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TESTOSTERONE PRODUCTION IS BETTER PRESERVED AFTER 16 THAN 20 GRAY IRRADIATION TREATMENT AGAINST TESTICULAR CARCINOMA *IN SITU* CELLS

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Purpose: To study the effect of 16 Gy radiotherapy (RT) vs. 20 Gy RT on Leydig cell function in men treated with radiotherapy against carcinoma *in situ* (CIS) of the testis.
Methods and Materials: Fifty-one men who were treated between 1985 and 2005 were included. Fourteen men had been treated with 20 Gy and 37 with 16 Gy RT. Measurements of sex hormone–binding globulin and basic and stimulated testosterone, as well as luteinizing hormone levels were performed.
Results: The follow-up periods for the patients treated without additional chemotherapy were for the 20 Gy and 16 Gy group mean/median/min-max: 9.0/10.0/1.0–20.3 years and 4.0/3.1/0.4–14.1 years, respectively. During the follow-up period, men treated with 16 Gy RT had stable testosterone levels (–1.1%/year, $p = 0.4$), whereas men treated with 20 Gy had an annual decrease of 2.4% ($p = 0.008$). For the latter group, the testosterone decrease was most pronounced in the first 5 years, leveling off during the following 5 years. Additionally, more men treated with 20 Gy needed androgen substitution treatment. Our study showed an increased luteinizing hormone level for the men treated with 16 Gy, although this was not significant ($p = 0.5$). We anticipated a similar increase in the patients treated with 20 Gy but instead observed a decrease (-3.1%, $p = 0.01$).
Conclusion: RT at 16 and 20 Gy seem to affect Leydig cell function differently, with 16 Gy RT better preserving testosterone levels and thus being preferred from an endocrinological point of view.

Testicular carcinoma *in situ*, Irradiation treatment, Testosterone production, Leydig cell function, Hypogonadism.

INTRODUCTION

Carcinoma *in situ* (CIS) testis can be treated either by radiotherapy (RT) to the affected testis or with orchidectomy. In a patient with CIS in a solitary testis, *e.g.*, after unilateral orchidectomy for testicular cancer, both treatments will result in permanent removal of sperm cell production. These patients will also be permanently testosterone depleted after bilateral orchiectomy, whereas radiotherapy will leave more than half of patients with a sufficient testosterone production (1). Various doses of RT have been studied to find a balance between complete eradication of CIS cells while maintaining sufficient testosterone production. It has been shown that RT with doses of 20 Gy (21 patients), 18 Gy (3 patients), and 16 Gy (10 patients) resulted in eradication of CIS. In contrast, radiation therapy with 14 Gy (14 patients) did not eradicate CIS cells completely in all patients (2). An annual decrease of testosterone levels by approximately 3.6% in the RT-treated men was observed independent of the RT dose.

Since 2001, testicular RT with a dose of 16 Gy has been the treatment for CIS in a solitary testis at our clinic. Patients irradiated for CIS have been followed prospectively with assessment of Leydig cell function. Thus, the aim of this study is to further determine the effect of various radiation doses on testosterone production.

METHODS AND MATERIALS

Study population

The investigation protocol was approved by the local ethical committee. Fifty-one men were included retrospectively, 14 with CIS treated with 20 Gy (in 10 fractionated doses) RT from June 1985 to May 2001 and 37 men treated with 16 Gy (in 8 fractionated doses) from March 1990 to February 2005. Nine men in the 16 Gy group

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and 2 men in the 20-Gy group were also treated with bleomycin, etoposide, and cisplatin (BEP). The staging procedure of the patients included CT scan of the abdomen, X-ray of the chest, and measurement of tumor markers (alpha-fetoprotein, beta subunit of human choriogonadotropin, and lactate dehydrogenase). Only patients with disseminated disease received BEP, either three or four cycles, depending on the prognostic group. Chemotherapy was given before RT in 10 patients and 3 months after RT in 1. For the 51 men, both the mean and median ages were 32 years. Fifty men had been orchidectomized for unilateral testicular cancer (27 seminomas and 20 nonseminomas) and were diagnosed with CIS cells in the contralateral testis. Histology of 3 tumors of the men treated with 20 Gy could not be retrieved. One man had unilateral CIS and no spermatogenesis in the contralateral, non-tumor-bearing testis. For 39 men, the number of cross-sectional seminiferous tubules harboring CIS cells had been quantified and ranged from 1% to 100% of tubules (mean and median 47% and 35%, respectively). We did not detect any development of new germ cell tumors in any of the patients during the follow-up period.

