Semen Quality and Sperm DNA Integrity in Patients With Severe Active Inflammatory Bowel Disease and Effects of Tumour Necrosis Factor-alpha Inhibitors

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Abstract

Background and Aims: The impact of severe inflammation on semen quality, including sperm DNA integrity, in men with inflammatory bowel disease [IBD] is unknown, as are the potential effects of anti-tumour necrosis factor-alpha [TNF-alpha] therapy. We investigated the influence of severe active IBD and anti-TNF-alpha treatment on semen quality.

Methods: We prospectively included 20 patients admitted with severe active IBD. Further, 19 patients who initiated and 17 who stopped anti-TNF-alpha therapy were included. Semen samples were obtained during active disease, and on/off treatment. For paired comparisons, samples were collected not less than 3 months after achieving remission, after treatment initiation, or after treatment cessation. Sperm DNA Fragmentation Index [DFI], concentration, morphology, and motility were evaluated. Sex hormones and seminal plasma anti-TNF-alpha drug levels were measured.

Results: In patients with severe disease, progressive sperm motility was impaired and increased significantly [from 28.4% to 37.4%, \( p = 0.045 \)] during remission. There was no difference in DFI [12.5% versus 12.0%, \( p = 0.55 \)], concentration [55.0 mill/ml versus 70.0 mill/ml, \( p = 0.39 \)], or normal morphology [4.7% versus 5.1%, \( p = 0.51 \)] in these patients. During active disease, testosterone was decreased, and normalised after obtaining remission. Patients who started anti-TNF-alpha therapy had a statistically significant, but clinically irrelevant, reduction in DFI after treatment initiation [12.8% versus 10.0%, \( p = 0.02 \)]. All other semen parameters were unaffected by therapy. Anti-TNF-alpha drugs were excreted in negligible amounts in semen.

Conclusions: Severe active IBD reduces progressive sperm motility and testosterone levels, but sperm DNA integrity is unaffected by active disease. Anti-TNF-alpha therapy does not impair sperm quality.

Key Words: Anti-TNF-alpha therapy; inflammatory bowel disease; sperm DNA integrity
1. Introduction

Inflammatory bowel disease [IBD] is often diagnosed during the reproductive years, and preconception counselling of men remains challenging.\(^1\) Anti-tumour necrosis factor-alpha [TNF-α] inhibitors are frequently required to obtain sustained remission.\(^2\) In addition to uncertainties about the potential toxic effects of active disease on spermatogenesis, anti-TNF-α agents may modulate the local cytokine milieu in the testis, where TNF-α exerts ambiguous effects on spermatogenesis.\(^3\,4\) A questionnaire survey found that men with recent disease activity were more likely to experience difficulty in conception than were patients in sustained remission.\(^5\) The authors speculated that systemic inflammation and adverse effects of medication were the main causes. Few studies have addressed the role of disease control in IBD. By nature, it is difficult to separate the effects of disease from those of medication, and a reduction in the inflammatory burden may pose a potential toxicity of the given drug.\(^5\) Further, male fertility and sexual function may be affected through a systemic effect on the hypothalamic-pituitary-gonadal axis. Firm conclusions have been limited by the inherent fluctuation and heterogeneity in semen parameters between individuals, emphasising the value of obtaining pre- and post-exposure samples.

Based on the results of epidemiological birth outcome data, paternal anti-TNF-α therapy may be continued during the preconception period.\(^6\) Only a limited number of studies have investigated effects on semen quality. Increased semen volume, disruption of sperm morphology, and a trend toward reduced motility were observed in 10 men with IBD following infliximab treatment.\(^7\) Conversely, better progressive motility and morphology of spermatozoa were observed with infliximab or adalimumab treatment in 10 men with Crohn’s disease [CD] compared with 20 CD patients not on anti-TNF-α treatment.\(^8\) In the only in vivo study addressing the impact of anti-TNF-α therapy on sperm DNA integrity, fluorescence in situ hybridisation revealed a significant decrease in sperm aneuploidy rate in 10 men with spondyloarthritits after 12 months on adalimumab treatment.\(^9\)

