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Sperm counts and endocrinological markers of spermatogenesis in long-term survivors of testicular cancer

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BACKGROUND: The objective of this study was to assess markers of spermatogenesis in long-term survivors of testicular cancer (TC) according to treatment, and to explore correlations between the markers and associations with achieved paternity following TC treatment.

METHODS: In 1191 TC survivors diagnosed between 1980 and 1994, serum-follicle stimulating hormone (s-FSH; $n = 1191$), s-inhibin B ($n = 441$), and sperm counts (millions per ml; $n = 342$) were analysed in a national follow-up study in 1998–2002. Paternity was assessed by a questionnaire.

RESULTS: At median 11 years follow-up, 44% had oligo- (< 15 millions per ml; 29%) or azoospermia (15%). Sperm counts and s-inhibin B were significantly lower and s-FSH was higher after chemotherapy, but not after radiotherapy (RT), when compared with surgery only. All measures were significantly more abnormal following high doses of chemotherapy (cisplatin (Cis) > 850 mg, absolute cumulative dose) compared with lower doses (Cis ⩽ 850 mg). Sperm counts were moderately correlated with s-FSH (-0.500), s-inhibin B (0.455), and s-inhibin B : FSH ratio (-0.524; all $P < 0.001$). All markers differed significantly between those who had achieved post-treatment fatherhood and those with unsuccessful attempts.

CONCLUSION: The RT had no long-term effects on the assessed markers of spermatogenesis, whereas chemotherapy had. At present, the routine evaluation of s-inhibin B adds little in the initial fertility evaluation of TC survivors.

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In testicular cancer (TC) survivors, spermatogenesis may be compromised either due to the cancer treatment or by factors related to the aetiology of TC (Wohlfahrt-Veje *et al*, 2009). Fertility is a concern for many TC survivors, and among those who have tried to father children following their cancer treatment conception and paternity rates of 49–82% have been reported (Spermon *et al*, 2003; Huyghe *et al*, 2004; Brydøy *et al*, 2005; Huddart *et al*, 2005; Matos *et al*, 2010).

Spermatogenesis is preferably assessed by sperm samples or testicular biopsies. Endocrinological measures may yield additional information (Islam and Trainer, 1998; Andersson *et al*, 2004) and are often tested in the initial evaluation of cancer survivors whose fertility is of concern. Follicle stimulating hormone (FSH), secreted by the pituitary gland, and inhibin B, a product of Sertoli cells, are considered endocrine markers of spermatogenesis (Islam and Trainer, 1998; Pierik *et al*, 2003). Serum (s)-inhibin B has been proposed to be a better marker of spermatogenesis than s-FSH (Jensen *et al*, 1997; Pierik *et al*, 2003; Mabeck *et al*, 2005), but based on the literature it is uncertain whether s-inhibin B gives sufficient additional information to s-FSH (Bohring *et al*, 2002; Andersson *et al*, 2004), to encourage its use in the initial evaluation of male cancer survivors with possibly impaired fertility.

Although markers of spermatogenesis have been addressed in several series of TC survivors with at least 100 cases (Lampe *et al*, 1997; Gerl *et al*, 2001; Eberhard *et al*, 2004; Bahadur *et al*, 2005; Huddart *et al*, 2005; Nuver *et al*, 2005; Gandini *et al*, 2006; Wiechno *et al*, 2007; Brydøy *et al*, 2010), only some include sperm parameters (Lampe *et al*, 1997; Eberhard *et al*, 2004; Bahadur *et al*, 2005; Gandini *et al*, 2006; Brydøy *et al*, 2010), all treatment modalities (Eberhard *et al*, 2004; Huddart *et al*, 2005), or concentrate on observations 5 years or more after treatment

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(Huddart *et al*, 2005; Nuver *et al*, 2005; Brydøy *et al*, 2010). Information on inhibin B is particularly sparse (Nuver *et al*, 2005; Romerius *et al*, 2010). Little is known about the inter-relationship between post-treatment endocrine markers of spermatogenesis, sperm counts, and paternity in TC survivors (Huddart *et al*, 2005).

The primary aim of this study was to assess sperm counts (millions per ml), s-FSH, and s-inhibin B according to treatment in long-term TC survivors. A secondary aim was to study in what manner the endocrinological markers reflected sperm counts and, specifically, whether s-inhibin B in this situation added appreciable information to s-FSH alone. Finally, we explored associations between sperm counts, endocrinological markers, and achieved paternity following TC treatment.