Some data from 22 of the 51 men has previously been reported (2, 3). In addition to the 51 men, another 18 patients also received RT during the study period but were not included in this study because they chose not to participate in the follow-up program.

RT was delivered as previously described (2), and all patients were treated with the same technique. The testis with CIS was placed in a wax-lined lead cup, and daily doses of 2 Gy were given as electron irradiation (9–18 MeV) 5 days a week up to a total dose of either 16 or 20 Gy.

Follow-up of endocrine function

Endocrine follow-ups were routinely carried out approximately 3 months, 1 year, 2 years, 3 years, 4 years, 5 years, 8 years, and 10 years after RT. At follow-up, blood samples were drawn to assess the levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone–binding globulin (SHGB). A gonadotropin-releasing hormone (GnRH) stimulation test was performed by intravenous injection of 100 μg of GnRH to determine LH and FSH serum levels 15, 30, 60, and 120 min after injection. After the GnRH test, the men had a human chorionic gonadotropin (HCG) stimulating test with an intramuscular injection of 5,000 IU HCG. Blood samples were taken 72 and 96 h later to assess testosterone and SHBG levels following injection.

Serum levels of LH, FSH, and SHBG were determined using a time-resolved immunofluorometric assay (Delfia, Wallac, Turku, Finland), and testosterone levels were determined using a time-resolved fluoroimmunoassay (Delfia). Free androgen index (FAI) was calculated as (Testosterone/SHBG) $\times$ 100. The intra- and inter-assay coefficient of variation (CV) for measurement of LH and FSH were 3% and 4.5%, respectively. CVs for both testosterone and SHBG were < 8% and < 5%, respectively.

Statistics

The hormone values were transformed with the natural logarithm to obtain variance stability and to normalize data. The development of hormone values over time after RT was described in a multivariate random coefficient model in which each individual was allowed a piecewise linear function in time. In the statistical model, the time course during the first 5 years and the subsequent years were included separately, which allowed for comparison between the groups despite different follow-up times. Apart from time, year of birth and age at RT were also entered into the model as covariates; however, the effect of both were nonsignificant. The model was also used to estimate mean hormone levels at different time points after RT.

Patients were excluded from the follow-up analysis if treatment with intramuscular or dermal androgen substitution was started. If a patient was excluded at a certain point in the analysis during the follow-up period either because of testosterone treatment or end of follow-up, the hormone development data up until that time was nonetheless included in the final analysis. The proportion of men without androgen substitution therapy at different time points during the follow-up period were compared with a log-rank test.

The analyses were performed using the statistical package SAS (SAS Institute, SAS/STAT User's Guide: Statistics, Version 6, 4th ed., vol. 2. Cary, NC, 1990).

RESULTS

Patients treated with irradiation only

The total follow-up period for patients treated with 20 Gy was 108 years (mean 9.0, median 10.0, minimum-maximum 1.0–20.3 years). For the patients treated with 16 Gy, the follow-up period was 112 years (mean 4.0, median 3.1, minimum-maximum 0.4–14.1 years). Table 1 summarizes the annual changes of the total testosterone, LH, and FAI after RT for both RT groups.

Testosterone

For the 20 Gy-treated group testosterone levels showed an annual decrease of 9.0% during the first 5 years (95% confidence interval [CI]: 3.2%–14.5%, $p = 0.004$), whereas the levels were stable the following 5 years (decrease 0.8 per year, $p = 0.5$). The decrease during the first 5 years and the following 5 years differed statistically significantly ($p = 0.02$). Patients treated with 16 Gy had no significant change in testosterone levels during the first 5 years ($p = 0.7$) or the following years ($p = 0.1$). The testosterone levels decreased significantly more in the 20 Gy than in the 16 Gy group during the first five years ($p = 0.006$). For the entire follow-up period, men treated with 20 Gy had an annual decrease of 2.4% (95% CI: 0.7%–4.1%, $p = 0.008$) and men treated with 16 Gy an annual decrease of 1.1% ($p = 0.4$), which is statistically significantly different ($p = 0.005$).

Figure 1A illustrates the actual hormone levels of testosterone at different time points after RT with 16 Gy or 20 Gy. The hormone levels are calculated to represent a man aged 30 years at the time of entry into the study. For men in the 20-Gy group, the average testosterone level changed from 10.8 to 6.6 nmol/L during the 10-year period, and for men treated with 16 Gy, the level changed from 10.6 to 9.2 nmol/L.