The World Health Organization [WHO] criteria for semen quality, namely semen volume, concentration, motility, and morphology of spermatozoa, have been applied for decades as the gold standard.\(^10\) However, the WHO criteria based on light microscopy do not include the important aspect of sperm DNA integrity, and the Sperm Chromatin Structure Assay [SCSA] and comet assay have evolved.\(^11\,12\) Intact sperm chromatin structure has been established as an independent factor in male fertility.\(^13\) Clinically, the SCSA parameter DNA Fragmentation Index [DFI, i.e. the extent of DNA fragmentation in a given sperm sample] predicts the probability of conception, and DNA double strand breaks detected by the neutral comet assay have been associated with recurrent miscarriages.\(^14\,15\)

In the present study, we prospectively recruited men who were admitted with severe active disease, to investigate the effects of severe intestinal inflammation on semen quality, including sperm DNA integrity, and on the hypothalamic-pituitary-gonadal axis. Further, we studied patients with active disease who started infliximab or adalimumab and patients in sustained remission who stopped therapy, to separately evaluate the impact of medication. Drugs in seminal plasma may exert an adverse effect on the fetus, and we measured infliximab and adalimumab levels to determine transmisison via the seminal ejaculate.

2. Materials and Methods

2.1. Patient population and setting

This prospective, observational cohort study was conducted at a tertiary IBD centre in Denmark. We included eligible patients who consented between August 2013 and December 2017. Inclusion criteria were male patients, 18–50 years of age, with an established diagnosis of CD or ulcerative colitis [UC]. We enrolled three patient groups: patients with severe active disease who required admission to intravenous corticosteroid treatment, patients seen in our outpatient clinic who initiated infliximab or adalimumab, and patients who stopped infliximab or adalimumab therapy. Exclusion criteria were vasectomy, known infertility, previous chemotherapy, and treatment with drugs known to affect male fertility [e.g. sulphasalazine], treatment with vedolizumab, ustekinumab, or tocilizumab, or the use of recreational drugs [e.g. marijuana]. All participants delivered two semen samples for paired analysis.

Patients with severe disease according to the physician’s global assessment [PGA]\(^16\,17\) were treated with intravenous methylprednisolone during admission.\(^18\) Baseline samples were procured while in hospital. To ensure one cycle of spermatogenesis [which is approximately 60–80 days]\(^19\) during remission, follow-up samples were collected after patients had been in clinical remission for at least 3 months, according to the PGA.

Patients who started infliximab or adalimumab delivered a sample before initiation and a follow-up sample after at least 3 months of clinical remission and therapy, to ensure drug exposure during one spermatogenic cycle. For those who stopped anti-TNF-α therapy, samples on and off treatment were collected while the patients were in clinical remission according to the PGA for at least 3 months. Further, duration of therapy was a minimum of 3 months before sampling, and patients were off anti-TNF-α therapy for at least 3 months before follow-up sampling. Concomitant treatment with non-phthalate-containing 5-aminosalicylic acid [5-ASA] or azathioprine/6-mercaptopurine was permitted.\(^20\) Further, steroids have no adverse impact on sperm quality, and were permitted for patients who were admitted with severe disease or initiated treatment with anti-TNF-α inhibitors.\(^21\)

The treating gastroenterologist noted the PGA, a composite assessment of disease activity integrating patient-reported symptoms, physical examination, biochemical assessment, and any imaging and endoscopic assessments available on the day patients were seen by the physician before sampling. The PGA categories were remission, mild-moderate disease activity, and severe disease activity. Remission meant that patients were without symptoms [i.e. normal stool frequency and no abdominal pain for CD patients; stool frequency less than four times a day and no rectal bleeding or urgency for UC patients] for 3 months with no treatment escalation and no need for steroids. Patients with PGA-classified severe disease required admission to intravenous corticosteroids. Patients between the two extremes were classified as having mild-moderate disease activity. Furthermore, on the day when the patients delivered the samples, the Harvey-Bradshaw Index [HBI] was recorded for patients with CD and the Montreal and Simple Clinical Colitis Activity Index [SCCAI] for those with UC.\(^22\,23\) Disease phenotype was classified according to the Montreal Classification.\(^24\) UC patients admitted for severe active disease underwent endoscopy before admission and were classified according to the Mayo Endoscopic Score for UC.\(^25\) Patients filled out general health and fertility questionnaires and reported the duration of the current period with active disease preceding admission or initiation of anti-TNF-α therapy.