MATERIALS AND METHODS

Population and study design

This study was part of a national multi-centre follow-up survey conducted during 1998–2002 by five collaborating units as described previously (Brydøy *et al*, 2005). Briefly, all men aged 18–75 years who were treated for unilateral germ cell TC in Norway in 1980 through 1994 were identified. Overall, 1814 eligible TC survivors were invited, and 1462 (81%) participated in the survey, which included a mailed questionnaire and an outpatient clinical examination with laboratory tests. The study sample for this report consists of 1191 men (Supplementary Figure S1, online only), excluding participants older than 65 years ($n=70$), those with missing hormone and sperm data ($n=158$), men using androgen replacement therapy ($n=36$), or having documented hyperprolactinaemia ($>1000\,\mathrm{mIU\,l^{-1}}$; $n=6$; Islam and Trainer, 1998; Mohr *et al*, 2005).

Data regarding histology, Royal Marsden Hospital system staging (Peckham *et al*, 1979), treatment, and relapse were retrieved from the patients' medical records. The Committee for Medical Research Ethics of the Southern Health Region of Norway approved the study, and all participants gave written informed consent.

Treatment

Treatment principles of TC applied in Norway in 1980–1994 are described previously (Brydøy *et al*, 2005). Men treated with chemotherapy mostly received cisplatin (Cis)-based combinations with bleomycin and vinblastine or etoposide (BEP). Some patients with metastases were treated with carboplatin-based chemotherapy only ($n=24$) or in addition to Cis-based chemotherapy ($n=8$). Carboplatin was not used as an adjuvant in seminoma stage I. Infradiaphragmatic radiotherapy (RT) was mostly administered to seminoma patients by dog-leg or L-fields, or in some ($n=39$) by para-aortic fields. The radiation dose was gradually reduced from 36–40 to 25.2–27 Gy.

The TC survivors were, in the present study, allocated to four groups according to their post-orchiectomy treatment including any relapse treatment as follows: (1) surgery only (surveillance or retroperitoneal lymph node dissection (RPLND); Surgery), (2) RT only (RT); (3) chemotherapy with a cumulative Cis dose $\leqslant 850$ mg, or any carboplatin-based treatment, if Cis was not applied, with or without RPLND or RT (Cis $\leqslant 850$ mg); and (4) chemotherapy with a cumulative Cis dose >850 mg, with or without RPLND or RT (Cis >850 mg). The cut-off point (850 mg) was chosen to approximately differentiate between standard four cycles or less, and more than four cycles.

Laboratory assessments

As part of the follow-up visit, non-fasting blood samples were drawn by venipuncture between 0800 and 1200 h in the majority of patients. The s-FSH was analysed in fresh serum, based on commercial immunoassay technology with similar reference ranges at each of the five hospital laboratories, and values $\geqslant 12\,\mathrm{IU\,l^{-1}}$ were considered elevated. The co-efficient variation (CV) was $<8\%$. The s-inhibin B was assessed at one institution only, in serum samples ($n=441$) stored at $-70\,^{\circ}\mathrm{C}$, until it was analysed in January 2007. An ELISA Kit (Diagnostic Systems Laboratories Inc., Webster, TX, USA) with a lower detection limit of $7\,\mathrm{ng\,l^{-1}}$ was used. The CV was $<10\%$. As published reference levels are not uniform (Jensen *et al*, 1997; Andersson *et al*, 2004; Sikaris *et al*, 2005; Myers *et al*, 2009), we used 80 and $140\,\mathrm{ng\,l^{-1}}$ when grouping s-inhibin B values into a categorical variable (Jensen *et al*, 1997; Mahmoud *et al*, 1998; Pierik *et al*, 1998; van Casteren *et al*, 2009; Romerius *et al*, 2010).

Sperm counts (millions per ml) were assessed at two units ($n=342$, 42% of the eligible participants at these centres). The samples were delivered during or in proximity with the follow-up visit. Oligozoospermia was defined as <15 millions per ml, and samples with no visible sperm were classified as azoospermic (World Health Organization, 2010). The recently updated WHO lower reference level of 15 millions per ml corresponds to the fifth centile in fertile men whose partners had a time to pregnancy (TTP) $\leqslant 12$ months (Cooper *et al*, 2010).

Post-treatment paternity

Data regarding attempts at conception and achieved paternity following TC treatment were retrieved from the questionnaire (Brydøy *et al*, 2005). Men who became fathers by pre-treatment cryopreserved semen were not included in the 'post-treatment paternity' group. Male partners of couples who received other post-treatment assistance with reproduction were included if fresh semen was used. Female fertility issues were not specifically addressed. Men who reported dry ejaculation were excluded from the analyses testing associations between markers of spermatogenesis and post-treatment paternity.