Besides a decline in basic testosterone levels, the HCG-stimulated testosterone increase leveled off during the entire follow-up period for the 20 Gy group ($p = 0.0005$). After 10 years, they could only be stimulated to increase 3.3 nmol/L. For the 16 Gy group, no significant change in stimulated testosterone increase was detected ($p = 0.5$). After 10 years, the Leydig cells were still able to increase testosterone levels with 8.9 nmol/L. The change of stimulated testosterone levels between the two groups was statistically significant ($p = 0.0004$).

Table 1. Annual change of total testosterone, FAI, and LH levels in men treated with either 16 or 20 Gy irradiation against testicular carcinoma *in situ* cells

	16 Gy (n = 28)		20 Gy (n = 12)	
	% Change (95% CI)	p	% Change (95% CI)	p
Testosterone				
< 5 years	0.69 (–2.72 to 4.23)	0.7	–9.01 (–14.49 to –3.19)	0.004
> 5 years	–3.46 (–7.49 to 0.74)	0.1	–0.76 (–2.96 to 1.48)	0.5
FAI				
< 5 years	1.39 (–2.84 to 5.80)	0.5	–3.79 (–11.10 to 4.11)	0.3
> 5 years	–3.51 (–8.42 to 1.67)	0.2	–2.77 (–5.48 to 0.01)	0.05
LH				
< 5 years	–0.41 (–4.91 to 4.30)	0.9	–3.63 (–11.35 to 4.77)	0.4
> 5 years	2.72 (–2.93 to 8.69)	0.4	–2.95 (–5.84 to 0.03)	0.05

Abbreviations: CI = confidence interval; FAI = Free1 androgen index; LH = luteinizing hormone.Hormonal changes during the first 5 years are indicated as < 5 years and the following years as > 5 years. All patients included here were treated with irradiation only and no additional chemotherapy.

In the calculation of the hormonal changes the ages of the men were taken into consideration as a statistical confounding factor.

Figure 1B illustrates the levels of FAI. Annual changes can be seen in Table 1. The changes in FAI levels had the same tendencies as the changes over time in total testosterone levels. During the entire follow-up period, men treated with 20 Gy had an annual decrease of 3.0% (95% CI: 0.8%–5.1%, p = 0.007), in FAI and those treated with 16 Gy had an annual decrease of 0.7% (p = 0.6).

Luteinizing hormone

LH seemed to increase during the follow-up period for men treated with 16 Gy; however, this increase was not statistically significant (p = 0.5). Patients treated with 20 Gy had an annual, statistically significant decrease of 3.1% (95% CI: 0.8%–5.3%, p = 0.01) during the entire follow-up. The estimated 3.6% annual decrease during the first 5 years was nonsignificant (p = 0.4), whereas the 3.0% annual decrease during the following 5 years was significant at the 5% level (p = 0.05, Table 1). The differences in the annual LH changes between the two groups were statistically significant (p = 0.04).

For men treated with 20 Gy, the estimated levels of LH decreased from 15.7 nmol/L to 11.3 nmol/L 10 years later. For men treated with 16 Gy, LH levels increased from 15.7 nmol/L to 17.6 nmol/L.

For both groups the GnRH-stimulated LH levels indicated that the increase during the stimulation became lower with increasing follow-up time. A significant change in stimulated LH was detected in the men treated with 16 Gy (p < 0.0001). Here, the stimulated LH levels decreased from 75.3 U/L at treatment to 44.6 U/L after 10 years. In men treated with 20 Gy, the buffer capacity decreased from 63.4 U/L at treatment to 39.4 U/L after 10 years (p = 0.17). The more pronounced annual LH decrease in the 20 Gy vs. the 16 Gy group during the first 5 years was statistically significant (p = 0.014). No notable difference was seen between 5 and 10 years of follow-up (p = 0.58).