2.2. Semen analyses

Semen samples were procured via masturbation into a plastic cup. The semen volume, sperm concentration, motility, and morphology were analysed according to the WHO 2010 recommendations.\(^26\) Sperm motility was categorised as progressively motile, non-progressively motile, and immotile, and values expressed as percentages. Sperm morphology was assessed by Kruger’s strict criteria.\(^27\) Following basic...
examination, raw semen was stored at -196°C for later DNA analysis. The raw sample was filtered through a 0.2-μm [pore size] filter [Nalgene, NY, USA] to separate the seminal plasma from spermatozoa, and stored at -196°C until infliximab/adalimumab analysis. Sperm DNA fragmentation was assessed by the SCSA and neutral comet assays. [13,14] SCSA measures existing DNA strand breaks as well as the susceptibility to acid-induced DNA denaturation which happens more often in sperm with poor DNA integrity [e.g. due to adducts or oxidative stress]. More than 5000 spermatozoa are analysed by flow cytometry. Analysis of the flow cytometric data involved calculation of the DFI, the percentage of fragmented sperm DNA. A DFI level above 20% is associated with a reduced fecundability. [20] The neutral comet assay is a sensitive method for detecting double-stranded DNA strand breaks in individual cells via electrophoresis, with approximately 150 spermatozoa being analysed in each sample. For each cell, the value of the tail intensity [i.e. the ratio of fragmented DNA migrated in the tail of the comet to total DNA] is assessed, and the mean tail intensity is calculated for each individual. Moreover, to quantify the percentage of sperm with abnormal DNA, a cut-off of 10% tail DNA was used to discriminate undamaged from damaged sperm.

2.3. Blood analyses
Blood samples were drawn for analysis of C-reactive protein [CRP], leukocytes, haemoglobin, thrombocytes, albumin, total testosterone, follicle-stimulating hormone [FSH], luteinising hormone [LH], and infliximab/adalimumab drug levels. A femal sample was obtained for analysis of follic letprotecin [FCP] within 2 weeks before or 2 weeks after sperm collection, unless collected in connection with admission or the start of anti-TNF-α therapy, where a maximum of 4 days after treatment initiation was accepted. [31]

2.4. Drug levels in blood and seminal plasma
Drug levels in the samples provided by patients during anti-TNF-α therapy were measured. Infliximab and adalimumab measurements in blood and seminal plasma samples were performed by sandwich enzyme-linked immunosorbent assay [Infliximab and Adalimumab level ELISA, Sanquin Blood Supply, Amsterdam, The Netherlands], according to the manufacturer’s instructions. The serum was diluted 1:2000–3000 and seminal plasma 1:20. As internal validation, possible matrix effects were evaluated by comparing the recovery of infliximab/adalimumab standards in serum and seminal plasma samples containing the same concentration. Seminal plasma infliximab/adalimumab standards were prepared to control for linearity across the standard curve. Furthermore, the patients served as their own control in the evaluation of seminal plasma levels, as the off-drug semen sample was used as a negative control to adjust for any matrix effects. All samples were run in duplicate. A control sample was run first and last on each plate and from plate to plate, to control for possible intra-assay and inter-assay drift.

2.5 Statistical analysis
Data were analysed using Stata version 13.1 [StataCorp LP, College Station, TX, USA]. Paired t-tests were applied to compare characteristics within subgroups at baseline and follow-up, and the unpaired sample t-test was employed to compare different patient groups [e.g. CD versus UC]. Where applicable, logarithmic transformation was applied to obtain normality; otherwise, data were analysed non-parametrically. All available data were included for mean/median values given at a specific time point [e.g. on anti-TNF-α therapy]. In the case of missing data at one time point, the patient was censored from the paired t-test in question. Tests were considered statistically significant when two-tailed p-values were <0.05.

2.6. Ethics approval
The study was approved by the Danish Data Protection Agency [reference 1-16-02-363-13] and the Regional Ethical Review Board [reference 1-10-72-405-12]. Subjects participated after informed written consent.

