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Sex hormones and sperm parameters after adjuvant oxaliplatin-based treatment for colorectal cancer

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ABSTRACT

Background: The incidence of colorectal cancer (CRC) in individuals of fertile age is increasing. Oxaliplatin is a cornerstone treatment in the adjuvant setting for stage III and high-risk stage II CRC. Limited data exist on possible side effects of oxaliplatin on fertility and gonadal function. More data is needed to guide possible fertility preservation procedures and aid evidence-based fertility counselling.

Patients and methods: The aim of this study (EudraCT2006-002832-10) was to prospectively investigate sex hormones and sperm parameters after oxaliplatin-based adjuvant chemotherapy to clarify the risk of infertility and hypogonadism. Twenty males aged ≤55 years and 16 females aged ≤40 years were recruited from five hospitals in the Nordic countries. All had undergone radical surgery due to CRC and were given adjuvant oxaliplatin in combination with 5-fluorouracil. Measurement of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, sex hormone binding globulin (SHBG) and semen analysis were done in males, while LH, FSH and oestradiol were measured in females. Measurements were done prior to chemotherapy, after completion of adjuvant treatment and at follow-up 1 and up to 5 years after end of treatment.

Results: FSH and testosterone levels increased in males after chemotherapy treatment but were restored at follow-up. No patients developed hypogonadism. There was a trend towards a decrease in sperm concentration during treatment ($p = 0.063$). When comparing sperm concentration and rapid progressive motility of sperms prior to chemotherapy and at follow-up, there were no differences, and no patients became permanently azoospermic by treatment. No distinct altering of gonadal function could be observed in females.

Conclusions: Oxaliplatin in combination with 5-fluorouracil seems to induce transient decrease in sperm concentration with recovery and a minor transient increase in FSH in males. No distinct altering of gonadal function was observed in females. The risk of infertility and hypogonadism in males and females after adjuvant oxaliplatin-based chemotherapy seems low.

1. Introduction

Colorectal cancer (CRC) is the third most common cancer in the world (third in males, second in females) with an estimated 1.9 million new cases each year [1]. CRC generally affects the elderly, with more than 90% above 50 years of age, but the absolute number of affected individuals of reproductive age is still substantial and the incidence among younger individuals in the western world is increasing [2–6].

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Adjuvant treatment following radical surgery of stage III and high-risk stage II colon cancer and rectal cancer consists of 5-fluorouracil (5-FU) and oxaliplatin [7]. 5-FU is considered to have a very modest and reversible effect on fertility [8,9].

There is limited knowledge of the effect of oxaliplatin on fertility and gonadal function in patients of fertile age. The NCCN (National Comprehensive Cancer Network) and ESMO (European Society for Medical Oncology) CRC clinical practice guidelines and guidelines for patients do not mention the fertility aspect concerning adjuvant chemotherapy [10,11]. ASCO guidelines state that health care providers should discuss the possibility of infertility and fertility preservation options with all reproductive-age patients diagnosed with cancer [12]. There is a need for further data to guide possible fertility preservation procedures and aid evidence-based fertility counselling of young CRC patients before receiving adjuvant chemotherapy with oxaliplatin and 5-fluorouracil. Among young women diagnosed with cancer, concerns regarding future fertility are secondary only to concern regarding survival [13,14]. Pre-treatment fertility counselling of women with cancer by an oncologist and fertility specialist has been shown to be associated with less remorse and greater quality of life [15]. A study from 2012 showed that only 34% of patients with newly diagnosed CRC had a fertility discussion [16].

The group of cytotoxic drugs that are most prone to induce gonadal failure in both women and men are alkylating agents, such as cyclophosphamide [17]. Susceptibility varies among individuals and by age, but the risk of gonadal damage increases with the dose given [18]. The administration of cisplatin, a platinum compound as oxaliplatin, has been observed to induce infertility in males in about 50% if given with doses >400 mg/m^2 [19]. In general, spermatogenesis is more prone to be affected by chemotherapy than testosterone production [20,21] and can lead to decreased sperm concentration and sperm motility. Follicle-stimulating hormone (FSH) secretion frequently increases because of a decrease of inhibin B, while luteinizing hormone (LH) and sex hormone binding globulin (SHBG) generally remains unchanged [22,23]. Testosterone levels may be affected, but not always. The ovaries seem to be affected by cytotoxic agents in a similar fashion, the dividing theca and granulosa cells are affected, whereas the non-dividing oocytes are more resistant [24]. Amenorrhea is often observed during treatment but is usually transient over time [25].

As mentioned above, knowledge regarding the effect of oxaliplatin on the gonads and fertility is more limited. Although similar to other platinum compounds, toxicity profiles of cisplatin, carboplatin and oxaliplatin differ. In a retrospective study patients who had undergone adjuvant oxaliplatin-based treatment (FOLFOX: folinic acid, 5-fluorouracil and oxaliplatin), 41% (20/49) of the women reported amenorrhea during chemotherapy, and 16% had persistent amenorrhea one year after treatment [26]. In a similar retrospective study, 3 of 72 women (4%) experienced persistent amenorrhea after adjuvant oxaliplatin-based treatment [27].

With increasing incidence of CRC in individuals of fertile age, expanded knowledge of the effect of oxaliplatin-based chemotherapy on fertility is needed. This study prospectively investigated gonadal function in males and females and sperm parameters in males with follow-up at 5 years in young CRC patients receiving adjuvant chemotherapy with oxaliplatin and 5-FU.

2. Methods

2.1. Study design and participants

This prospective study (EudraCT2006-002832-10) was carried out at five centres in Norway, Sweden and Finland. Patients were recruited from November 2006 to November 2013. The protocol was approved by the ethical committees at the respective centres/countries and all participants signed a written informed consent. Patients with CRC stage II and III were eligible for inclusion, with age limits set at ≤55 years for males and ≤40 years for females at the time of 5-FU-oxaliplatin-based chemotherapy initiation. Total cumulative dose and cycles of oxaliplatin administered for each patient was recorded.