Statistical analyses

Kruskal–Wallis test (exact using Monte Carlo method), Student's *t*-test or one way ANOVA were used for group comparisons of continuous data, and exact χ^2-, Kruskal–Wallis or Mann–Whitney (exact using Monte Carlo method) tests were used for categorical data. The s-FSH was skewed and log-transformed before analyses. Non-parametric methods were used for analyses of sperm counts and s-inhibin B. A s-inhibin B : FSH ratio was calculated as s-inhibin B ($\mathrm{ng\,l^{-1}}$) : s-FSH ($\mathrm{IU\,l^{-1}}$).

Linear regression analysis was used in multivariate testing of s-FSH, whereas tobit censored linear regression was applied for s-inhibin B because of the lower limit of detection ($7\,\mathrm{ng\,l^{-1}}$; Tobin, 1958). For s-FSH, the analysis was performed on the logarithmic value, and reported values are the exponentially transformed regression co-efficients. In addition to treatment, age at follow-up and self-reported cryptorchism (at least one undescendent testis at birth), were included as covariates (Perheentupa and Huhtaniemi, 2009; Wohlfahrt-Veje *et al*, 2009). Bootstrapping did not show any substantial problems with these models. A proportional odds ordinal logistic regression model was used in multivariate testing of sperm counts with five levels of sperm counts (azoospermia, Few visible sperm-1. 1.1–9.9, 10–14.9 and $\geqslant 15$ millions per ml), including treatment, age, follow-up time, and cryptorchism. The assumption of proportional odds was checked (Harrell, 2001). To compare the two chemotherapy groups, the multivariate analyses were repeated using Cis <850 mg as reference.

Spearman's correlation was used for assessing correlations between endocrinological markers and sperm counts. To explore the positive predictive values (PPVs) for sperm counts of s-FSH and s-inhibin B, we used specified cut-off levels previously

reported to predict oligo- (s-inhibin B $<80\,ng\,l^{-1}$ and s-FSH $>10\,IU\,l^{-1}$) and azoospermia ($<50\,ng\,l^{-1}$ and $>10.9\,IU\,l^{-1}$, respectively; Jensen *et al*, 1997; Romerius *et al*, 2010). As oligozoospermia was defined as <20 millions per ml in these reports, we also report the results for this prior definition of oligozoospermia (Cooper *et al*, 2010).

The data were analysed by the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA) and R (The R Foundation for Statistical Computing, Vienna, Austria). All tests were two-sided, and *P*-values <0.05 were considered significant.

RESULTS

Study population

The interval between orchiectomy and follow-up among the 1191 men forming the study population was a median of 11 (range 4–22) years. Inhibin and sperm counts were assessed in subgroups where the interval from orchiectomy to sampling was a median of 11 (range 5–21) years for inhibin and 9 (5–20) years for sperm counts. Clinical characteristics including treatment are described in Supplementary Table S1 (online only). There were no significant differences regarding treatment group or age at orchiectomy and follow-up between the study sample and the remaining invited men below 65 years ($n=533$, data not shown).

Endocrinological markers of spermatogenesis

The geometric mean of s-FSH was 10.9 (Table 1). Overall, 42% had elevated s-FSH ($\geqslant 12\,IU\,l^{-1}$), varying from 30% in the Surgery group to 75% in the Cis >850 mg group ($P<0.001$; Figure 1A). In the Cis >850 mg group, one out of three had s-FSH values above twice the upper normal level ($\geqslant 24\,IU\,l^{-1}$). According to the linear regression model, the two chemotherapy groups ($P<0.001$), but not the RT group, had significantly higher s-FSH than the Surgery group. The Cis >850 mg group had 89% higher levels of s-FSH compared with the Surgery group (Table 2), and 56% higher s-FSH levels than the Cis $\leqslant 850$ mg group ($P<0.001$).

The median s-inhibin B level was $57\,ng\,l^{-1}$ (Table 1). Only 10% had values above $140\,ng\,l^{-1}$, and in the Cis >850 mg group 60% had levels below the detection limit (Figure 1B). Both chemotherapy groups ($P=0.031$ and <0.001), but not the RT group, had significantly lower s-inhibin B values than the Surgery group in the multivariate model (Table 2). The s-inhibin B was significantly lower in the Cis >850 mg than the Cis $\leqslant 850$ mg group ($-42\,ng\,l^{-1}$, $P=0.006$).

Sperm counts

Overall, 193 (56%) had normospermia ($\geqslant 15$ millions per ml), 98 (29%) oligozoospermia, and 51 (15%) had azoospermia. Normospermia varied with treatment from 65% in the Surgery group to 29% in the Cis >850 mg group, and azoospermia from 6 to 43% (Figure 1C).