Follicle stimulating hormone

FSH showed the same tendencies as LH. For men treated with 16 Gy, there was a nonsignificant increase of 2.1%

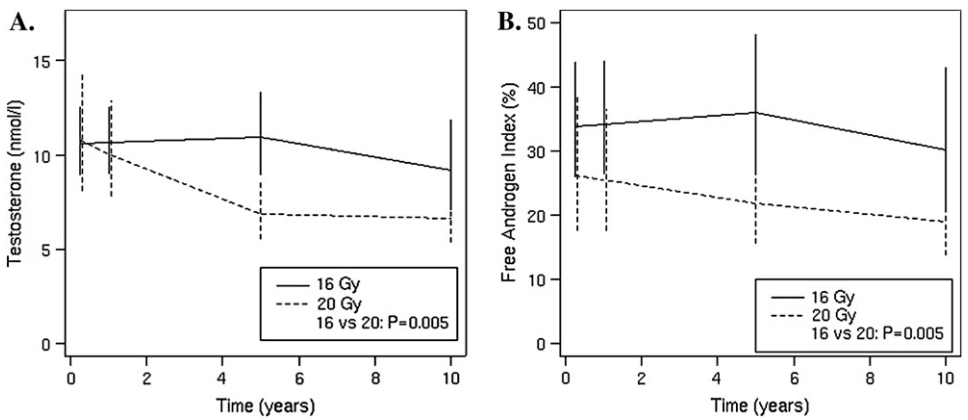

Fig. 1. Estimated testosterone (A) and free androgen index levels (B) at different time points after radiotherapy with 16 or 20 Gy for testicular carcinoma *in situ* are shown. In the estimations, patient age at time of study entry is taken into account, and the hormone levels are calculated to represent a man being 30 years of age at the time of entry. The vertical lines indicate the 95% confidence interval of the estimates.

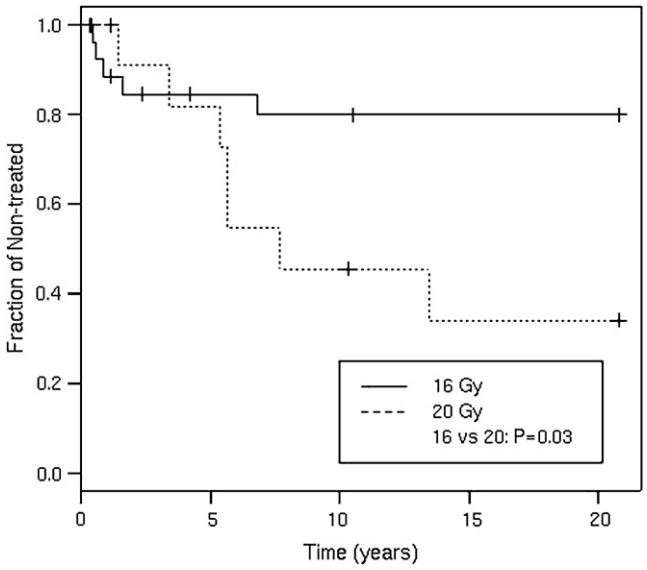

Fig. 2. The proportion of men without androgen substitution therapy at different time points during the follow-up period. The men were treated with radiotherapy only (16 or 20 Gy).

annually during the follow-up ($p = 0.1$). The men treated with 20 Gy had a statistically significant decrease of 2.6% annually (95% CI: 0.2%–5.0%, $p = 0.04$). The difference in FSH levels between men treated with 16 Gy and 20 Gy was statistically significant ($p = 0.01$).

For men treated with 20 Gy, the estimated levels of FSH decreased from 44.1 U/L to 30.6 U/L 10 years later. For men treated with 16 Gy FSH increased from 44.3 U/L to 59.1 U/L.

Patients treated with irradiation and additional chemotherapy

Testosterone: Two men received 20 Gy RT and chemotherapy. The testosterone level in one of these patients was 10.7 nmol/L, 10.7 nmol/L, and 6.1 nmol/L at 3 months, 5 years, and 20 years, respectively, after RT; in the other, it was 8.5 nmol/L and 9.7 nmol/L at 1 month and 3 years after RT, respectively. For the 9 men who received both 16 Gy RT and chemotherapy, the mean/median follow-up period was 2.6/2.3 years. They had a 5.9% annual decrease in testosterone during the follow-up period (95% CI: 0.5%–11.0%, $p = 0.04$). The levels of FAI followed the same trend as testosterone—a decrease of 4.5% annually ($p = 0.2$) during the follow-up period.

Luteinizing hormone: In the two men who had 20 Gy RT and chemotherapy, the LH levels were 15.7 nmol/L, 17.2 nmol/L, and 15.3 nmol/L at 3 months, 5 years, and 20 years, respectively, after RT, and 9.7 nmol/L and 11.0 nmol/L at 1 month and 17 months after RT, respectively. For the men who received 16 Gy RT and chemotherapy, LH levels were stable during the entire follow-up period ($p = 0.98$).

