



## Original Contribution

# Fertility Treatment and Reproductive Health of Male Offspring: A Study of 1,925 Young Men from the General Population

Tina Kold Jensen<sup>1,2</sup>, Niels Jørgensen<sup>1</sup>, Camilla Asklund<sup>1</sup>, Elisabeth Carlsen<sup>1</sup>, Mette Holm<sup>1</sup>, and Niels E. Skakkebaek<sup>1</sup>

<sup>1</sup> Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark.

<sup>2</sup> Department of Environmental Medicine, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark.

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Little is known about the reproductive health of offspring after fertility treatment. In 2001–2005, the authors approached young Danish men attending a compulsory physical examination to determine their fitness for military service. A total of 1,925 men volunteered, delivered a semen sample, had a physical examination performed and a blood sample drawn, and responded to a questionnaire. Their mothers were questioned about whether they had received fertility treatment in order to conceive their sons. Forty-seven mothers reported having received fertility treatment to conceive the index subject. After control for confounders, men whose mothers had received fertility treatment to conceive them had a 46% lower sperm concentration (95% confidence interval (CI): –63, –20) and a 45% lower total sperm count (95% CI: –64, –16). They had a smaller testis size (–0.9 ml, 95% CI: –2.2, 0.4), fewer motile sperm (–4.0%, 95% CI: –8.0, –0.1), and fewer morphologically normal spermatozoa (–2.0%, 95% CI: –4.1, 0.0). They also had a lower serum testosterone level and free androgen index (results not statistically significant). These findings should be viewed in light of the increasing use of fertility treatments. Although the cause of these findings is unknown, they raise concern about possible late effects of fertility treatment. Larger-scale studies of children born after fertility treatment should be performed.

fertilization in vitro; infertility; infertility, male; semen; spermatozoa; sperm count; testis; testosterone

Abbreviations: CI, confidence interval; SD, standard deviation.

Fertility treatment has become a major health issue and an increasing economic burden in many Western countries. Approximately 7 percent of Danish children born in 2005 were conceived through the use of assisted reproductive technology (1). However, little is known about the reproductive health of offspring after fertility treatment with regard to transfer of genetic factors related to the fertility of the next generation. In addition, data on the possible long-term effects of various hormone treatments given to the infertile female partner on the reproductive system of the fetus are lacking.

Controversial results have been obtained with regard to long-term effects of in-utero exposure to diethylstilbestrol and other steroids during early pregnancy (2–8). However, there is some evidence that exposure to estrogens and estrogenic substances may cause genital abnormalities in newborn boys (9–11) and perhaps also testicular cancer in the sons of exposed mothers (12–23). Therefore, we studied the association between maternal fertility treatment around the time of conception and reproductive health (cryptorchidism, serum levels of reproductive hormones, semen quality, and testis size) among Danish young men from the general population.

Correspondence to Dr. Tina Kold Jensen, University Department of Growth and Reproduction, GR 5064 Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark (e-mail: tkjensen@health.sdu.dk).

**TABLE 1. Reproductive and sociodemographic characteristics of 1,925 Danish military conscripts and their mothers, according to maternal receipt of fertility treatment for conception of the index subject, 2001–2005**

