations, a unique value corresponding to a single participant must not be reported, and thus, all medians and percentiles were calculated as pseudomedians and percentiles based on the average of 5 individuals (-sumat- command in STATA).

For all semen parameters, testicular volume as well as hormone levels, we applied negative binomial regression analyses (-nbreg- package in STATA) to estimate the crude and adjusted percentage difference with 95% confidence interval (CI) in semen parameters and reproductive hormone levels comparing the different exposure categories with the reference group (TTP $\leq$ 5 months). All models were adjusted for the potential confounding factors as well as the selected precision variables described above. Men with azoospermia ($n = 17$) were excluded from the analyses on motility and morphology. Further, men who reported spillage during the semen sample collection ($n = 186$) were excluded from models on semen volume and total sperm count.

We performed the following subanalyses: First, we compared sons conceived after IVF or ICSI treatment with sons conceived after ovulation induction or IUI treatment, to explore the potential impact of IVF or ICSI treatment on the reproductive health of sons. These treatment modalities are used mostly when needed due to male factor infertility but IVF and ICSI are considered more invasive than IUI. Second, we excluded participants reporting use of donor sperm in the MAR treatment ($n = 2$).

In all analyses, selection weights were applied to consider potential bias due to selective nonparticipation (22). The selection weights were calculated as the inverse probability of participation, estimated by using multivariable logistic regression, modeling a range of factors possibly associated with participation. Further, to account for clustering of siblings within the cohort, robust standard errors were used in all analyses (22).

Assumptions for negative binomial regression models were checked by comparing the observed distributions against the model-based distributions using Q-Q plots. Then, standardized deviance residuals were plotted against model-based predictions. This did not raise concerns about model fit (data not shown). All statistical analyses were conducted using STATA 15.0 MP software.

Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki. The Committee for Biomedical Research Ethics in

TABLE 1

Baseline characteristics according to parental subfertility, Denmark, 2017–2019.

	Waiting TTP			MAR			
N (%)	≤5 mo 632 (60%)	6–12 mo 110 (11%)	> 12 mo 72 (7%)	IUI or OI 40 (2%)	IVF or ICSI 23 (4%)	Unplanned pregnancies 175 (16%)	Missing 6 (0.6)
Parental educational level							0 (0.0)
High grade professional	217 (34)	38 (34)	18 (25)	19 (47)	12 (52)	52 (30)	
Low grade professional	214 (34)	35 (32)	27 (38)	<16 (<30)	7 (30)	53 (30)	
Skilled/unskilled worker	172 (27)	<39 (<35)	<30 (<36)	9 (22)	<5 (<20)	53 (30)	
Student	29 (5)	<5 (<2)	<5 (<2	<5 (<2)	<5 (2)	17 (10)	
Maternal age[a]	30.3 (27.8; 37.2)	31.0 (27.8; 37.9)	31.7 (28.6; 38.5)	32.6 (29.9; 36.2)	34.3 (20.8; 40.9)	30.9 (27.2; 39.2)	<5 (<1)
Maternal prepregnancy BMI[a]	22.2 (20.5; 24.1)	21.9 (20.5; 24.3)	22.1 (20.6; 25.2)	21.9 (20.1; 24.5)	21.0 (18.7; 23.3)	21.8 (19.9; 24.2)	25 (2.4)
Maternal smoking in first trimester							0 (0.0)
Nonsmoker	516 (82)	76 (71)	43 (60)	19 (87)	35 (80)	122 (70)	
0–10 cigarettes/d	100 (15)	24 (22)	23 (32)	<5 (<7)	9 (21)	45 (26)	
>10 cigarettes/d	16 (3)	7 (7)	6 (8)	<5 (<7)	<5 (<2)	8 (5)	
Parity							23 (2.2)
Nulliparous	260 (42)	51 (48)	41 (59)	24 (64)	<20 (85)	71 (41)	
Multiparous	361 (58)	56 (52)	29 (41)	14 (36)	<5 (15)	101 (59)	
Precision variables							
Place of semen sample collection							10 (1.0)
At home	81 (13)	15 (14)	7 (10)	7 (14)	<5 (<20)	21 (12)	
At the clinic	544 (87)	94 (86)	64 (90)	64 (86)	<20 (<85)	153 (88)	
Abstinence time[a]	2.0 (1.0; 3.0)	2.0 (1.0; 3.0)	2.0 (1.2; 3.1)	2.6 (1.7; 3.4)	2.8 (1.6; 3.6)	2.0 (1.0; 3.0)	5 (0.5)
Spillage							9 (0.9)
No	507 (81)	95 (86)	61 (85)	29 (75)	20 (87)	151 (86)	
Yes	117 (19)	15 (14)	11 (15)	10 (25)	3 (13)	24 (14)	
Time from ejaculation to analysis							12 (1.1)
0–60 min	473 (76)	83 (76)	53 (75)	29 (73)	18 (77)	172 (73)	
>60 min	150 (24)	27 (24)	17 (24)	11 (27)	5 (23)	47 (27)	

Due to Danish data regulations, it is not allowed to report numbers or percentages in cells <5 observations. Abbreviations: BMI, body mass index; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, in vitro fertilization; MAR, medically-assisted reproduction; N, number; TTP, time to pregnancy

[a] Presented as pseudomedians with 25th and 75th percentiles

Arendt. Parental subfertility and male reproductive health. Fertil Steril 2022.