Androgen substitution therapy

During the follow-up period, androgen substitution therapy was initiated in 29 (56.9%) of the 51 men because of biochemical androgen insufficiency and symptoms of androgen insufficiency. Androgen substitution therapy was initiated in 11 (78.6%) of 14 patients treated with 20 Gy, including 2 patients treated with additional chemotherapy. For the 16 Gy group, 18 (48.7%) of the 37 patients, including 6 patients treated with additional chemotherapy, were started on androgen substitution. The difference between men treated with 16 Gy vs. 20 Gy was statistically significant ($p = 0.03$). Figure 2 illustrates the proportion of men without androgen substitution therapy at different time points.

DISCUSSION

We have shown that radiation therapy of CIS of the testes with doses of 16 Gy and 20 Gy affect Leydig cell function differently. Testicular radiation therapy for CIS with 20 Gy in 10 fractions results in a larger decrease of testosterone production compared with irradiation with 16 Gy. This difference has direct clinical implication because that a higher proportion of the men who received 20 Gy needed androgen substitution therapy. The impairment of testosterone production in the 20 Gy group started immediately following treatment and continued throughout the first 5 years. The decrease during the following 5 years was much smaller. In addition to the decrease in basic testosterone, HCG-stimulated values also showed that the capacity of testosterone production in the Leydig cells decreased in the 20 Gy group. In fact, the stimulated testosterone levels after 10 years were similar to the unstimulated values at the start, emphasizing decreased Leydig capacity. Contrary to these findings, patients treated with 16 Gy had almost stable levels of both basic testosterone and stimulated testosterone through the entire 10-year period, and they did not show the same tendency of decreasing testosterone levels during the first 5 years as the 20 Gy treated patients. Additionally, the fact that statistical analyses have focused on the time course during both the first 5 years and subsequent years make comparisons between the groups possible.

Body mass index tends to increase with age (4). This may lead to a reduced SHGB levels and thereby a reduced total testosterone to keep free testosterone levels unchanged (5). We detected the same tendencies for total testosterone and FAI, which indicates that the decrease in total testosterone, as we have shown, reflects a decreased Leydig cell function rather than an adaptation because of changed SHGB levels.

Although we did not find any significant change in either testosterone or LH levels for men treated with 16 Gy, a subclinical Leydig cell dysfunction, indicated by a decrease in testosterone and an increase in LH, may occur. In the patients receiving 20 Gy, we saw an unexplainable statistically significant decrease in LH during the entire follow-up period despite dropping testosterone levels. The decreasing basic LH levels were also associated with a decrease in stimulated LH levels. This finding seems not to be an artifact because we saw the same tendencies for FSH levels. This decrease in the gonadotropin levels indicates a reduction in pituitary function. However, none of the patients appeared to be pituitary insufficient, although we were unable to explore this further. In postmenopausal women, an initial increase in LH as well as FSH levels occurs during the early years after

menopause. This is, however, followed by a decrease caused by a blunted response to GnRH stimulation as menopause progresses (6, 7). Our findings seem not to reflect a change parallel to what is seen in postmenopausal women, however, because the decrease in gonadotropin levels in the men had already begun in the early years after RT.

Age as well as year of birth were considered possible confounders of the hormone levels. Because these variables were tested as explanatory variables in the statistical model and were found to be nonsignificant, the different effects of 16 vs. 20 Gy cannot be ascribed to difference in age or year of birth between the two groups.

We have based our conclusions on results from the group of patients treated without additional chemotherapy because it cannot be excluded that chemotherapy affects Leydig cell function. Treatment with cisplatin-based chemotherapy has been reported to result in normal testosterone levels but elevated LH, indicating a subclinical decrease in Leydig cell function (8). Another study detected a persistent and significant decrease in the testosterone/LH ratio compared with pretreatment levels up to 60 months after treatment (9). In our study, we found that 8 (72%) of 11 of the chemotherapy-treated patients ended up receiving androgen substitution therapy, compared with only 21 (52%) of the 40 patients treated with RT only. This is in line with additional damage to Leydig cell function by chemotherapy, but the group of men receiving chemotherapy was too small to allow for final conclusions regarding this issue.

The effect of RT on Leydig cells can best be evaluated by hormone assessments. The number of men started on substitution therapy points toward the same direction as the hormonal evaluations, but the criteria of when to start a man on substitution therapy have probably changed gradually over the years, despite of the same principal indication. In our clinic, men tend to be started on androgen substitution at milder degrees of androgen insufficiency than they were 10–15 years ago. Thus, conclusions regarding Leydig cell function based on when androgen substitution therapy was started will most likely be flawed by changing clinical practice.