The design of the study included measuring levels of FSH (IE/l), LH (IE/l), testosterone (nmol/l) and SHBG (nmol/l) in males and FSH (IE/l), LH (IE/l) and oestradiol (pmol/l) in females. Longitudinally easurements were done before and after chemotherapy as well as at follow-ups with yearly intervals until normalization, or if not normalized up to 5 years after completion of chemotherapy. In females, the samples were collected 3–4 days into the menstrual cycle. In males, testosterone/SHBG-ratio was calculated, as this is a marker for the bioavailable testosterone. Semen analysis (according to the most recent WHO-criteria) was performed and carried out at the same time points. Included in the sperm analysis was volume (ml), concentration (10^6/l), number alive (percentage), rapid progressive motility (percentage) and normal morphology (percentage). All samples were analyzed at each centre's certified fertility lab according to local routines regarding sample collection and analysation.

2.2. Statistical analysis

Statistical analysis was performed using the SPSS 24 (Armonk, NY, USA). We compared values of FSH, LH and testosterone for men and FSH, LH and oestradiol for women after surgery before chemotherapy, immediately after chemotherapy completion and at follow-up 1, 2 and 3 years (some patients were tested after 4 and 5 years as well) post chemotherapy. Of the follow-up values we chose the highest value for each patient. We also compared values at the same time points for sperm concentration and rapid progressive motility. We used Wilcoxon rank test to – pairwise - evaluate for statistically significant altering of the values between the individual time points, two-sided p-values below 0.05 was considered statistically significant.

3. Results

Patient characteristics are shown in Table 1. Sixteen women and 20 men were included. All patients received 5-FU and oxaliplatin with the regimens FOLFOX, CAPOX (capecitabine orally twice a day for two weeks combined with oxaliplatin every three weeks) or Nordic FLOX (5-FU bolus, folic acid and oxaliplatin) [31]. One male did not provide any hormone or sperm samples. None of the patients received preoperative or adjuvant radiotherapy.

Table 1
. Patient characteristics and treatment in colorectal cancer patients given adjuvant oxaliplatin in combination with 5-FU.

	Male ***n*** **= 20**	**Female** ***n*** **= 16**
Median age in years (range)	36 (27–55)	35 (20–40)
Primary tumor (n) Colon cancer stage II	2 (10%)	0
Colon cancer stage III	16 (80%)	13 (81.25%)
Rectal cancer stage II-III	2 (10%)	1 (6.25%)
Colorectal cancer stage IV	0	1 (6.25%)
Appendiceal adenocarcinoma	0	1 (6.25%)
Chemotherapy regimen (n) FLOX	17 (85%)	10 (62,5%)
FOLFOX	0	3 (18,75%)
CAPOX	3 (15%)	3 (18,75%)
Median body surface area (m^2) (range)	1.98 (1.65–2.23)	1.75 (1.65–2.05)
Median number (range) of cycles		
FOLFOX/FLOX two-weekly	10 (6–12)	10 (6–12)
CAPOX three-weekly	5 (2–8)	8 (7–8)
Median total dose (range) of oxaliplatin/m^2 (mg/m^2)	753 (261–1074)	869 (630–1038)

3.1. Hormone levels in males

Hormone levels from 19 males were analyzed before start of chemotherapy. Fifteen of them provided samples after chemotherapy and 9 during follow-up, whereas 4 did not provide any further samples (Fig. 1a). The hormone levels at the different time points are illustrated in Fig. 2. FSH increased from median 4 (2–27) before start to 7 (4–30) IU/L ($p = 0.002$) after the completion of chemotherapy. The median value then decreased at follow-up to 4 (2–10) IU/L ($p = 0.024$), thus to the same level as before chemotherapy. For LH, no statistically significant changes with time were observed. Testosterone levels increased from median 14.5 (8–23) at baseline to 18 (9–36) nmol/L nmol/L) ($p = 0.002$) after completion of chemotherapy, and at follow-up the median level decreased to 14 (8–21) nmol/L ($p = 0.063$), thus to similar level as before start of treatment.

After chemotherapy only two individuals had increased levels above the reference values of FSH of which one also had levels of LH above the reference (20 IU/L and 30 IU/L for FSH, 16 IU/L for LH). One of them had a low sperm concentration before chemotherapy, but increased after chemotherapy and at follow-up (2, 15, 28 × 106/mL at the respective time points), and the other had azoospermia. After completion of chemotherapy, the testosterone levels increased, and only one had a level below 10 nmol/l (9 nmol/l).

SHBG levels increased significantly from a median 27 (12–42) at baseline to 51 (27–89) nmol/L at completion of treatment ($p = 0.001$) whilst the testosterone/SHBG ratio decreased significantly from a median 0.53 (0.41–0.88) to 0.38 (0.18–0.56) ($p = 0.002$, respectively) (Fig. 2b). Both SHBG levels and testosterone/SHBG ratio was restored at follow-up after one year, with a significant difference between the values after chemotherapy and at follow-up ($p = 0.018$ and $p = 0.018$, respectively). When comparing the levels of SHBG and testosterone/SHBG ratio before chemotherapy and at follow-up, there were no statistically significant differences. There were no pathological SHBG levels before chemotherapy or at follow-up, and only two slightly elevated