3. Results
3.1. Patient characteristics
A total of 56 patients were enrolled in the study and divided into three subgroups [Figure 1]. We included 20 patients [18 UC and two CD] with severe disease, of whom 11 [55%] achieved remission on high-dose steroids and nine [45%] required additional infliximab therapy to achieve remission. The latter patient group was furthermore combined with 19 outpatients who initiated anti-TNF-alpha therapy [n = 28]. Finally, 17 patients who discontinued anti-TNF-alpha treatment were enrolled. The patient demographics for all subgroups are presented in Table 1. In total, 38 patients received infliximab and seven patients received adalimumab. Smoking habits and alcohol consumption did not change during the study period, and there was no difference in abstinence duration between baseline and follow-up samples [data not shown].

The 20 patients admitted with severe disease were treated with intravenous methylprednisolone 40 mg twice a day. A median of 80 mg [range 0–160 mg] had been administered to the patients before samples were collected [i.e. median 12 H [range 0–48 H] of steroid therapy before sampling]. Endoscopy was performed before admission on 17 of the 18 UC patients [94%]; of these, 14 [82%] had Mayo endoscopic score 3, and three [18%] had Mayo endoscopic score 2. The two patients with CD had both lost 20 kg body weight before admission, and small bowel magnetic resonance imaging [MRI] showed severe inflammation and stenosis in the terminal ileum. Due to steroid-refractory disease, they both subsequently underwent ileal resection, and one of the patients was additionally started on infliximab. No patient had been treated with oral corticosteroids before admission. The median patient-reported duration of the current active disease period preceding admission was 64 days [range 9–311].

The 28 patients who started infliximab or adalimumab treatment all had clinically active disease at the time of sampling, and the median patient-reported duration of the current active disease period before sampling was 77 days [range 14–311]. Three patients were on or recently tapered off systemic corticosteroids before admission. At the time of their follow-up sampling, all 20 patients with severe disease and all 28 patients who initiated anti-TNF-α therapy were in remission according to all disease activity indices, and had been for at least 3 months [Table 1]. The patients who started anti-TNF-α therapy had been treated for a median of 5.4 months [range 83 days–11.6 months] at the follow-up sampling.

For all 17 patients who stopped anti-TNF-α therapy, drug cessation was due to sustained remission. They had been treated with anti-TNF-α therapy for a median of 13.5 months [7.5–51 months] when providing the initial sample, and had been off anti-TNF-α therapy for a median of 4.3 months [3–29 months] before follow-up. They were all on concomitant therapy [tiapourines and/or 5-ASA] at both sampling times, to ensure sustained remission after anti-TNF-α cessation.

3.2. Semen parameters
Semen quality was evaluated by the standard WHO analysis, the SCSA, and the neutral comet assay [Table 2]. In patients with severe active disease and those who started anti-TNF-α therapy, mean progressive motility values were below the WHO lower reference limits and increased
to normal levels after the patients achieved remission; the increase was statistically significant in the severe-disease patients [progressive motility 28.4% versus 37.4%, \( p = 0.045 \), Table 2]. All other WHO semen parameters, i.e. semen volume, sperm concentration, total number, total motility, immotile sperm, and normal sperm morphology, were above the lower reference levels and did not differ significantly between baseline and follow-up samples for any of the three subgroups [Table 2]. We observed no change in DFI in the samples collected from the period with active disease compared with samples obtained during remission in patients admitted with severe disease [12.5 versus 12.0, \( p = 0.55 \)]. In those who initiated treatment, a statistically significant reduction in DFI was observed after the start of anti-TNF-\( \alpha \) therapy [median DFI 12.8 off therapy versus 10.0 on therapy, \( p = 0.02 \)]. There were no significant changes in DFI in patients who stopped anti-TNF-\( \alpha \) therapy [16.0 on therapy versus 11.0 off therapy, \( p = 0.48 \), Table 2]. Sperm DNA integrity evaluated by the neutral comet assay showed no difference in the mean tail intensity or the percentage of damaged sperm between baseline and follow-up samples for any of the three subgroups [Table 2].