Variable	No. of subjects ( <i>N</i> = 1,925)	%	Mother received infertility treatment to conceive index subject		
			"No" ( <i>n</i> = 1,702)	"Yes" ( <i>n</i> = 47)	"Don't know" ( <i>n</i> = 176)
Semen quality and physical examination					
Mean testis size (ml)*	1,890	98.2	20.2 (4.6)†	19.3 (4.5)	19.3 (4.7)
Mean semen volume (ml)	1,925	100.0	3.3 (1.4)	3.6 (1.6)	3.1 (1.4)
Median sperm concentration (million/ml)	1,914	99.4	48 (23–83)	33 (13–66)	47.0 (23–89)
Median total sperm count (million)	1,915	99.5	152 (72–263)	129 (50–226)	144.8 (145–275)
Mean percentage of motile sperm	1,921	99.8	65.5 (13.0)	61.8 (18.7)	65.0 (14.2)
Mean percentage of morphologically normal spermatozoa‡	94		10.5 (4.5)	8.6 (5.2)	
Mean time to motility assessment (minutes)	1,913	99.4	33 (25–44)	34 (26–45)	33 (25–45)
Period of abstinence from sexual activity prior to sampling (hours)					
0–95	1,576	82.0	82.6	87.2	75.6
≥96	345	18.0	17.4	12.8	24.4
Season					
April–September	142	7.4	7.3	8.5	7.4
October–March	1,783	92.6	92.7	91.5	92.6
Body temperature above 38°C (100.4°F) during previous 3 months					
No	1,733	95.4	95.7	90.9	93.9
Yes	84	4.6	4.3	9.1	6.1
Diseases of reproductive organs found at physical examination§					
No	1,641	87.4	87.5	89.1	86.0
Yes	237	12.6	12.5	10.9	14.0
Questionnaire information					
City					
Copenhagen	1,818	94.4	94.7	97.9	91.5
Aalborg	107	5.6	5.3	2.1	8.5
Age (years)					
<20	1,580	82.4	82.3	93.6	80.9
≥20	337	17.6	17.7	6.4	19.1

Table continues

## MATERIALS AND METHODS

### Population

Because of the military drafting system in Denmark, all 18-year-old men are required to undergo a compulsory physical examination to determine their fitness for military service. Men who suffer from chronic diseases (10–14 per-

cent of the population) are not summoned by the military board and therefore were not approached for this study. From June 1996 onward, trained staff personally approached all of these young men when they appeared for this compulsory physical examination in two cities in Denmark (Copenhagen and Aalborg) and asked them to complete a questionnaire. From September 2001 onward, the questionnaire included

TABLE 1. Continued

Variable	No. of subjects (N = 1,925)	%	Mother received infertility treatment to conceive index subject		
			"No" (n = 1,702)	"Yes" (n = 47)	"Don't know" (n = 176)
Body mass index¶					
<20	293	15.3	15.3	12.8	15.5
20–25	1,247	65.1	64.4	66.0	71.3
>25	376	19.6	20.2	21.3	13.2
Alcohol intake (units#/week)					
0–19	1,457	78.2	78.7	66.7	76.8
≥20	405	21.8	21.3	33.3	23.2
Smoking					
No	1,148	59.9	60.4	65.2	53.4
Yes	768	40.1	39.6	34.8	46.6
Born with testicles in scrotum**					
No	173	9.0	9.0	12.8	8.0
Yes	1,752	91.0	91.0	87.2	92.0
Self-reported diseases of reproductive organs††					
No	1,683	95.2	95.6	93.3	91.9
Yes	89	4.8	4.4	6.7	8.1
Maternal questionnaire information					
Mother helped to fill in the questionnaire					
No	532	27.6	22.8	14.9	85.5
Yes	1,493	72.4	77.2	85.1	16.5
Mother waited more than a year to conceive index subject					
No	1,110	83.6	85.7	25.6	69.2
Yes	218	16.4	14.3	74.4	30.8
Mother smoked during pregnancy					
No	1,097	59.8	60.2	50.0	59.3
Yes	736	40.2	39.8	50.0	40.7

\* Calculated as the mean of right and left testis size.

† Numbers in parentheses, standard deviation or interquartile range (25th–75th percentiles).

‡ Counted in the 47 sons of mothers who received treatment to conceive and 47 randomly selected sons of mothers reporting no treatment.

§ Varicocele, hydrocele, or abnormally hard or soft testes found at the physical examination.

¶ Weight (kg)/height (m)<sup>2</sup>.

# 1 unit = 12 g of alcohol.

\*\* Missing information was counted as being born with testicles in the scrotum (see text).

†† Self-reported information about undergoing an operation for torsion of the testis or having had epididymitis or chlamydia.

a detailed section for the participants' mothers' about their living and working conditions before, during, and after their pregnancy with the index subject. (For a detailed description of the study, see the article by Andersen et al. (24).) Participation required that the man fill in the questionnaire, deliver

a semen sample, have a blood sample drawn, and undergo a physical examination. The participants received economic compensation (500 kroner; ~US\$80). Ethical approval was obtained from the local ethical committee, which is common practice for clinical studies in Denmark.