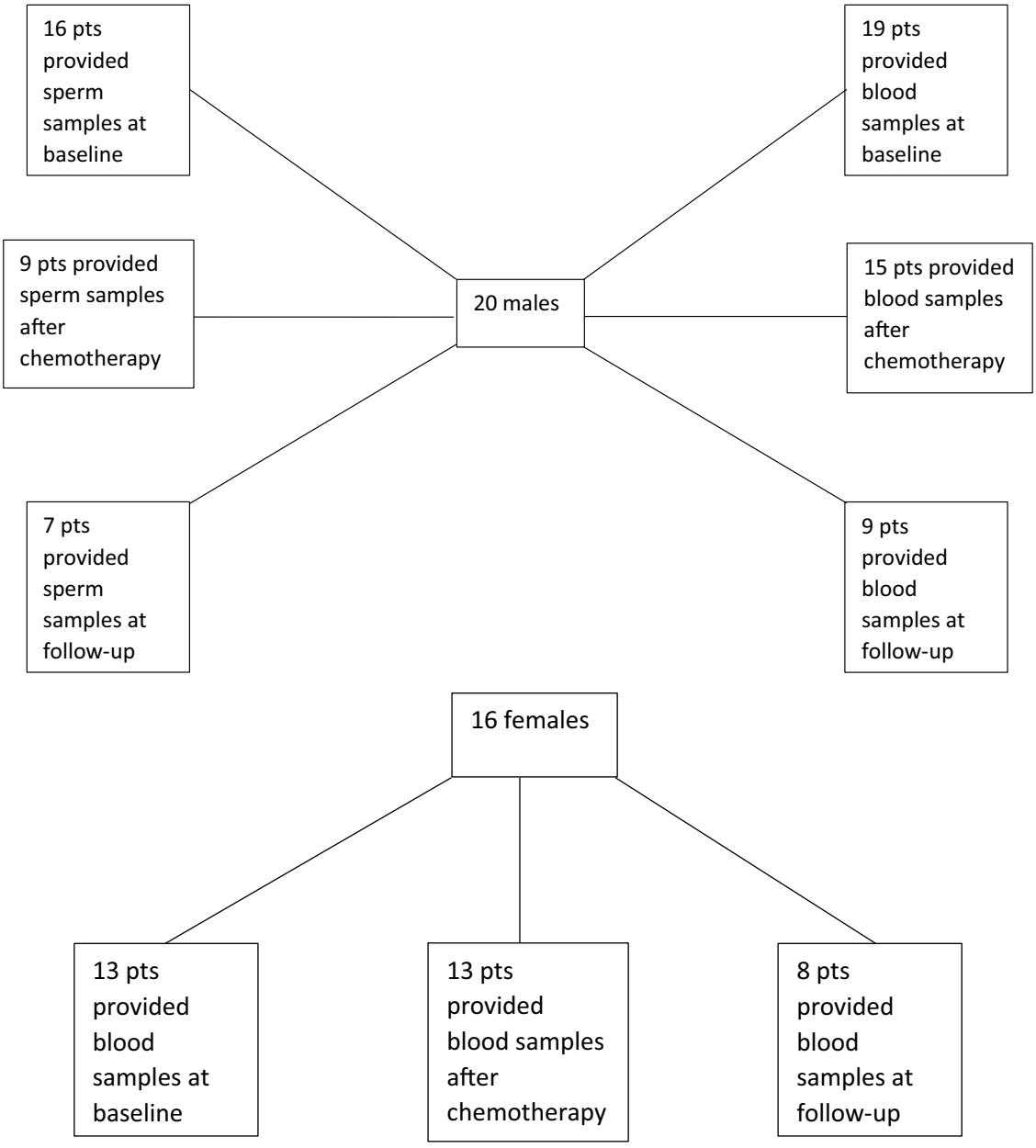


Fig. 1. (a)Diagram illustrating number of male patients providing sperm samples (to the left) and blood samples for hormonal analysis (to the right).b Diagram illustrating number of female patients providing blood samples for hormonal analysis.