Denmark approved data collection in the DNBC ([KF] 01-471/94). The establishment of the FEPOS cohort was approved by the Scientific Research Ethics Committee for Copenhagen and Frederiksberg, Denmark (No.: H-16015857) and registered at the Danish Data Protection Agency (No.: 2012-58-0004). Further, this study was permitted by the DNBC Steering Committee (Ref. No.: 2016-08). Upon recruitment, informed written consent was obtained from all participants. The funders had no role in study design, data collection, data analysis, or data interpretation or presentation.

RESULTS

The final study population consisted of 1,058 men born between 1998 and 2000. Among these, 72 (6.8%) men were born to couples who conceived spontaneously, but with a TTP > 12 months. In total, 63 men were conceived by MAR, among them 40 (2%) by IUI or ovulation induction and 23 (4%) by IVF or ICSI.

Table 1 presents the characteristics of the study population stratified by parental subfecundity status. Overall, mothers with a TTP > 12 months on average had a lower educational level, a slightly higher body mass index, were slightly older, and more often were nulliparous or smoked ≥10 cigarettes/day compared with mothers in the most fecund couples with a TTP ≤ 5 months. Contrary, mothers who conceived following any type of MAR had on average a higher educational level, but they also were slightly older and more often nulliparous than mothers in couples with a TTP ≤ 5 months. Furthermore, sons conceived by any type of MAR had slightly longer abstinence time than other sons.

The crude distribution of semen parameters and reproductive hormone levels according to parental subfertility status is presented in Supplemental Tables 1 and 2 (available online); main results are presented in Tables 2 and 3. Overall, we found no association between a longer TTP and any of the semen characteristics. Further, sons conceived by IUI or ovulation induction had similar semen quality as sons born to the most fecund couples with a TTP ≤ 5 months (reference). When comparing sons conceived after IVF or ICSI treatment with sons of couples with a TTP ≤ 5 months, we found 29% (95% CI, −7%–79%) with a higher semen concentration and 20% (95% CI, −8%–56%) with morphologically normal spermatozoa but with 95% CI overlapping null. In the subanalyses comparing sons conceived after IVF or ICSI treatment with sons conceived after IUI treatment, the association attenuated (24%; 95% CI, −18%–88%).

The association between parental subfertility or MAR and reproductive hormone levels is presented in Table 3. Compared with sons of the most fecund couples with a TTP ≤ 5 months, sons conceived after IVF or ICSI treatment had 30% (95% CI, 7%– 57%) higher estradiol levels and higher FAI (15%; 95% CI, 0%–15%). In the subanalysis comparing sons conceived after IVF or ICSI treatment with sons conceived after IUI treatment, the association attenuated toward no difference (19%; 95% CI, −7%–53%) for estradiol levels and other hormonal levels remained statistically nonsignificant (data not shown). The absolute differences seen were small, and the clinical significance of these differences are unknown. Finally, excluding <5 participants reporting use of donor sperm in the IUI treatment did not change the results (data not shown).

DISCUSSION

In this nationwide population-based cohort study, we found no major difference in semen quality or reproductive hormones in sons conceived by subfertile couples or with the use of MAR. Thus, these data do not support the hypothesis that parental subfecundity was inherited, thus, affecting the semen quality of male offspring or that reproductive health in sons was affected by parental MAR treatment.

Our results on semen characteristics controvert the few previous studies in the area reporting an overall support for impaired spermatogenesis in sons born to subfecund couples (16) and in sons conceived after MAR (15, 17). The Belgium cohort study published by Belva et al. (17) is, to our knowledge, the only previous study specifically exploring male reproductive health conceived following ICSI and found evidence of lower total sperm count in exposed sons. Further, they found a higher risk of having a sperm concentration below the WHO reference limit of <15 million/mL as well as extremely low sperm concentration < 5 million/mL. When exploring hormonal levels, they found a tendency toward lower inhibin B levels and higher FSH levels in men conceived by ICSI compared with men resulting from spontaneous conception. Inhibin B and FSH are markers of spermatogenesis (23), and men with defective spermatogenesis may have decreased serum levels of inhibin B and higher FSH levels (24). Direct comparison may be problematic, as we did not have the statistical power to distinguish between IVF and ICSI, but we found no indication of lower semen quality in this group. Further, we had no data on inhibin B levels.