We evaluated all samples from patients in remission on anti-TNF-\( \alpha \) maintenance therapy, i.e. 28 patients in remission after initiation and 17 patients before cessation \( [n = 45] \), and found no difference in sperm DNA fragmentation or WHO parameters between those with UC \( [n = 24] \) and CD \( [n = 21] \), as well as no difference between infliximab- \( [n = 38] \) and adalimumab-treated \( [n = 7] \) patients [data not shown]. Furthermore, there was no correlation between DFI and duration of anti-TNF-\( \alpha \) therapy \( [r = 0.13, p = 0.41] \). Further, we stratified patients with severe disease into patients obtaining remission on steroids \( [n = 11] \) versus patients requiring additional infliximab \( [n = 9] \). There was no difference in sperm quality at admission, and the change in sperm quality between activity and remission samples did not differ between the two subgroups. Within each subgroup, progressive motility increased significantly in patients treated with steroids only but not in patients requiring additional infliximab, and all other semen characteristics did not significantly change within the two groups [data not shown]. Furthermore, we observed no difference in progressive motility between smokers and non-smokers with severe disease [data not shown].

### 3.3 Inflammatory markers and the hypothalamic-pituitary-gonadal axis

In both patients who had severe active disease at inclusion and those who started anti-TNF-\( \alpha \) therapy, plasma levels of CRP, thrombocytes, and leukocytes and FCP all significantly decreased from the time of inclusion to remission [Table 1]. Plasma albumin and haemoglobin increased concomitantly. Additionally, inflammatory markers remained unchanged in patients who stopped anti-TNF-\( \alpha \) therapy. We also investigated correlations between inflammatory markers, i.e. CRP, thrombocytes, albumin, and FCP, and sperm parameters. In patients admitted with severe disease activity, we found a moderate negative correlation between the thrombocyte level and progressive motility \( [r = -0.5, p = 0.01] \) and between albumin and DFI \( [r = -0.7, p < 0.001] \), and a moderate positive correlation between thrombocytes and DFI \( [r = 0.6, p < 0.01] \). There was no correlation between FCP or CRP and sperm parameters.

In patients with severe disease and those who started anti-TNF-\( \alpha \) therapy, we observed parallel significant increases in plasma levels of total testosterone and decreases in LH after achieving remission, and FSH decreased in patients with severe disease after achieving remission [Table 1]. All patients with total testosterone levels below the lower reference level normalised their testosterone levels when remission was achieved.

### 3.4 Infliximab and adalimumab measurements

Both infliximab and adalimumab were detected in serum and seminal plasma collected while patients were on maintenance treatment, but were undetectable in the off-drug semen samples, indicating a sufficient wash-out period for patients who stopped therapy. Seminal infliximab levels were 0.01–0.54 \( \mu \text{g/mL} \) and adalimumab levels were 0.01–0.12 \( \mu \text{g/mL} \). The blood/semens drug level ratio varied from 83 to 725 for infliximab and 53 to 453 for adalimumab.

### 4. Discussion

We investigated the effects of severe active disease and the use of anti-TNF-\( \alpha \) therapy on semen quality in men with IBD. We evaluated semen quality by sperm DNA integrity assessment as well as conventional WHO analysis. To account for variation between individuals, we monitored the effects of disease activity and infliximab/adalimumab therapy in a paired design. In order to separately analyse the effect of therapy, we obtained samples from patients in sustained remission, who stopped therapy. Our findings indicate that neither severe active IBD nor anti-TNF-\( \alpha \) therapy impair sperm DNA integrity, and that only sperm motility is moderately affected by severe disease.
### Table 1. Disease and medication characteristics of patients with inflammatory bowel disease admitted with severe active disease or who start or stop anti-TNF-α treatment.