## Semen analysis

Each man provided a semen sample by masturbating into a wide-mouthed plastic container in a room close to the semen laboratory. The period of abstinence from sexual activity prior to sampling was recorded, and the semen sample was analyzed according to the World Health Organization's 1992 guidelines (25), modified in accordance with Jørgensen et al. (26). Smears from all semen samples were stained and preserved, but as of this writing they had not yet been analyzed. Therefore, we nested a matched exposure cohort within the cohort. The morphologic slides for the 47 men exposed in utero were found, and morphology was assessed. We randomly selected an unexposed control group by assessing the morphology of each man included in the study just after an exposed man. A single experienced technician blindly assessed all morphologic slides (including approximately 400 other samples) within 4 working weeks, using strict criteria (27).

## Serum hormone analysis

A blood sample was drawn from a cubital vein of each participant and centrifuged. The serum was separated and frozen. All samples were analyzed in the Department of Growth and Reproduction, Rigshospitalet (Copenhagen, Denmark). Serum levels of follicle-stimulating hormone, luteinizing hormone, sex hormone-binding globulin, testosterone, and inhibin B were measured. (For details, see Andersen et al. (24).) The free androgen index was calculated as  $(\text{total androgen} \times 100) / \text{sex hormone-binding globulin}$ . Only serum samples collected through spring 2004 had been analyzed at the time of publication of this article ( $n = 1,384$ ; 72 percent).

## Physical examination

All physical examinations were performed by one of four physicians. The Tanner stage of pubic hair and genital development, testicular volumes (determined using a Prader orchidometer; the mean of the volumes of both testes was calculated), the possible presence of a varicocele or hydrocele, the location of the testes in the scrotum, and the consistency of the testis and epididymis were recorded. Weight was measured in kilograms using only one weighing scale in each center; height was measured in centimeters; and body mass index was calculated as weight in kilograms divided by squared height in meters.

## Questionnaire

The questionnaire included information on previous and/or current diseases and genital diseases such as inguinal hernia, varicocele, epididymitis, gonorrhea, chlamydia, and surgery for testicular torsion. The men were asked whether they were born with both testicles in the scrotum. In addition, they reported whether their body temperature had exceeded 38°C (100.4°F) during the previous 3 months.

From September 2001 onward, the protocol was extended to include a questionnaire section about the mother's living

conditions before, during, and after her pregnancy with the index subject. The man was asked whether his mother had helped him fill in this section (but he could participate without his mother's knowledge). The section included information about maternal exposures incurred during her pregnancy with the index subject. The mother was asked whether she had received any form of fertility treatment in order to conceive her son; if so, in response to an open-ended question, she had the option of specifying what form of treatment she had received. These open-ended replies were categorized into "receipt of hormone treatment" versus "no receipt of treatment."

## Statistics

Outcome variables related to semen quality included testis size, semen volume, sperm concentration, total sperm count, and percentages of motile and morphologically normal spermatozoa. Data on sperm concentration and total sperm count were not normally distributed, so median values and interquartile ranges (25th–75th percentiles) were calculated for these variables. Data on testes size, semen volume, and motile and morphologically normal spermatozoa were all normally distributed, so mean values and standard deviations were calculated for these variables.

First, semen quality was compared for men whose mothers reported that they had received treatment to conceive and men whose mothers had not received treatment. Then we compared the distributions of information obtained from the questionnaires and physical examinations among these groups of mothers in order to identify possible confounders. Finally, we performed a multiple linear regression analysis taking into account confounders found to affect outcome variables and to be differently distributed among men whose mothers had received/not received treatment in order to conceive (entered as a dummy variable). Normally distributed outcome variables were entered directly as continuous variables in the linear multiple regression analysis, whereas sperm concentration and total sperm count were transformed by use of the natural logarithm to obtain normality. Confounders were excluded stepwise if they were not statistically significant at the 10 percent level. The results are presented with 95 percent confidence intervals. We evaluated the fit of the regression models by testing the residuals for normality and by inspecting the residual plots.