In the previous studies, the number of children born following MAR was small, which may explain the diverging results. Further, the studies investigated men born earlier than participants in the present study, which also could contribute to the diverging results, as it has become more and more common to use IVF and ICSI in couples without male factor infertility as the underlying reason. The previous 2 Danish cohort studies included men born in the 1980s, the Belgium cohort was born between 1992 and 1996 and in the present study, participants were born from 1998 and 2000. During these years, MAR treatment, the population seeking MAR treatment as well as indications for this treatment have changed considerably.

The major strength of this study was the longitudinal setup with detailed information on prenatal factors, including parental TTP and type of MAR and the rather large sample of young men providing a semen and blood samples.

As in many other studies on semen quality, the participation rate was low (19%), constituting a major limitation of this study due to the risk of selection bias. The participants were young and unaware of their semen quality, and therefore, participation was most likely unrelated to their reproductive health. One could speculate that men born to parents who had difficulties conceiving and were treated with MAR were more prone to participate to explore their own fertility

TABLE 2

Adjusted relative difference (in percentage, %) in semen characteristics according to parental subfertility status, FEPOS, 2017–2019.

	Volume (mL) N = 834		Concentration (mill/mL) N = 1,007		Total sperm count (mill) N = 834		Motility (NP+IM) N = 984		Morphology (% normal) N = 984		Testicular size (mL) N = 1,005	
	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[b]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[c]
TTP ≤5 mo	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
TTP 6–12 mo	−11	−9 (−17; 0)	−2	−1 (−17; 17)	−13	−10 (−25; 8)	4	5 (4; 13)	7	7 (−8; 24)	−4	−5 (−12; 2)
TTP ≥12 mo	3	2 (−9; 16)	10	21 (−5; 57)	−5	0 (−19; 22)	0	2 (−9; 12)	−17	−13 (−28; 6)	0	1 (−8; 11)
IUI	6	8 (−10; 29)	1	4 (−21; 37)	−6	9 (−18; 46)	9	9 (−6; 23)	3	6 (−15; 34)	−10	−8 (−17; 2)
IVF or ICSI	7	−3 (−23; 20)	38	29 (−7; 79)	32	5 (−28; 54)	3	2 (−19; 20)	17	20 (−8; 56)	−6	−5 (−19; 11)
Unplanned pregnancy	9	12 (3; 22)	−5	−2 (−15; 22)	−5	5 (−11; 23)	3	4 (−3; 11)	−2	−2 (−13; 11)	3	1 (−5; 8)

Abbreviations: CI, confidence interval; IM, immotile; N, number of observations; NP, Nonprogressive motile; TTP, time to pregnancy; IUI, intrauterine insemination; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.
[a] Adjusted for maternal age at delivery, maternal body mass index, maternal cigarette smoking in first trimester, highest educational level of parents, place of semen sample collection, spillage, and abstinence time
[b] Adjusted for maternal age at delivery, maternal body mass index, maternal cigarette smoking in first trimester, highest educational level of parents, place, place of semen sample collection, spillage, abstinence time, and interval from ejaculation to analysis.
[c] Adjusted for maternal age at delivery, maternal body mass index, maternal cigarette smoking in first trimester, highest educational level of parents, and abstinence time.

Arendt. Parental subfertility and male reproductive health. Fertil Steril 2022.

TABLE 3

Adjusted relative difference (in percentage, %) in reproductive hormone levels according to parental subfertility status, FEPOS, 2017–2019.

	FSH (IU/L) N = 1,012		LH (IU/L) N = 1,012		Testosterone (nmol/L) N = 1,013		SHBG (nmol/L) N = 1,012		Estradiol (pmol/L) N = 1,013		Free Androgen Index (%) N = 1,012	
Parental subfertility	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]	Crude	Adjusted (95 CI)[a]
TTP ≤5 mo	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
TTP 6–12 mo	−6	−5 (−16; 6)	0	−1 (−9; 7)	−4	−2 (−8; 5)	−9	2 (−7; 11)	7	9 (−5; 25)	−3	−3 (−11; 4)
TTP ≥12 mo	15	13 (−7; 37)	8	8 (−2; 19)	−3	−3 (−10; 4)	−16	−15 (−22; −7)	−6	−10 (−21; 3)	12	11 (2; 20)
IUI	−5	−6 (−21; 11)	6	5 (−9; 20)	3	5 (−6; 17)	1	1 (−10; 14)	7	8 (−9; 29)	−3	−1 (−9; 8)
IVF or ICSI	−2	−15 (−33; 9)	−2	−5 (−18; 9)	5	4 (−9; 19)	−12	−10 (−22; 4)	30	30 (7; 57)	13	15 (0; 32)
Unplanned pregnancy	−6	−7 (−17; 4)	2	3 (−4; 10)	0	0 (−4; 6)	−1	−1 (−7; 6)	5	5 (−5; 16)	4	4 (−6; 15)

Abbreviations: CI, confidence interval; IM, immotile; N, number of observations; NP, Nonprogressive motile; TTP, time to pregnancy; IUI, intrauterine insemination; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.
[a] Adjusted for maternal age at delivery, maternal body mass index, maternal cigarette smoking in first trimester, highest educational level of parents, and time at blood sample collection.