<table>
<thead>
<tr>
<th></th>
<th>Patients with severe disease [n = 20]</th>
<th>Patients who start anti-TNF-α therapy [n = 28]*</th>
<th>Patients who stop anti-TNF-α therapy [n = 17]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at inclusion [years]</strong></td>
<td>30.8 [19.1–47.9]</td>
<td>27.5 [19.7–49.8]</td>
<td>29.3 [18.8–45.5]</td>
</tr>
<tr>
<td><strong>Body mass index [kg/m²]</strong></td>
<td>21.7 [18.2–29.4]</td>
<td>24.0 [18.0–35.7]</td>
<td>23.8 [18.9–29.8]</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>3 [15.0]</td>
<td>8 [28.6]</td>
<td>4 [23.5]</td>
</tr>
<tr>
<td><strong>Crohn’s disease</strong></td>
<td>2 [10.0]</td>
<td>14 [50.0]</td>
<td>0 [0.0]</td>
</tr>
<tr>
<td><strong>L1/L1+L4/L2/L3/L3+L4</strong></td>
<td>1/0/0/0/0</td>
<td>2/2/4/4/2</td>
<td>0/0/2/4/1</td>
</tr>
<tr>
<td><strong>Ulcerative colitis</strong></td>
<td>18 [90.0]</td>
<td>14 [50.0]</td>
<td>10 [58.8]</td>
</tr>
<tr>
<td><strong>Type of treatment</strong></td>
<td>Infliximab/adalimumab</td>
<td>9 [45.0] / 0</td>
<td>23 [82.1] / 5 [17.9]</td>
</tr>
<tr>
<td><strong>Concomitant medication</strong></td>
<td>Thiopurine therapy</td>
<td>9 [45.0]</td>
<td>11 [39.3]</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>C-reactive protein [&lt;8.0 mg/L]</td>
<td>9.6 [1.0–69.4]</td>
<td>6.8 [0.5–50.6]</td>
</tr>
<tr>
<td></td>
<td>Albumin [36–45 g/L]</td>
<td>37.6 [30–43]</td>
<td>40 [30–45]</td>
</tr>
<tr>
<td></td>
<td>Thrombocytes [145–350 x 10^9/L]</td>
<td>232 [124–329]</td>
<td>8.7 [5.2–10.6]</td>
</tr>
<tr>
<td></td>
<td>Haemoglobin [8.3–10.5 mmol/L]</td>
<td>9.2 [8.2–9.8]</td>
<td>9.2 [6.6–10.5]</td>
</tr>
<tr>
<td></td>
<td>Leukocytes [3.50–10.0 10^9/L]</td>
<td>7.9 [3.1–19.0]</td>
<td>5.8 [3.1–10.2]</td>
</tr>
<tr>
<td></td>
<td>Faecal calprotectin [μg/g]</td>
<td>816 [263–3600]</td>
<td>897 [263–3600]</td>
</tr>
<tr>
<td></td>
<td>Total testosterone [10.3–27.4 nmol/L]</td>
<td>13.3 [5.6–21.2]</td>
<td>16.6 [5.6–26.0]</td>
</tr>
<tr>
<td></td>
<td>Luteinising hormone [1.7–8.6 IU/L]</td>
<td>6.2 [3.6–13.4]</td>
<td>5.5 [3.1–13.4]</td>
</tr>
<tr>
<td></td>
<td>Follicle-stimulating hormone [1.2–15.8 IU/L]</td>
<td>6.0 [1.8–9.4]</td>
<td>4.9 [2.1–9.5]</td>
</tr>
</tbody>
</table>

Values are provided as \[n [%]\] or the median \[range\].

*Patients who start anti-TNF-α therapy, including nine admitted with severe active disease.

*p <0.05; **p <0.01; ***p <0.001; ****p <0.0001 for paired t-tests between baseline and follow-up samples.

N/A, not available; PGA, physician’s global assessment; TNF-α, tumour necrosis factor-alpha.
### Table 2. Comparison of semen parameters, with 95% CIs, between baseline and follow-up samples from patients with inflammatory bowel disease admitted with severe active disease, or who start or stop anti-TNF-alpha treatment