In general, it is believed that CIS and the development of testicular germ cell cancer is part of the testicular dysgenesis syndrome caused by abnormal fetal testicular development (10). The disrupted testicular development can also affect the Leydig cells, observed as Leydig cell nodules and impaired Leydig cell function (11, 12). Accordingly, several studies have found that Leydig cell function in testes harboring CIS cells was impaired even before treatment (1–3). Petersen *et al*. (2) hypothetically explained this as resulting from direct CIS inhibition of Leydig cell function, through Sertoli cells or because of germ cell depletion. Thus, it is not unexpected that some men with CIS will eventually require testosterone supplement. However, men receiving 20 Gy RT had a more pronounced decrease in testosterone levels and more of them required testosterone substitution than men receiving 16 Gy. This indicated that the lower RT dose resulted in a milder adverse effect of Leydig cells. The shorter follow-up period of men treated with 20 Gy is also in line with this hypothesis.

RT with doses between 16 and 20 Gy will normally eradicate CIS; however, relapses have been observed at all dose levels (13–15). We did not detect any relapses, and our data suggest that radiation treatment with 16 Gy is better than treatment with 20 Gy for conservation of Leydig cell function. Thus, from an endocrinological perspective, 16 Gy appears to be the treatment of choice.

REFERENCES

1. Petersen PM, Giwercman A, Daugaard G, *et al*. Effect of graded testicular doses of radiotherapy in patients treated for carcinoma-*in-situ* in the testis. *J Clin Oncol* 2002;20:1537–1543.
2. Petersen PM, Giwercman A, Hansen SW, *et al*. Impaired testicular function in patients with carcinoma-in-situ of the testis. *J Clin Oncol* 1999;17:173–179.
3. Giwercman A, von der Maase H, Berthelsen JG, *et al*. Localized irradiation of testes with carcinoma in situ: Effects on Leydig cell function and eradication of malignant germ cells in 20 patients. *J Clin Endocrinol Metab* 1991;73:596–603.
4. Schubert CM, Rogers NL, Remsberg KE, *et al*. Lipids, lipoproteins, lifestyle, adiposity and fat-free mass during middle age: The Fels Longitudinal Study. *Int J Obesity* 2006;30:251–260.
5. Andersson AM, Jensen TK, Juul A, *et al*. Secular decline in male testosterone and sex hormone binding globulin serum levels in Danish population surveys. *J Clin Endorinol Metab* 2007;92:4696–4705.
6. Rossmanith WG, Scherbaum WA, Lauritzen C. Gonadotropin secretion during aging in postmenopausal women. *Neuroendocrinol* 1991;54:211–218.
7. Genazzani AD, Petraglai F, Sgarbi L, *et al*. Difference of LH and FSH secretory characteristics and degree of concordance between postmenopausal and aging woman. *Maturitas* 1997;26:133–138.
8. Hansen SW, Berthelsen JG, von der Maase H. Long-term fertility and Leydig cell function in patients treated for germ cell cancer with cisplatin, vinblastin, and bleomycin versus surveillance. *J Clin Oncol* 1990;8:1695–1698.
9. Brennemann W, Stoffel-Wagner B, Helmers A, *et al*. Gonadal function of patients treated with cisplatin based chemotherapy for germ cell cancer. *J Urol* 1997;158:844–850.
10. Skakkebæk NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: An increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16:972–978.
11. Hoei-Hansen C, Holm M, Rajpert-De Meyts, *et al*. Histological evidence of testicular dysgenesis in contralateral biopsies from 218 patients with testicular germ cell cancer. *J Pathol* 2003;200:370–374.
12. Holm M, Rajpert-De Meyts E, Andersson A-M, *et al*. Leydig cell micronodules are a common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreased testosterone/LH ratio. *J Pathol* 2003;199:378–386.
13. Dötsch M, Brauers A, Büttner R, *et al*. Malignant germ cell tumor of the contralateral testis after radiotherapy for testicular intraepithelial neoplasia. *J Urol* 2000;164:452–453.
14. Dieckmann K-P, Lauke H, Michl U, *et al*. Testicular germ cell cancer despite previous local radiotherapy to the testis. *European Urol* 2002;41:643–650.
15. Classen J, Dieckmann K, Bamberg M, *et al*. Radiotherapy with 16 Gy may fail to eradicate testicular intraepithelial neoplasia: Preliminary communication of a dose reduction trial of the German Testicular Cancer Study Group. *Brit J Cancer* 2003;88:828–831.