## RESULTS

A total of 6,035 men attended the physical examination and 1,925 participated (32 percent) in the study, of whom 107 were from Aalborg. The participation rates in Copenhagen and Aalborg were 31 percent and 36 percent, respectively. Ninety-one percent of the mothers responded to the question on fertility treatment, and 47 mothers reported having received fertility treatment in order to conceive the index subject (2.4 percent). Semen quality among sons whose mothers did not report whether they had received treatment to conceive ( $n = 176$ ) was similar to that of

men whose mothers did not receive treatment (table 1). In addition, their distribution of confounders was more like that of the unexposed men (table 1).

Men whose mothers had received fertility treatment to conceive them had a lower sperm concentration (33 million/ml vs. 48 million/ml) and total sperm count (129 million vs. 152 million), smaller testicles (19.3 ml vs. 20.2 ml), and fewer motile and morphologically normal spermatozoa (61.8 percent vs. 65.5 percent and 8.6 percent vs. 10.5 percent, respectively). Thirty percent of exposed men had a sperm count below 20 million/ml (the lower World Health Organization limit), as compared with 20 percent among unexposed men.

A total of 173 men reported not being born with both testicles in the scrotum (cryptorchidism) (10.6 percent), but this information was missing for 293 men. Nonresponders had semen quality almost identical to that of men who reported not being born with cryptorchidism; therefore, we decided to categorize them as not having cryptorchidism in order to increase the sample size. Six (12.8 percent) men whose mothers had received fertility treatment to conceive them were born with cryptorchidism. Among unexposed men, 153 (9.0 percent) were born with cryptorchidism (table 1).

Men whose mothers had received treatment to conceive them had shorter periods of abstinence and more often reported having a high body temperature during the previous 3 months (table 1). Diseases were less often found among these men at the physical examination. They more often reported diseases of the reproductive organs, were more often from Copenhagen, were younger, drank more alcohol, and smoked less. Their mothers more often had waited more than a year to conceive them and more often had smoked during pregnancy (table 1).

Linear regression was performed using semen quality and testis size as dependent variables and insertion of a dummy variable for maternal treatment as an explanatory variable. After control for confounders, men whose mothers had received treatment to conceive them had a lower sperm concentration (−45.7 percent, 95 percent confidence interval (CI): −63.1, −20.4), a lower total sperm count (−44.5 percent, 95 percent CI: −63.5, −15.6), and a smaller testis size (−0.9 ml, 95 percent CI: −2.2, 0.4) (table 2). In addition, they had fewer motile sperm (−4.0 percent, 95 percent CI: −8.0, −0.1) and a reduced number of morphologically normal spermatozoa (−2.0 percent, 95 percent CI: −4.1, 0.0) (table 2).

The analyses were repeated for men without cryptorchidism and for sons whose mothers provided the pregnancy-related information, and the same associations were found (table 2).

In response to an open-ended question, the mothers had the option of stating what fertility treatment they had received. Of the 47 mothers who had received treatment, 25 stated that their treatment was hormonal. After control for confounders, sperm concentration (−58.7 percent, 95 percent CI: −76.1, −28.5), total sperm count (−58.6 percent, 95 percent CI: −77.2, −24.7), testis size (−1.9 ml, 95 percent CI: −3.7, −0.1), percentage of motile sperm (−2.0 percent, 95 percent CI: −7.4, 3.4), and percentage of

**TABLE 2. Semen quality and testis size among men whose mothers received fertility treatment to conceive them as compared with men whose mothers did not (reference group), Denmark, 2001–2005\***

	Sperm concentration (%)		Total sperm count (%)		Semen volume (ml)		Testis size (ml)		Percentage of morphologically normal spermatozoa		Percentage of motile spermatozoa	
	Difference	95% CI†	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI	Difference	95% CI
Unadjusted	−40.0	−58.9, −12.3	−37.1	−58.3, −5.0	0.2	−0.2, 0.6	−0.9	−2.2, −0.5	−1.9	−3.9, 1.0	−3.6	−7.5, 0.2
Adjusted	−45.7‡	−63.1, −20.4	−44.5‡	−63.5, −15.6	0.3§	−0.2, 0.7	−0.9¶	−2.2, 0.4	−2.0#	−4.1, 0.0	−4.0**	−8.0, −0.1
Among men without cryptorchidism	−48.9‡	−65.4, −24.6	−47.6‡	−65.8, −19.7	0.3§	−0.1, 0.8	−0.8¶	−2.2, 0.6	−2.2#	−4.3, 0.0	−4.1**	−8.2, −0.1

\* Both unadjusted and adjusted results were obtained from linear regression analyses.