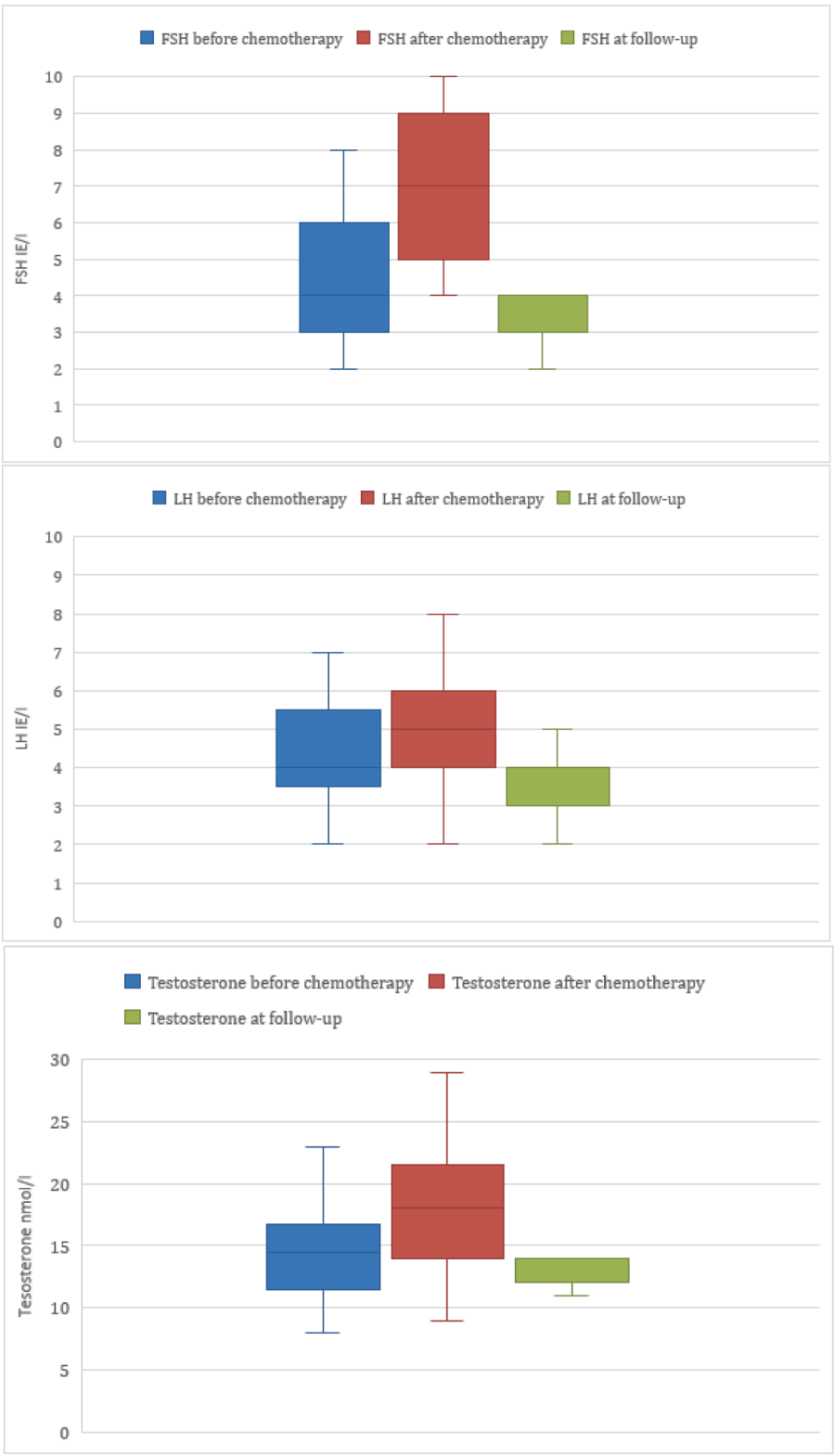


Fig. 2. (a) Levels of FSH (IE/l, top panel), LH (IE/l, middle panel) and testosterone (nmol/l, bottom panel) in male colorectal cancer patients given adjuvant oxaliplatin-based chemotherapy. Hormone levels before start of chemotherapy, after completion of chemotherapy, and at follow-up (1–3 years after chemotherapy) is shown. The increase from pre-treatment in FSH after chemotherapy was statistically significant ($p = 0.002$), and also the decrease in FSH at follow-up ($p = 0.024$). (b) Ratio testosterone/SHBG and levels of SHBG before chemotherapy, after chemotherapy and at follow-up (1–3 years after chemotherapy) in male colorectal cancer patients given adjuvant oxaliplatin-based chemotherapy. The ratio testosterone/SHBG decreased and SHBG increased, both significantly ($p = 0.001$ and $p = 0.002$, respectively) after chemotherapy treatment. The values were restored at follow-up ($p = 0.018$ and $p = 0.018$, respectively).

levels (82 and 89) after chemotherapy. A similar pattern was found in regards of the testosterone/SHBG ratio, with no pathological values before chemotherapy, four slightly low ratios after chemotherapy (0.18, 0.27, 0.27 and 0.29), and one value just below normal at follow-up (0.28).

3.2. Sperm parameters

Sixteen patients provided one or more sperm samples. Five patients provided samples on all three time points, 4 before and after chemotherapy, 2 before chemotherapy and at follow-up, 4 before chemotherapy, and 1 did only provide sample at follow-up (Fig. 1a). Sperm

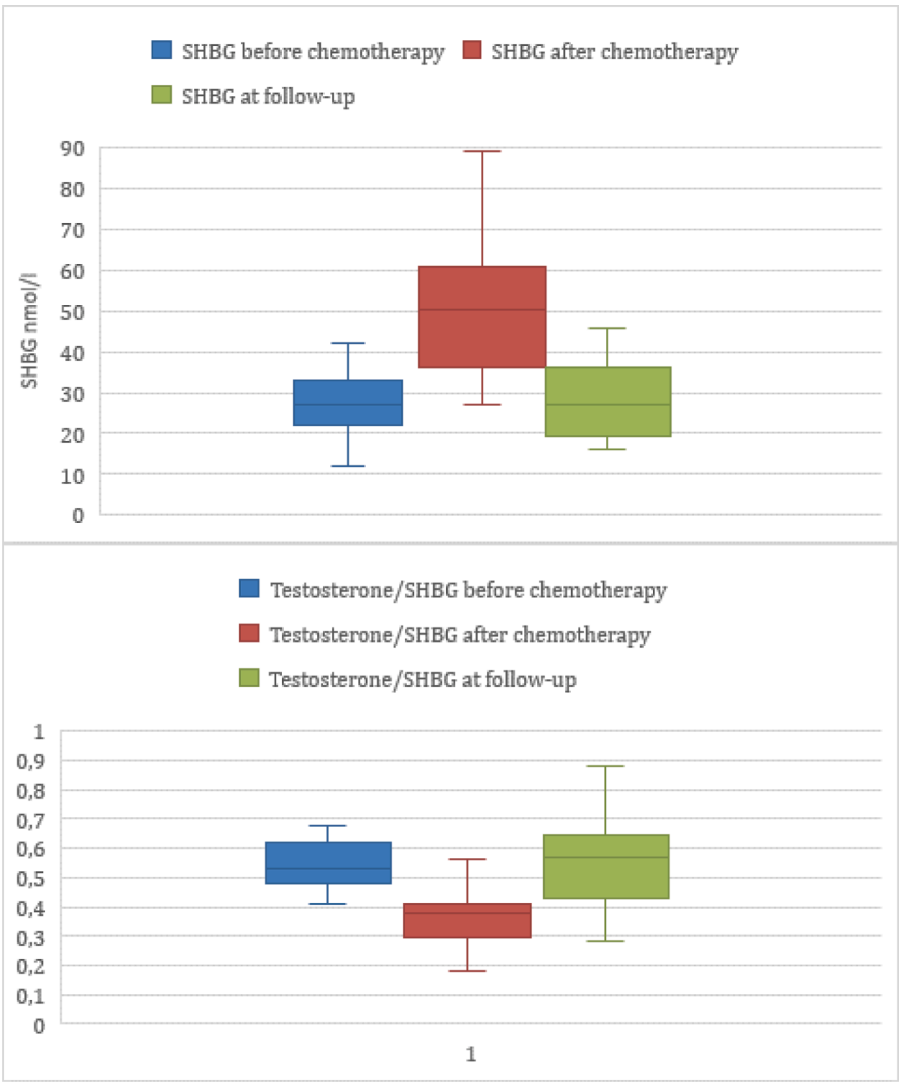


Fig. 2. (*continued*).