In the multivariate model, the two chemotherapy groups (OR 0.54–0.18), but not the RT group (OR 0.76), had significantly lower sperm counts compared with the Surgery group ($P<0.001$ (Cis >850 mg), $P=0.04$ (Cis $\leqslant 850$ mg) and $P=0.37$ (RT); Figure 2). The Cis >850 mg group also had significantly lower sperm counts than the Cis $\leqslant 850$ mg group ($P=0.014$).

Table 1 Hormone values and sperm counts according to treatment group[a]

	Surgery ($n=232$)	RT ($n=485$)	Cis $=850$ mg ($n=381$)	Cis >850 mg ($n=93$)	Total ($n=1191$)	*P*-value
s-FSH, geometric mean (range), $IU\,l^{-1}$	9.6 (2.8–60.9)	10.3 (1.9–86.5)	11.5 (0.5–64.4)	16.9 (1.3–66.3)	10.9 (0.5–86.5)	<0.001[b]
s-Inhibin B, median (range), $ng\,l^{-1}$	70 (<7–203)	62 (<7–256)	41 (<7–271)	<7 (<7–211)	57 (<7–271)	<0.001[c]
Sperm count, median (range), millions per ml	30 (0–293)	24 (0–283)	18 (0–480)	1 (0–98)	24 (0–480)	0.001[c]

Abbreviations: ANOVA = analysis of variance; Cis = cisplatin; FSH = follicle stimulating hormone; s = serum; RT = radiotherapy. [a]s-Inhibin B available in 441 cases. Sperm counts available in 342 cases. [b]One-way ANOVA (performed on the log-transformed value due to skewness). [c]Kruskal–Wallis test.

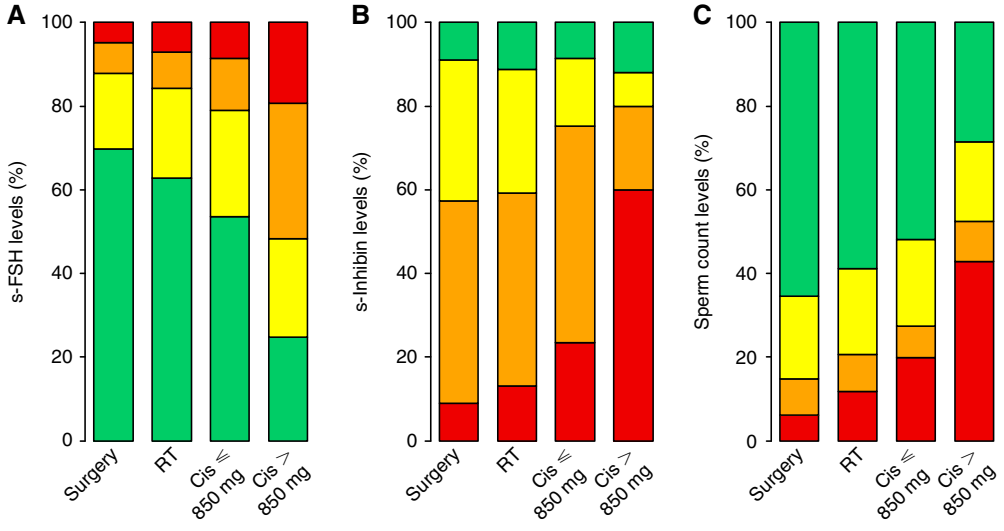

Figure 1 Fraction of men with grouped variables of s-FSH (**A**), s-inhibin B (**B**), and sperm counts (**C**) according to treatment. The colour range illustrates increasing pathological levels from normal (green) to most pathological (red). (**A**) For s-FSH ($IU\,l^{-1}$): green <12, yellow 12–23.9, orange 24–36, red $\geqslant 36$. (**B**) For s-inhibin B ($ng\,l^{-1}$): green $\geqslant 140$, yellow 80.01–139.9, orange 7–80, red <7, and (**C**) for sperm counts (millions per ml): green normospermia ($\geqslant 15.0$), yellow oligozoospermia (2.0–14.9), orange oligozoospermia (few visible sperm-1.9), and red azoospermia. ($P<0.001$ for s-FSH and s-inhibin B, $P=0.001$ for sperm counts, Kruskal–Wallis test, exact using Monte Carlo method).