† CI, confidence interval.

‡ Adjusted for period of abstinence from sexual activity prior to sampling (transformed by the use of the natural logarithm), in utero exposure to smoking, body temperature exceeding 38°C during the previous 3 months, cryptorchidism, self-reported diseases of the reproductive organs, and diseases of the reproductive organs found at the physical examination.

§ Adjusted for period of abstinence from sexual activity prior to sampling (transformed by the use of the natural logarithm).

¶ Adjusted for cryptorchidism, self-reported diseases of the reproductive organs and diseases of the reproductive organs found at the physical examination, examining physician, and body mass index.

# Adjusted for cryptorchidism and body temperature exceeding 38°C during the previous 3 months.

\*\* Adjusted for period of abstinence (transformed by the use of the natural logarithm), body temperature exceeding 38°C during the previous 3 months, and cryptorchidism.

**TABLE 3. Mean serum levels of reproductive hormones and adjusted differences in hormone levels among 1,385 Danish military conscripts, according to maternal receipt of fertility treatment for their conception, 2001–2005**

Reproductive hormone	No. of subjects	%	Mother received fertility treatment to conceive index subject		Adjusted* difference in reproductive hormone levels	95% confidence interval
			No ( <i>n</i> = 1,222)	Yes ( <i>n</i> = 39)		
Follicle-stimulating hormone (IU/liter)	1,385	72.0	3.3 (2.5)†	3.2 (1.8)	0.0	−16.2, 21.6‡
Luteinizing hormone (IU/liter)	1,385	72.0	3.7 (1.6)	3.5 (1.5)	−0.2	−0.7, 0.3
Testosterone (nmol/liter)	1,384	71.9	23.1 (7.0)	21.7 (6.1)	−1.4	−3.6, 0.8
Sex hormone-binding globulin (nmol/liter)	1,385	72.0	29.3 (10.6)	28.3 (9.5)	−1.1	−4.5, 2.3
Free androgen index	1,384	71.9	86 (32)	84 (37)	−1.3	−11.5, 8.9
Inhibin B (pg/ml)	1,385	72.0	180 (66)	186 (68)	7	−14, 28
Percentage of serum samples collected before 12:00 p.m.	1,384	92.3	92.1	94.9		

\* Adjusted for time of sampling (before or after 12:00 p.m.).

† Numbers in parentheses, standard deviation.

‡ Data were transformed by the use of the natural logarithm.

morphologically normal spermatozoa (−1.7 percent, 95 percent CI: −4.1, 0.7) were lower among these 25 men.

Mean serum levels of luteinizing hormone, follicle-stimulating hormone, testosterone, sex hormone-binding globulin, free androgen index, and inhibin B were 3.7 IU/liter (standard deviation (SD), 1.8), 3.3 IU/liter (SD, 3.3), 23.2 nmol/liter (SD, 6.9), 29.3 nmol/liter (SD, 10.6), 86 (SD, 32), and 179 pg/ml (SD, 66), respectively. Men whose mothers had received fertility treatment to conceive them had a higher follicle-stimulating hormone level, a lower testosterone level, and a lower free androgen index than the other men. Serum hormone levels were affected by the time of sampling; after control for this (a dummy variable was inserted for sampling before or after 12:00 p.m.), men whose mothers had received treatment to conceive them had a lower serum testosterone level (−1.4 nmol/liter, 95 percent CI: −3.6, 0.8) and a lower free androgen index (−1.3, 95 percent CI: −11.5, 8.9), although these results were not statistically significant (table 3).

## DISCUSSION

We found significantly reduced sperm counts, fewer motile sperm, more morphologically abnormal sperm, and smaller testes in offspring of women who had received fertility treatment, including hormone administration. The sons of the treated women also had a nonsignificant reduction in serum testosterone levels. Our questionnaire data did not allow us to distinguish between different forms of treatment. However, analysis of the data originating from men whose mothers provided information about hormone treatment showed an even stronger association with poorer semen quality.