<table>
<thead>
<tr>
<th>Patients with severe disease [n = 20]</th>
<th>Patients who start anti-TNF-α therapy [n = 28]</th>
<th>Patients who stop anti-TNF-α therapy [n = 17]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe disease</td>
<td>Remission</td>
<td>Within group p-value</td>
</tr>
<tr>
<td>Mean [95% CI]</td>
<td>Mean [95% CI]</td>
<td>Mean [95% CI]</td>
</tr>
<tr>
<td>WHO 2010 [lower reference limits]</td>
<td></td>
<td></td>
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<tr>
<td>WHO 2010 [lower reference limits]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semen volume [min. 1.5 mL]</td>
<td>3.3 [2.6; 4.1]</td>
<td>4.0 [3.0; 4.9]</td>
</tr>
<tr>
<td>Total motility [min. 40%]</td>
<td>52.2 [41.7; 62.6]</td>
<td>61.2 [51.4; 71.0]</td>
</tr>
<tr>
<td>Progressive motility [min. 32%]</td>
<td>28.4 [19.8; 36.9]</td>
<td>37.4 [28.9; 45.9]</td>
</tr>
<tr>
<td>Non-progressive motility [%]</td>
<td>23.8 [13.9; 33.7]</td>
<td>23.9 [18.9; 28.8]</td>
</tr>
<tr>
<td>Immotile [max. 60%]</td>
<td>47.8 [37.3; 58.3]</td>
<td>38.8 [29.0; 48.7]</td>
</tr>
<tr>
<td>Sperm morphology [min. 4% normal forms]</td>
<td>4.7 [3.2; 6.2]</td>
<td>5.1 [3.7; 6.5]</td>
</tr>
<tr>
<td>Sperm concentration [min. 15 x 10^6/mL]</td>
<td>55.0 [4.7–177]</td>
<td>70.0 [21.4–217]</td>
</tr>
<tr>
<td>Total sperm number [min. 39 x 10^6/ejaculate]</td>
<td>1.55 [12.2–671]</td>
<td>249 [78.1–781]</td>
</tr>
<tr>
<td>Sperm DNA fragmentation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>SCSA, DFI, DNA fragmentation index</td>
<td></td>
</tr>
<tr>
<td>SCSA, DFI, DNA fragmentation index</td>
<td>12.5 [4.0–60.0]</td>
<td>12.0 [7.0–32.0]</td>
</tr>
<tr>
<td>Comet, mean tail intensity [%]</td>
<td>0.8 [0.2–4.9]</td>
<td>0.9 [0.2–6.7]</td>
</tr>
<tr>
<td>Comet, percentage of damaged sperm [%]</td>
<td>3.0 [0.0–17.2]</td>
<td>2.7 [0.0–21.9]</td>
</tr>
</tbody>
</table>

CI, confidence interval; DFI, DNA fragmentation index; SCSA, sperm chromatin structure assay; TNF-α, tumour necrosis factor-alpha; WHO, World Health Organization; min., minimum; max., maximum.

<sup>a</sup>Patients who start anti-TNF-α therapy, including nine with severe disease.

<sup>b</sup>Fresh samples with recorded spillage were excluded [n]: severe disease [5], patients who start anti-TNF-α therapy [4], patients who stop anti-TNF-α therapy [2].

<sup>c</sup>Missing values: comet analysis performed on 15 paired samples from patients with severe disease, on 22 paired samples from patients who initiate treatment, and on 16 paired samples from patients who stop treatment.
We investigated the effect of active disease on sperm quality by including patients with severe mucosal inflammation who required hospitalisation. In this group of IBD patients, we observed no difference in sperm DNA integrity compared with samples collected during remission. Nonetheless, DFI levels did correlate with clinical markers of inflammation, i.e., thrombocytes and albumin levels, during active disease. Furthermore, sperm progressive motility was significantly reduced in severe disease, correlating negatively with thrombocytes, whereas there was no association with FCP or CRP. This indicates that only severe and prolonged systemic inflammation affects spermatogenesis. The lack of a significant impact on sperm DNA integrity likely reflects the resilience of spermatogenesis. In accordance with our findings, reduced motility was observed in the majority of untreated men with spondyloarthritis, whereas anti-TNF-α treated patients in remission had sperm quality comparable to those of healthy controls. Further, motility and morphology correlated negatively with disease activity in patients with active spondyloarthritis. Others also showed impaired semen quality in patients with untreated psoriasis, whereas patients with active ankylosing spondylitis and CRP elevation had normal WHO semen quality that remained unaffected by initiation of anti-TNF-α inhibitors.