Table 2 Variations in s-FSH (%) and s-inhibin B (absolute values) with 95% CI according to age, follow-up time, treatment group, and cryptorchism

	s-FSH			s-Inhibin B		
	Increase (%)[a]	**95% CI**	***P*-value**	**Regression co-efficient[b]**	**95% CI**	***P*-value**
Age (10-year interval)	22	16, 27	<0.001	−15	−21, −8	<0.001
Follow-up time (10-year interval)	−1	−10, 9	0.9	5	−10, 19	0.5
Treatment			<0.001			<0.001
Surgery	Reference			Reference		
RT	−0.01	−10, 10	0.9	2	−12, 16	0.8
Cis⩽850 mg	21	9, 34	<0.001	−19	−36, −2	0.031
Cis>850 mg	89	61, 121	<0.001	−61	−90, −32	<0.001
Cryptorchism			<0.001			0.6
No	Reference			Reference		
Yes	24	11, 37		−5	−22, 12	

Abbreviations: CI = confidence interval; Cis = cisplatin; FSH = follicle stimulating hormone; RT = radiotherapy. [a]On the basis of linear regression analyses, the analysis was performed on the logarithmic value of s-FSH; numbers stated as % are the exponentially transformed regression co-efficients. [b]On the basis of tobit censored linear regression, the regression co-efficient corresponds to change in absolute values of s-inhibin B ($ng\,l^{-1}$).

Age, follow-up time, and cryptorchism were non-significant ($P=0.7$, $P=0.13$, and $P=0.07$, respectively).

Relations between sperm counts, hormones, and post-treatment paternity

There was a moderate correlation between sperm counts *vs* s-FSH, s-inhibin B, and the s-inhibin B : FSH ratio, with correlation co-efficients of −0.500, 0.455, and −0.524, respectively (all $P<0.001$). The correlation between s-FSH and s-inhibin B was −0.656 ($P<0.001$).

The vast majority of men with azoospermia had elevated s-FSH and undetectable or low s-inhibin B levels (Figure 3). However, five had normal s-FSH and s-inhibin B levels of approximately or above $80\,ng\,l^{-1}$, possibly indicating obstructive azoospermia in some. The majority of men with normospermia had normal or only slightly elevated s-FSH levels, in some contrast to s-inhibin B, which was $<80\,ng\,l^{-1}$ in more than half of these men.

The endocrinological markers could not reliably predict oligo- or azoospermia. For oligozoospermia, the PPV for s-inhibin B $<80\,ng\,l^{-1}$ was 52% and for s-FSH $>10\,IU\,l^{-1}$ was 64%. When combined, the PPV increased to 73%. For counts <20 millions per ml, the PPV was slightly higher (59%, 69%, and 78%, respectively). For azoospermia, the PPV for s-inhibin B $<50\,ng\,l^{-1}$ was 32%, for s-FSH $>10.9\,IU\,l^{-1}$ was 36%, and 57% when combined.

Overall, 486 men (41%) reported attempts at conceiving a child following TC treatment. At follow-up, 66% (320 out of 486) had become post-treatment fathers without using pre-treatment banked semen (missing data in one). When men reporting dry ejaculation were excluded ($n=46$), 70% (308 out of 439) had been successful in their attempts. However, 28 reported that the couple had received some kind of medical assistance with reproduction. Post-treatment paternity varied significantly according to treatment (Surgery 80%, RT 66%, Cis ⩽850 mg 73%, and Cis>850 mg 42%; $P=0.001$). The men treated with the highest doses of chemotherapy were thus about half as likely to succeed in their attempts than men treated with surgery only, whereas the paternity rate following more limited chemotherapy was considerably closer to the rate observed in the Surgery group.

The s-FSH, s-inhibin B, and sperm counts all varied significantly with paternity outcome (Table 3). Among men with normal s-FSH, 79% had achieved paternity compared with 23% if grossly elevated s-FSH ($\geqslant 36\,IU\,l^{-1}$). Paternity rate was doubled among men with s-inhibin B levels $>140\,ng\,l^{-1}$ (83%) compared with undetectable levels ($<7\,ng\,l^{-1}$; 41%). In men with oligozoospermia (visible sperm-14.9 millions per ml), 71% had achieved paternity. Four men recorded with post-treatment paternity had azoospermia at follow-up. Three of them also had elevated s-FSH ($13-42\,IU\,l^{-1}$) and low s-inhibin B ($\leqslant 25\,ng\,l^{-1}$). The interval between the birth of their first child and follow-up was 7 − 16 years, allowing time for intercurrent episodes that further might have compromised spermatogenesis.

DISCUSSION

In this large cohort of long-term TC survivors, spermatogenesis was significantly impaired by all assessed markers in both chemotherapy groups, but not in the RT group when compared with the Surgery group. Among those who provided a semen sample, 44% had oligo- or azoospermia. The s-FSH, s-inhibin B, and sperm counts assessed at follow-up were all associated with post-treatment paternity.