concentration decreased after chemotherapy compared to after surgery, from a median 29 (0–365) to 9 (0–16) $\times$ 10^6/mL, but not statistically significant (p = 0.063) (Fig. 3). The concentration then increased at follow-up to a median 105 (6–606) $\times$ 10^6/ml, but again, not statistically significant (p = 0.08). When comparing sperm concentration before chemotherapy and at follow-up the median value increased, but not statistically significant (p = 0.2). The rapid progressive motility (%) assessment varied in a similar fashion, with a median after surgery at 30% (0–76%), with a decrease after chemotherapy to 6% (0–29%), though not statistically significant (p = 0.2). At follow-up, the median increased to 33% (8–48%), though not statistically significant (p = 0.1). When comparing the rapid progressive motility after surgery and at follow-up, there were no difference (p = 0.6). An increase of testosterone occurred with the decline in sperm concentration, whereas an increase of sperm concentration normalized testosterone levels (Fig. 4a). Sperm concentration was frequently higher at follow-up than before chemotherapy (Fig. 4a+b).

The values for sperm concentration for the individuals providing samples are illustrated in Fig. 4b. Two men had azoospermia after surgery, one of which did not provide any more samples, and the other had azoospermia also after chemotherapy. Four men who had normal concentration of sperm (WHO reference range 15–259 $\times$ 10^6/mL) after surgery had subnormal values after chemotherapy. Two of them did not provide any more samples, and the other two had normal concentration values at follow-up. Four men had subnormal values after surgery and after chemotherapy. Of these, two had normal values at follow-up, one still had a subnormal value, and the fourth did not provide any sample at follow-up. Amongst the eight men included in the follow-up, only one had a sperm concentration below normal according to WHO (6 $\times$ 10^6 /mL). Rapid progressive motility showed a similar pattern, with a transient decline after chemotherapy, but with recovery at follow-up. Of the eight men providing samples at follow-up, four had subnormal motility (8, 15, 20 and 27%), whilst the other four men had normal values (38, 39, 47 and 48%). Three men reported at follow-up that they had conceived a child.

3.3. Hormone levels in females

Hormone levels were analyzed in 16 women. The hormone levels in 7 females were analyzed before chemotherapy, after chemotherapy and at follow-up, in 4 before and after chemotherapy, in one female after chemotherapy and at follow-up, whereas 2 only provided samples before chemotherapy and 1 only after chemotherapy (Fig. 1b). Three women with very low levels of oestradiol but with normal levels of FSH/LH were excluded from analysis because we interpreted their values as represented by intake of oral contraceptives, which was unfortunately not recorded. One woman had postmenopausal values before start of chemotherapy and was excluded from analysis. We could not demonstrate changes in neither FSH, LH nor oestradiol comparing values before chemotherapy, after chemotherapy or at follow-up (Fig. 5). One