In order to specifically analyse the effect of treatment, we obtained paired samples from patients with quiescent IBD who stopped anti-TNF-α therapy and remained in remission. The DFI decreased from median 12.8% to 10.0% after obtained remission, and intriguingly decreased from 16.0% to 11.0% after cessation of anti-TNF-α therapy. A DFI level above 20% is associated with a reduced fecundability, and the changes in the DFI interval observed in patients who started therapy are not clinically relevant even though they reached statistical significance. Furthermore, sperm DNA integrity evaluated by the neutral comet assay was unaffected by initiation of therapy. When applying conventional semen analysis, we found no influence on semen volume, sperm concentration, morphology, or motility in those who started or stopped anti-TNF-α therapy. We detected low levels of infliximab and adalimumab in the seminal plasma, generally 1–2% of the blood plasma levels. The drugs did not accumulate in semen, and paternal transmission of anti-TNF-α drugs via seminal ejaculate is negligible.

In the present study, total testosterone increased significantly in parallel with a decrease in LH and FSH after remission was achieved, whereas there were no changes after drug cessation. This indicates that inflammation, rather than therapy, has an adverse effect on the hypothalamic-pituitary-gonadal axis. We do not believe that the hypogonadism was attributed to steroid use, because no one with severe disease had been treated with systemic steroids before admission. Low testosterone levels do not invariably result in a reduced sperm quality. The observed low testosterone levels may therefore be a marker of severe inflammation alongside the decrease in progressive motility, or potentially the cause of an impaired motility.

Taken together, the clinical implications of our findings are reassuring because the moderate changes in sperm quality were temporary and caused by severe active disease. The observed decreased sperm motility may in part explain the US patient survey among 256 men indicating a prolonged time to conception in men with recent active IBD.

Vale et al. observed a trend toward poorer semen quality in men with CD compared with UC, indicating that the nature and localisation of inflammation are important. In our population of 21 CD and 24 UC patients in remission, we observed no differences between the two disease entities.

The strength of our study is the paired study design in a well-characterised population of patients with IBD, with application of state-of-the-art analyses of semen quality. Semen samples were collected at specific time points to ensure relevant exposure during spermatogenesis. As an important limitation, our results may have been influenced by concomitant medications. The evaluation of severe disease activity alone is not possible, and in clinical practice, patients rarely have drug-free intervals. Most patients have received an array of different local and systemic concomitant treatments. However, thiopurines do not have significant impact on semen quality, and patients admitted with severe disease in our cohort were not treated with thiopurines before admission. Because the study was powered to detect differences in DFI levels within groups, it may have had insufficient power to detect significant differences in WHO sperm parameters. Reassuringly, all mean/median WHO parameters, except for progressive motility, were above lower reference limits at all times.

In conclusion, our study indicates that active IBD has limited impact on semen quality, including sperm DNA integrity. Sperm motility may be affected in severe cases with a high burden of systemic inflammation. Treatment with anti-TNF-α inhibitors has no negative impact on sperm DNA integrity, and may improve sperm quality due to a reduction in the inflammatory load. Despite disease flares, patients who wish to father children should not fear alarming impairment of sperm quality and may continue anti-TNF-α treatment in the periconception period.

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Conflict of Interest
LAC has received speaker’s fees from Janssen and Takeda. OHL has served on advisory boards for Shire and received speaker’s fees from Shire and Baxterla. MJ has served on the advisory board of Tillotts Pharma AB and Janssen, has received consultation fees from Ferring, and has received speaker’s fees from MSD, Ferring, and Takeda. CLH received consultation fees from Ferring and has received speaker’s fees from MSD, Takeda, Tillotts Pharma AB, Novartis, and AbbVie A/S. The remaining authors have nothing to declare.

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Author Contributions
AG: study design, collection of samples, interpretation of data, preparation of the manuscript, critical review. MB: laboratory analysis, interpretation of data, preparation of the manuscript, critical review. LAC: study design, interpretation of data, preparation of the manuscript, critical review. EC: laboratory analysis, interpretation of data, preparation of the manuscript, critical review. OHL: laboratory analysis, interpretation of data, preparation of the manuscript, critical review. GL: laboratory analysis, interpretation of data, preparation of the manuscript, critical review. MJ: preparation of the manuscript, critical review. TV: preparation of the manuscript, critical review. PV: laboratory analysis, interpretation of data, preparation of the manuscript, critical review. CLH: study design, interpretation of data, preparation of the manuscript, critical review. JK: study design, collection of samples, interpretation of data, preparation of the manuscript, critical review. All authors read and approved the final manuscript.
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