To our knowledge, this is the largest study performed, evaluating long-term effects on spermatogenesis in TC survivors, and the first to report and relate sperm counts and the endocrinological markers s-FSH and s-inhibin B within the same men. The population-based cohort, long follow-up, and the possibility to compare different treatment groups are strengths of the study. Limitations include the cross-sectional study design, with only one assessment per man. In men treated with two to four cycles of BEP, the sperm counts ($n=71$) and s-FSH results ($n=316$) were included in a previous publication (Brydøy *et al*, 2010). Paternity has previously been described in detail (Brydøy *et al*, 2005), and are therefore only briefly reported here.

In line with previous studies, spermatogenesis was significantly impaired in the Cis>850 mg group when compared with the Surgery group (Hansen *et al*, 1990; Palmieri *et al*, 1996). However, spermatogenesis, as evaluated by all three assessed measures, was also significantly impaired in the Cis⩽850 mg group. This is in contrast to most previous reports from smaller studies among TC survivors treated with up to four chemotherapy cycles (Nijman *et al*, 1985; Aass *et al*, 1991; Pont *et al*, 1996). Elevated s-FSH and/or low s-inhibin B have, however, been reported 5 years or more following chemotherapy in more recent studies, in which the chemotherapy doses were not specified (Huddart *et al*, 2005; Nuver *et al*, 2005).

Interestingly, no significant impairment for any of the spermatogenic markers was found following RT compared with the Surgery group in multivariate analyses. This is supported by

similar non-significant results for s-FSH in the study by Huddart *et al* (2005). The long follow-up in these studies has likely yielded sufficient time to repair (Hansen *et al*, 1990). Shielding of the remaining testicle during radiation has probably also been an important factor, as probability and time to recovery of spermatogenesis are dose-dependent (Petersen *et al*, 1998). Although long-term spermatogenesis seem to be largely restored following RT, a prolonged period of recovery, the first years following treatment, may impair fertility during a more limited reproductive period of the couple, and thus reduce the chance of becoming a father (Huyghe *et al*, 2004; Brydøy *et al*, 2005).

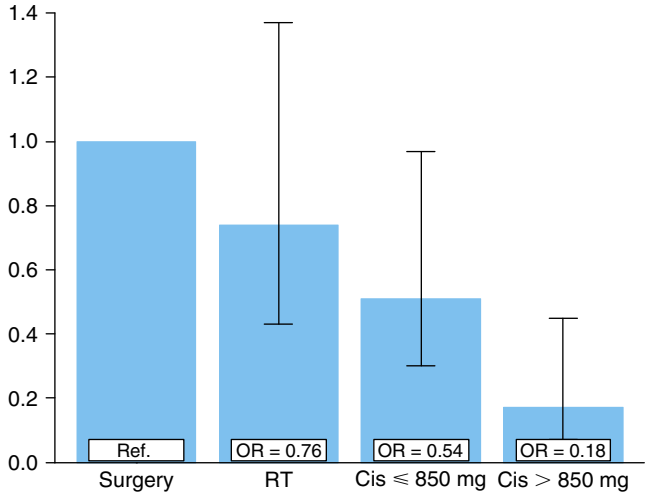

Figure 2 Odds ratios for increasing levels of sperm counts according to treatment group (overall $P=0.003$ compared with the Surgery group: $P<0.001$ (Cis > 850 mg), $P=0.04$ (Cis ⩽ 850 mg), and $P=0.37$ (RT)). Bars indicate 95% confidence intervals. In the proportional odds ordinal logistic regression model, sperm counts were grouped in five categories (azoospermia, few visible sperm to 1 million per ml, 1.1–9.9, 10–14.9, and ⩾15 millions per ml). The model was adjusted for age at follow-up, follow-up time, and self-reported cryptorchism (all non-significant).

Table 3 s-FSH, s-inhibin B, and sperm counts (millions per ml) at follow-up by post-treatment paternity[a]

	Achieved post-treatment paternity		
	Yes ($n=308$)	**No ($n=131$)**	***P*-value[b]**
s-FSH, IU l^{-1}, N *(%)*			<0.001
<12.0	223 (79%)	60 (21%)	
12.0–23.9	69 (60%)	46 (40%)	
24.0–35.9	13 (46%)	15 (54%)	
⩾36.0	3 (23%)	10 (77%)	
Geometric mean, IU l^{-1}	8.7	12.7	
s-Inhibin B, ng l^{-1}, N *(%)*[c]			0.003
<7.0	7 (41%)	10 (59%)	
7.0–79.9	52 (70%)	22 (30%)	
80.0–139.9	40 (82%)	9 (18%)	
⩾140.0	15 (83%)	3 (17%)	
Median (ng l^{-1})	78	57	
Sperm counts, millions per ml, N *(%)*[d]			<0.001
0	4 (18%)	18 (82%)	
Visible–1.9	9 (56%)	7 (44%)	
2.0–14.9	21 (81%)	5 (19%)	
⩾15.0	77(78%)	22 (22%)	
Median (millions per ml)	33	4	

Abbreviations: FSH = Follicle stimulating hormone. [a]Among 439 men without dry ejaculation who reported attempts at conception. [b]Mann–Whitney (exact using Monte Carlo method). [c]Available in 158 cases. [d]Available in 163 cases.