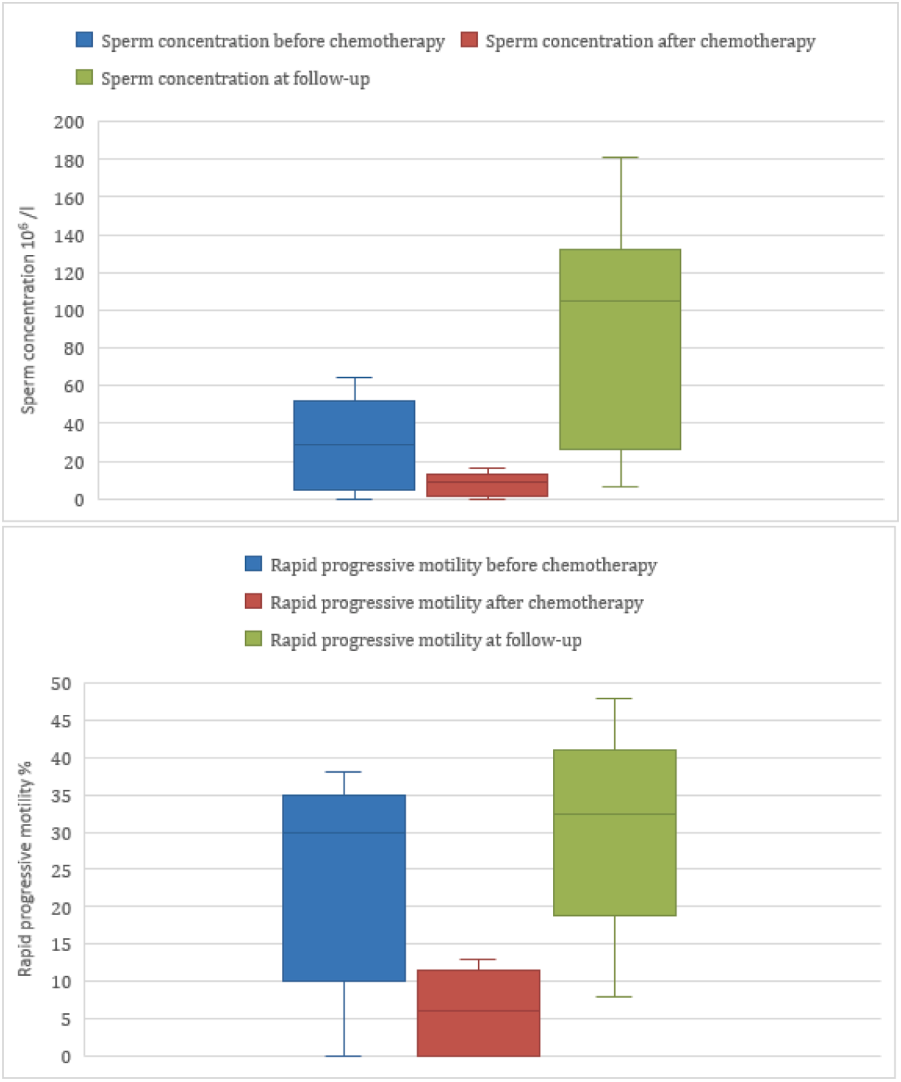


Fig. 3. Sperm concentration (106/l) and rapid progressive motility (%) measured before chemotherapy, after chemotherapy and at follow-up (1–3 years after chemotherapy) in male colorectal cancer patients given adjuvant oxaliplatin in combination with 5-FU. The changes in sperm concentration were not significant after surgery compared to after chemotherapy, (p = 0.63) or after chemotherapy compared to follow-up, (p = 0.08). The p-values for rapid progressive motility were higher (p = 0.17 and 0.138) for the respective time comparisons).

woman had slightly elevated FSH (14) and normal LH (10) before chemotherapy, both had increased to pathological values after chemotherapy (26 and 32 for FSH and LH, respectively) and were normalized at follow-up (13 for both). Oestradiol was normal at all three time points. None of the other women had pathological values of FSH or LH after chemotherapy or at follow-up. Two women that had normal oestradiol values before chemotherapy had subnormal values after chemotherapy but did unfortunately not provide any samples at follow-up. All five women who provided values at follow-up had normal values. Three women reported at follow-up that they had conceived a child.

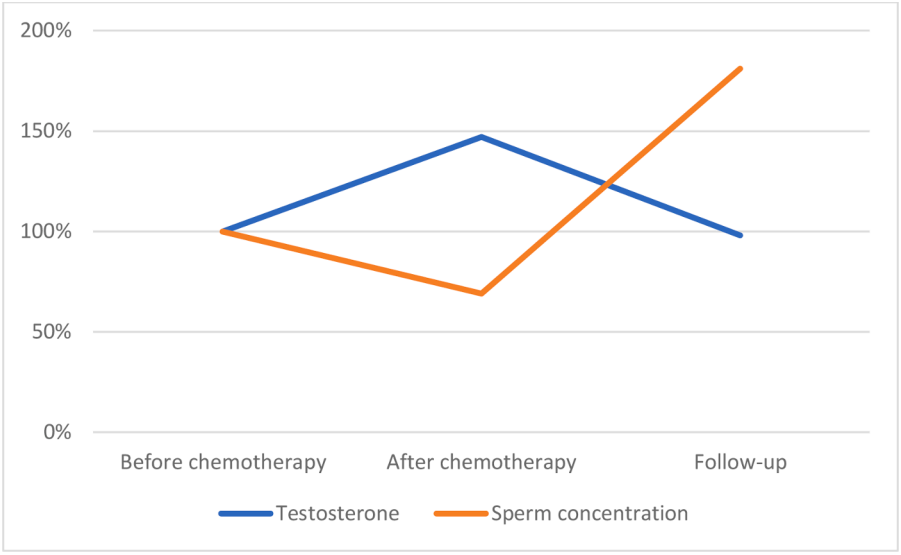
No correlations were found between total dose oxaliplatin exposure and any of the above-mentioned alterations in hormone or sperm parameters.

4. Discussion

The increase in CRC among younger age groups has led to more frequent adjuvant oxaliplatin-based treatment in fertile patients. Knowledge of possible side-effects of oxaliplatin treatment on fertility is therefore needed to guide pre-treatment fertility procedures and give relevant information to patients on this issue. In this study, adjuvant chemotherapy with oxaliplatin/5-FU in CRC patients led to a transient increase in FSH and reduced sperm concentration and motility in the majority of male patients, while the treatment did not seem to affect female sex hormone values. The risk of infertility and hypogonadism in males and females after adjuvant oxaliplatin/5-FU thus seems low. The possible individual impact of 5-FU and oxaliplatin, respectively, was not evaluated in this study, as all patients received both components.

Our study did not find any evidence that oxaliplatin in combination with 5-FU induced testosterone deficiency, and LH was not altered in males. The observed transient increase in FSH in many patients, was likely due to reduced spermatogenesis. In Levi et al's study from 2015, only minor changes in male sex hormones levels given adjuvant oxaliplatin-based chemotherapy for CRC was demonstrated. This study also found that FSH and testosterone level increased transiently [28].

Our study did not find any evidence that oxaliplatin/5-FU affected female sex hormones or induce menopause. Levi et al. measured AMH (anti-Müllerian hormone), inhibin B and oestradiol in females given adjuvant oxaliplatin-based chemotherapy for CRC [28]. At follow-up 6 months after treatment, levels of oestradiol had declined, whereas data on AMH and inhibin B were not collected at this point. In our study, information on AMH and inhibin B were not collected, but our longer follow-up did not show any decline in oestradiol or FSH levels. The incidence of amenorrhea after oxaliplatin treatment was reported by Cercek et al. [26] and Wan et al. [27] in two retrospective studies, in which 16% and 4% of the women experienced persistent amenorrhea after one year of follow-up, respectively. The reason why the incidence was so different between the studies could not be explained by the