Arendt. Parental subfertility and male reproductive health. Fertil Steril 2022.

potential. If participation was related to semen quality, our findings could be affected by selection bias in both directions. However, we do not consider this a major risk, as participation in the FEPOS cohort was not related to parental subfertility or fertility treatment (data not shown), and the risk of selection bias due to nonparticipation within this cohort has previously been minimal (25). Further, to address potential selective mechanisms, we adjusted for prespecified selection weights in all analyses.

Information on TTP and MAR was reported in early pregnancy at approximately gestational week 17. We consider this information valid with limited risk of misclassification, which also was supported by a Danish validation study showing a high positive predictive value of self-reported information on MAR (26). We were able to differentiate between specific types of MAR treatment, but unfortunately, we did not have the statistical power to distinguish between IVF and the more invasive ICSI method. Further, we could not define whether longer TTP was due to male or female factors, or specifically study the influence of male factor infertility, which is an important limitation of this study.

We assessed semen quality and reproductive hormone levels based on the World Health Organization manual; we relied, however, on a single semen or blood sample. Knowing that between-day variations exist, this is a potential drawback of the study. We had the possibility to adjust for several potential confounding factors as well as important precision variables, for example, abstinence time, but we cannot rule out the risk of unknown or residual confounding.

In conclusion, we found no evidence to support the hypothesis that parental subfecundity or use of MAR negatively affects reproductive health in male offspring measured by semen quality or testicular volume. However, we found a slightly altered hormonal profile of men conceived following IVF or ICSI. These results are reassuring for the many couples worldwide struggling with subfecundity, but contradict previous findings. The findings of this study thus need replication in future independent studies.

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Calidad espermática y hormonas reproductivas en los hijos de parejas subfértiles: un estudio de cohorte.

Objetivo: Estudiar las asociaciones entre la subfertilidad de los padres, evaluada por el tiempo hasta el embarazo y el uso de técnicas de reproducción médico-asistida, y la salud reproductiva de los hombres jóvenes.

Diseño: Estudio de cohortes

Lugar: Dinamarca

Pacientes(s): Un total de 1,058 hombres en la cohorte del Programa Fetal de calidad Espermática, una subcohorte de la Cohorte Nacional Danesa de Nacimiento.

Intervención(es): Entre 2017 y 2019, los hombres fueron reclutados y proporcionaron muestras de semen y sangre. Información sobre el tiempo de los padres hasta el embarazo y el uso médico de reproducción asistida (incluido el tipo de tratamiento), así como factores demográficos, de salud y de estilo de vida estaban disponibles. Se estimaron las diferencias porcentuales relativas crudas y ajustadas con intervalos de confianza (CIs) del 95% en los resultados según el tiempo hasta el embarazo y el uso de reproducción asistida, usando un análisis de regresión binomial negativa con ajuste múltiple.

Principal(es) medida(s) de resultado(s): Características del semen (volumen del semen, concentración de espermatozoides, recuento total de espermatozoides, movilidad espermática y morfología), volumen testicular y niveles de hormonas reproductivas (hormona foliculoestimulante, hormona luteinizante, testosterona, estradiol, globulina transportadora de hormonas sexuales e índice de andrógenos libres).

Resultado(s): En general, la calidad del semen y los niveles de hormonas reproductivas no fueron más bajos entre los hijos de padres subfértiles que reportaron un tiempo hasta el embarazo $>$ 6 meses o uso de inseminación artificial. Hijos concebidos después de fecundación in vitro o inyección intracitoplasmática de esperma, tenían una concentración de semen más alta (29 %; CI 95 %, 7%–79 %) y un porcentaje más alto de espermatozoides con morfología normal (20 %; CI 95 %, 8 %–56 %), pero con un CI 95 % que solapa con el 0. Además, estos hijos tenían niveles ligeramente más altos de estradiol (30 %; IC 95 %, 7%–57 %). Las diferencias absolutas observadas fueron pequeñas y la relevancia clínica de estas diferencias es desconocida.

Conclusión(es): No encontramos diferencias importantes en la calidad espermática o las hormonas reproductivas en hijos concebidos por parejas subfértiles o con uso de reproducción médico-asistida.