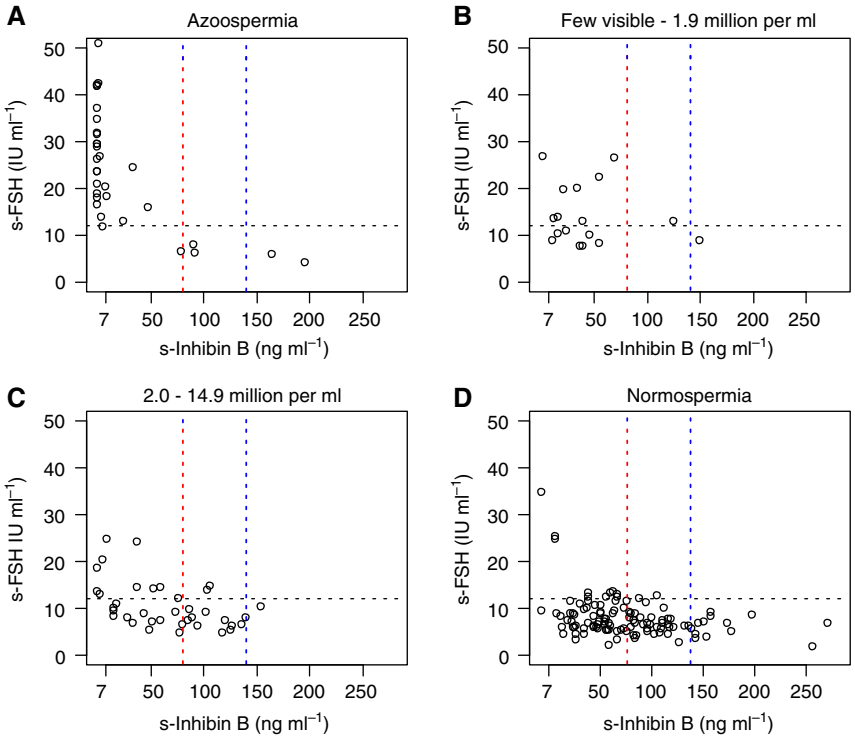

Figure 3 The s-inhibin B and s-FSH according to levels of sperm counts. Each circle represents one individual of the 199 participants, where all three measures were available. Horizontal black lines represents s-FSH 12 IU l^{-1} and separate normal from elevated values. Vertical lines represent s-inhibin B of 80 (red) and 140 ng l^{-1} (blue).

Following surgery only, 65% had normospermia and 6% azoospermia, compared with about 90% and 1%, respectively, in the general population (Cooper *et al*, 2010; Romerius *et al*, 2010). This difference may reflect an inherent testicular defect related to aetiological factors for TC (Wohlfahrt-Veje *et al*, 2009) and the consequence of having only one testicle. Jacobsen *et al* (2001) reported a higher frequency of azoospermia (13%) 2 years following orchiectomy only. Compared with the results for sperm counts in the RT and Cis $\leqslant$850 mg groups, Bahadur *et al* (2005) observed a similar or slightly lower prevalence of azoospermia (12%) and oligozoospermia (38%) following gonadotoxic treatment for TC (not further specified. Others report azoospermia to be less prevalent (Eberhard *et al*, 2004; Gandini *et al*, 2006). In the Cis $>$ 850 mg group, 43% had azoospermia, very similar to that reported by Petersen *et al* (1994) in their high-dose group (47%). This also corresponds well with the prognostic model for prediction of recovery to at least oligozoospermia defined by Lampe *et al* (1997) who described an estimated probability of $\sim$60% recovery 10 years after $>$4 cycles of Cis-based chemotherapy.