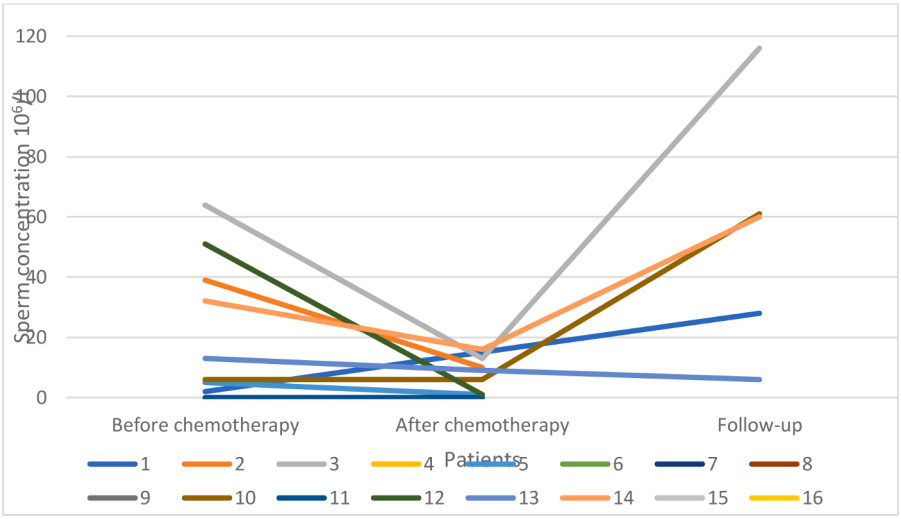


Fig. 4. (a)Relative values of testosterone (blue line) and sperm concentration (red line) for patients providing samples before and after treatment, and at follow up. Values before chemotherapy are set at 100%. Median change from base line is illustrated after chemotherapy and at follow-up. The range for the change in value for testosterone before and after chemotherapy was 86–164% and before chemotherapy and at follow-up 72–156%. The range for the change in value for sperm concentration before and after chemotherapy was 20–750% and before chemotherapy and at follow-up 46–1400%.b. Each line represents the values for sperm concentration (106/l) from one individual at the different time-points. Sixteen patients provided sperm samples, but all did not do so on all three time-points (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

authors. In comparison, from a study observing frequency and recurrence of amenorrhea in breast cancer patients receiving neoadjuvant or adjuvant chemotherapy, no patients ≤39 years of age experienced persistent amenorrhea, whereas 38% of patients 40–44 years, 81% of patients 45–49 years and 100% of patients aged over 50 year did. With a 4 year follow-up time only 11 of the 40 women with chemotherapy-induced amenorrhea with subsequent resumption, experienced recurrence of menses within 1 year [29]. Our study was not designed to evaluate amenorrhea, however from the hormone levels obtained, we did not see any patient with treatment induced menopause. The risk for amenorrhea is likely to increase with age and chemotherapy dose given, but the number of patients were too low in both above mentioned studies to evaluate this [26,27]. Obtaining a baseline fertility evaluation before initiation of cancer therapy may however be useful [30].

As far as we know, no prior studies have evaluated sperm analyzes after adjuvant oxaliplatin-based chemotherapy in CRC patients. This has however been studied more extensively for patients undergoing treatment for testicular cancer. Testicular cancer patients, however, frequently show signs of impaired spermatogenesis before initiation of any treatment [19]. Cisplatin is generally a component in the treatment of testicular cancer, and its deteriorating effect on the spermatogenesis is well established. With doses exceeding 400 mg/m^2 or cumulative doses >850 mg, the risk of irreversible impairment of spermatogenesis is 50% [19]. Our results suggest that sperm concentration and motility are affected by oxaliplatin/5-FU, but normalized with time in all our tested patients.

According to summary guidelines from the FertilPROTEKT Network, a network society of physicians and biologist specialized in fertility and preservation, chemotherapy-induced risk for CRC has been considered low to moderate [32]. Use of fertility preservation procedures before adjuvant oxaliplatin-based chemotherapy is not mentioned in ESMO or NCCN guidelines [10,11]. Some experts recommend considering embryo or oocyte cryopreservation before chemotherapy treatment alone in CRC patients [12,30]. In our Nordic experience, most centres do cryopreservation of sperm in males whereas fertility preservation is not done in females. Our study indicates that the present clinical routine is sufficient. Although we did not observe induction of permanent azoospermia in males, our numbers are too few to conclude that fertility preserving measures before administration of oxaliplatin/5-FU- is unnecessary in males. We believe our study offers some further knowledge that will help clinicians to inform patients about infertility risk before they start oxaliplatin-based chemotherapy.

There are some limitations in our study. All samples before start of chemotherapy were collected after the patients had undergone colorectal surgery. The surgery itself and the overall stress on the body might affect sex hormones and sperm values compared to if they would have been collected before surgery. The number of patients was relatively