Couples with infertility problems often show “double pathology” (28). Therefore, our findings do not necessarily indicate that female infertility treatment induces a risk of reproductive problems for the next generation. Our findings could, in fact, be due to genetic factors inherited through the mother or even the father.

There is large inter- and intraindividual variation in semen quality, and therefore large study populations are required in order to determine effects of exposures. We included only 47 exposed men, and thus our estimates may have been imprecise; they were, however, statistically significant. To our knowledge, there have been no other studies including almost 2,000 young men from the general population, and the reductions in semen quality and testis size were larger than the effect of previous exposures affecting semen quality. For example, current smoking and exposure to smoking in utero have been found to reduce sperm concentration approximately 15–20 percent (29, 30), whereas we found an almost 50 percent reduction among men whose mothers had received fertility treatment to conceive them.

It is well-known that interobserver variability in semen analysis between different laboratories exists. However, all analyses were done blindly, our laboratory participated in an external quality control program (26), and a single technician performed all of the morphologic assessments. In addition, the changes in the various semen parameters (count, motility, and morphology) all pointed in the same direction: The sons of the treated mothers showed the poorest results.

As in other studies involving collection of semen samples, the participation rate was low (31 percent). However, the men were young and had essentially no prior knowledge of their own fertility potential (only 3 percent had been responsible for a pregnancy and only 0.8 percent had deliberately tried to conceive), and therefore this is unlikely to have affected their motivation to participate. Nine percent of the participating men reported having been born with cryptorchidism; this figure is higher than the 8 percent reported in a recent Danish study (31). Therefore, one could speculate that the men with diseases of the reproductive organs were more likely to participate. However, we compared semen quality among men with exposure and men without exposure, so it is of less importance whether the groups of men in fact represented the general population.

We relied on retrospectively self-reported data on fertility treatment. Mothers who reported receiving treatment were

older and had a lower parity and a longer waiting time to pregnancy than the other mothers. In addition, the sons of mothers who reported receiving treatment more often reported having been born without both testicles in the scrotum, which is in accordance with previous literature and suggests that our data are valid (10, 11). Most importantly, the mothers responded to the questionnaire before the results of their sons' semen analyses were known; therefore, recall bias is unlikely.

Most mothers of the young men in our study only stated whether they had received fertility treatment in order to conceive, not which type of treatment they had received. They were generally treated before the introduction of in vitro fertilization and intracytoplasmic sperm injection and most likely were treated for anovulation with clomiphene, human menopause gonadotropin, and human chorion gonadotropin. Clomiphene is an anti-estrogen with a suggested half-life of up to 33 days (32). The long half-life of this hormone suggests that it is biologically plausible that some of these men had been exposed to it during critical stages of gonadal development in the first trimester. Overall, no increased risk of malformations among offspring of clomiphene-treated women has been reported (33). However, animal studies have suggested increased risk of genital and urinary tract anomalies after maternal clomiphene treatment (34, 35), and human genital tract tissue exposed to clomiphene in culture has been shown to undergo estrogenic proliferation (36). Some women may have had progesterone treatment during pregnancy to prevent spontaneous abortion and reported that as fertility treatment. If so, the reduced semen quality observed among the sons of these women may have been due to some abnormality of the pregnancy instead of the hormonal treatment. Some investigators have reported an increased risk of cryptorchidism and hypospadias among sons of mothers receiving progestin treatment in early pregnancy, but the results have been inconsistent (10, 11, 37, 38). To our knowledge, no long-term effects on the offspring of progestin-treated women have been reported.

Our findings should be seen in light of the current low fertility rates in Europe and elsewhere, the widespread infertility problems in Western societies, and the increasing use of fertility treatments. Although we do not know whether our findings are due to hormone administration, genetic problems, or other exposures, these findings generate some concern about late effects of the use of assisted reproductive techniques. We suggest that larger-scale, multicenter follow-up studies of children born after the use of assisted reproductive technology be performed.

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