The observed s-inhibin B levels are considerably lower than that reported among the proven fertile men (Jensen *et al*, 1997; Andersson *et al*, 2004; Sikaris *et al*, 2005; Myers *et al*, 2009) and in the general population (Andersson *et al*, 2004). They also seem lower than that reported by Nuver *et al* (2005) in the TC survivors. They did, however, also report significantly lower levels following chemotherapy (median 63 ng l^{-1}) compared with surgery only (125 ng l^{-1}), as well as healthy controls (212 ng l^{-1}). Male inhibin B has mainly been assessed for research purposes, and uniform reference intervals are lacking (Jensen *et al*, 1997; Andersson *et al*, 2004; Sikaris *et al*, 2005; Myers *et al*, 2009). The cut-off levels applied in this report have previously been reported to discriminate competent from impaired spermatogenesis evaluated by testicular biopsies (140 ng l^{-1}; Pierik *et al*, 1998) and normospermia from oligozoospermia ($<$20 millions per ml) in ejaculates (80 ng l^{-1}; Jensen *et al*, 1997; Mahmoud *et al*, 1998). On the basis of a large study, Jørgensen *et al* (2010) recently suggested 150 ng l^{-1} as a relevant cut-off value for epidemiological studies, where lower levels may indicate subfertility and impaired semen quality. The s-inhibin B levels observed in this study ($<$140 ng l^{-1}) should thus indicate that $\sim$90% of the TC survivors have impaired semen quality. Although the sperm counts were normal in more than half of the available samples, other semen parameters that may influence quality were not assessed.

The correlations between sperm counts, s-inhibin B, and s-FSH were similar or slightly lower than that reported in men with impaired fertility, but higher than that reported in the general population (Jensen *et al*, 1997; Pierik *et al*, 1998; Andersson *et al*, 2004). The s-inhibin B has been proposed to be a better marker of spermatogenesis than s-FSH (Jensen *et al*, 1997; Pierik *et al*, 2003; Mabeck *et al*, 2005), and van Casteren *et al* (2010) found that s-inhibin B, but not s-FSH, significantly correlated with sperm concentration in cancer patients referred for sperm cryopreservation before treatment. We found that the correlation with sperm counts was slightly higher for s-FSH than for s-inhibin B, although highest for the s-inhibin B : FSH ratio. Some authors support combined testing for better prediction of spermatogenic function (von Eckardstein *et al*, 1999; Bohring *et al*, 2002; Andersson *et al*, 2004).

We applied cut-off levels for s-inhibin B and s-FSH that previously have been reported to be of predictive value for oligozoospermia ($<$20 millions per ml) in the general population or subfertile men (Jensen *et al*, 1997; Mahmoud *et al*, 1998), and for azoospermia in childhood cancer survivors (Romerius *et al*, 2010). We found the predictive abilities of these cut-off levels to be inferior to the findings in these reports (Jensen *et al*, 1997; Mahmoud *et al*, 1998; Romerius *et al*, 2010), but in line with a previous study for oligozoospermia (Meeker *et al*, 2007). Although the majority of participants with azoospermia had very low s-inhibin B and elevated s-FSH, this pattern was also seen in a few men with subnormal or normal sperm counts (Figure 3). Combined testing may thus give some indications of, but do not reliably predict, the sperm counts of TC survivors.

According to our opinion, we have not proven a definitive clinical benefit of evaluating s-inhibin B in the follow-up of TC survivors, where infertility might be a concern. There are also cost issues not further discussed here. Serum levels of luteinising hormone and testosterone are considered of limited value in the assessment of spermatogenesis (Jezek *et al*, 1998).

The fraction of men with achieved post-treatment paternity decreased with decreasing levels of s-inhibin B and, particularly, with increasing levels of s-FSH measured at follow-up. These associations must be interpreted with caution due to the unknown and varying interval between attempts at conceiving a child and follow-up. With normal s-FSH, 79% had succeeded in their attempts, somewhat lower than that reported by Huddart *et al* (2005) (91%). On the contrary, only about one in four of men with grossly elevated s-FSH ($\geqslant$36 IE) had achieved paternity. Post-treatment paternity seemed to vary less with s-inhibin B. As the 15 millions per ml reference value corresponds to the 5th centile in men whose partners conceived within a year of attempts, it is of interest that 71% of the men with oligozoospermia (excluding azoospermia) had become fathers. The TTP is, however, unknown, and some had received assistance with reproduction. Four men with azoospermia at follow-up reported post-treatment paternity. Although it is possible that some men were not the biological father, cautions should be made with such speculations as only one sample was analysed, and other incidents affecting sperm counts may have occurred between conception and sperm analysis years later (Islam and Trainer, 1998; Petersen and Hansen, 1999).

In conclusion, RT had no long-term effects on the assessed markers of spermatogenesis, whereas chemotherapy had. At present, the routine evaluation of s-inhibin B has not shown significantly better predictive value compared with FSH and other traditional fertility evaluations in the initial fertility assessment of TC survivors.

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