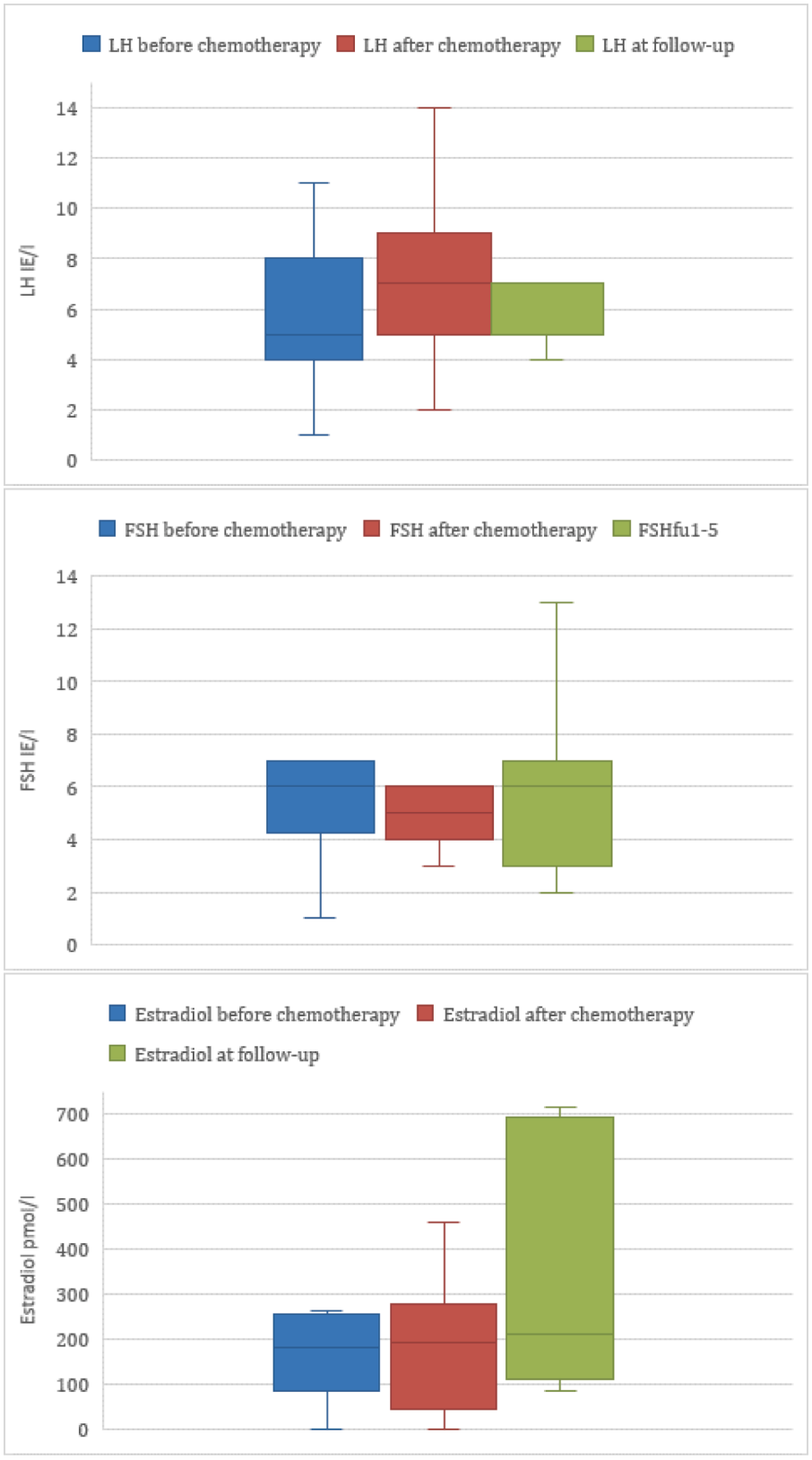


Fig. 5. Levels of (from top to bottom) FSH (IE/l), LH (IE/l) and estradiol (pmol/l) measured before chemotherapy, after chemotherapy and at follow-up (1–5 years after chemotherapy) in female colorectal cancer patients given adjuvant oxaliplatin in combination with 5-FU.

small and information at follow-up was incomplete especially for sperm analyzes, as males frequently declined, thus limiting paired comparisons. Furthermore, to evaluate male sperm fertility, repeated sperm analysis is generally recommended, as inter-individual variation is common [33], which was not found feasible in our study. We had no detailed information regarding duration of abstinence before semen collection, however, the common recommendation for this was according to the relevant fertility department. Regarding female fertility, hormonal levels can give clues for gonadal dysfunction, but even with normal levels woman can still be infertile. AMH can be used as a marker for the oocyte reserve, however we did not measure AMH in our study. Some men and women reported on attempts of pregnancy and children born, but even though success in childbirth confirms fertility, a lack of success can be due to many more reasons, and many fertile women and men do not desire pregnancy. All the above-mentioned limitations obviously affect the power of our study and thus conclusions. Cut off values for evaluating fertility or hypogonadism can be arbitrary and difficult to translate. Our focus in this study was to observe change between the different time points in the individual patients and interpreting the individual values in each patient to assess treatment effect on fertility and the gonads. The results should be interpreted with some caution due to the small sample and the significant decline of repeat testing among study participants

The strength of our study is its prospective design and that sperm analysis was carried out, which to our knowledge has previously not been performed to evaluate the effect of oxaliplatin-based chemotherapy. The initial recruitment of patients was fair, but to obtain samples at follow-up proved to be difficult. This is not surprising, especially considering sperm samples, as collection often is inconvenient for the patients and the benefit to the individual patient in providing samples is low.

If a similar future study could be conducted, one would have to find some way to ensure that patients would provide semen sample at all follow-up time points. Furthermore, analysis of AMH and recovery of ovulation should be recorded. Self-reported regularity of the menstruation periods was present in our questionnaire, but the number of women answering this category was sparse. A long follow-up period would be useful to document attempt and success rate of pregnancy efforts.

5. Conclusion

Adjuvant chemotherapy with oxaliplatin and 5-FU in CRC patients only transiently affected sex hormones and sperm function in males and did not seem to affect female sex hormone values. The risk of infertility and hypogonadism in male and female CRC patients after adjuvant oxaliplatin-based chemotherapy seems low.

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CRediT authorship contribution statement

Philip Falk: Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Mira Severin:** Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Åke Berglund:** Resources, Data curation, Writing – review & editing. **Marianne G. Guren:** Resources, Data curation, Writing – review & editing. **Eva Hofsli:** Resources, Data curation, Writing – review & editing. **Pia Österlund:** Resources, Data curation, Writing – review & editing. **Anne Tandberg:** Conceptualization, Methodology, Writing – review & editing. **Jakob Eberhard:** Resources, Data curation, Writing – review & editing. **Halfdan Sorbye:** Conceptualization, Methodology, Resources, Data curation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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