

# Effect of denosumab on semen quality in infertile men selected by serum level of antimüllerian hormone: a randomized controlled trial

Sam Kafai Yahyavi, M.D.,<sup>a,b</sup> Rune Holt, M.D., Ph.D.,<sup>a</sup> Mads Joon Jorsal, M.D.,<sup>a</sup> Gustav Wall-Gremstrup, M.D.,<sup>a</sup> Frederikke Bay Toft, M.D.,<sup>a</sup> Li Juel Mortensen, M.D., Ph.D.,<sup>b,c,d</sup> Bugge Nøhr, M.D., Ph.D.,<sup>e</sup> Linda Magnusson Melsen, M.Sc.,<sup>e</sup> Lisbeth Prætorius, M.D., Ph.D.,<sup>f</sup> Henriette Svarre Nielsen, M.D., Ph.D.,<sup>f,g</sup> Anja Pinborg, M.D., Ph.D.,<sup>g,h</sup> Jens-Erik Beck Jensen, M.D., Ph.D.,<sup>i</sup> Peter Schwarz, M.D., Ph.D.,<sup>f,j</sup> Finn Noe Bennedbæk, M.D., Ph.D.,<sup>k</sup> Lise Aksglaede, M.D., Ph.D.,<sup>c,d</sup> Anne Jørgensen, M.Sc., Ph.D.,<sup>a</sup> Niels Jørgensen, M.D., Ph.D.,<sup>c,d</sup> Jørgen Holm Petersen, M.Sc., Ph.D.,<sup>d,l</sup> Anders Juul, M.D., D.M.Sc.,<sup>c,d,g</sup> and Martin Blomberg Jensen, M.D., D.M.Sc.<sup>a,g</sup>

<sup>a</sup> Division of Translational Endocrinology, Department of Endocrinology and Internal Medicine, Copenhagen University Hospital—Herlev and Gentofte, Denmark; <sup>b</sup> Group of Skeletal, Mineral and Gonadal Endocrinology, Department of Growth and Reproduction, Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark; <sup>c</sup> Department of Growth and Reproduction, Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark; <sup>d</sup> International Centre for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark; <sup>e</sup> Department of Obstetrics and Gynecology, Copenhagen University Hospital—Herlev and Gentofte, Herlev, Denmark; <sup>f</sup> Department of Obstetrics and Gynecology, Copenhagen University Hospital—Hvidovre Hospital, Hvidovre, Denmark; <sup>g</sup> Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; <sup>h</sup> The Fertility Clinic, Department of Gynaecology, Fertility and Obstetrics, Copenhagen University Hospital—Rigshospitalet, Copenhagen, Denmark; <sup>i</sup> Department of Endocrinology, Copenhagen University Hospital—Hvidovre Hospital, Hvidovre, Denmark; <sup>j</sup> Department of Endocrinology and Metabolism, University of Copenhagen—Rigshospitalet, Denmark; <sup>k</sup> Department of Endocrinology, Copenhagen University Hospital—Herlev-Gentofte Hospital, Copenhagen, Denmark; and <sup>l</sup> Section of Biostatistics, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

**Objective:** To determine whether denosumab could improve sperm concentration in infertile men selected by serum antimüllerian hormone (AMH)  $\geq 38$  pmol/L. Mouse models, human testicular tissue models, and clinical intervention studies have suggested that the RANKL-inhibitor denosumab, normally used to treat osteoporosis, can improve semen quality in a sub-population of infertile men.

**Design:** A double-blinded, placebo-controlled, single-center, randomized clinical trial.

**Subjects:** The study was designed to include 282 infertile men with a planned interim analysis after inclusion of 170 men.

**Intervention:** The groups were randomized 1:1 to receive either a single subcutaneous injection of denosumab 60 mg or a placebo treatment for 80 days.

**Main Outcome Measure:** Sperm concentration.

**Results:** The trial was terminated after the interim analysis ( $n = 179$ ), with 90 men assigned to denosumab treatment and 89 men to placebo treatment. No difference in sperm concentration was found at day 80 between denosumab and placebo-treated men (difference of  $-1.0$  million/mL [95% CI  $-2.7, 1.1$ ]). Moreover, no differences in percentages of motile, progressively motile, or morphologically

Received February 18, 2025; revised May 9, 2025; accepted May 13, 2025.

Supported by Rigshospitalet, Novo Nordisk Foundation, Candys Foundation, EIFO, and The Innovation Foundation.

The subjects in this trial have not concomitantly been involved in other randomized trials. Data regarding any of the subjects in the study have not been previously published unless specified. Data will be made available to the editors of the journal for review or query on request.

The data that support the findings of this study are available from the corresponding author, M.B.J., on reasonable request.

S.K.Y. and R.H. should be considered similar in author order.

Correspondence: Martin Blomberg Jensen, M.D., D.M.Sc., Copenhagen University Hospital, Herlev-Gentofte, 54K1 Division of Translational Endocrinology, Borgmester Ib Juuls Vej 1, Entrance 7, 2730 Herlev, Denmark (E-mail: [martin.blomberg.jensen@regionh.dk](mailto:martin.blomberg.jensen@regionh.dk)).

Fertil Steril® Vol. ■, No. ■, ■ 2025 0015-0282/\$36.00

Copyright ©2025 American Society for Reproductive Medicine, Published by Elsevier Inc.

<https://doi.org/10.1016/j.fertnstert.2025.05.151>

normal spermatozoa were found. An interaction analysis identified a subgroup of men with testis size  $\leq 16$  mL and no history of cryptorchidism who had an increase in sperm concentration of 29% (8.5 million/mL at baseline to 11.0 million/mL at day 80) after treatment with denosumab.

**Conclusions:** Denosumab did not improve semen quality in infertile men selected on the basis of their serum AMH concentrations. The positive effect of denosumab in a subgroup can only be considered exploratory and needs verification in additional prospective studies.

**Trial ID:** ClinicalTrials.gov: NCT05212337. Registered 14-01-2022. Registered on 14 January 2022. EudraCT 2021-003451-42. Registered on 23 June 2021. Ethical committee H-21040145. Registered on 23 December 2021. (Fertil Steril® 2025; ■:■-■. ©2025 by American Society for Reproductive Medicine.)

**Key Words:** Denosumab, male infertility, semen quality, randomized controlled trial, RANKL

**A**lthough infertility is often perceived as primarily a female concern, male sub-fecundity is a significant contributing factor in around half of the infertile couples, and the only etiology in up to 30% of all infertile cases (1, 2). Male fertility potential (fecundity) is evaluated by semen analysis, and a low sperm concentration is a prognostic factor for male fertility potential (3). Today, no medical treatment options exist to improve semen quality for most infertile men (4, 5), except for a few specific cases, such as men with hypogonadotropic hypogonadism that can be treated with hormonal therapy (6), or varicocele patients who can be helped surgically (7). Instead of addressing the males with impaired semen quality, their healthy female counterparts often undergo treatment in fertility clinics with intrauterine insemination or more invasive assisted reproductive technology (ART) such as in vitro fertilization and intracytoplasmic sperm injection (8). Understanding and addressing male infertility is, therefore, crucial to addressing the etiology of the infertility problem and potentially reducing the need for injectable gonadotropins, egg retrieval, and the associated risks that follow these procedures.

Denosumab, a biological drug that is administered subcutaneously every 6 months, was initially approved by the Food and Drug Administration (FDA) for female osteoporosis and later also to prevent skeletal-related events in both male and female cancer patients, and eventually for male osteoporosis (9, 10). Denosumab blocks the receptor activator of the nuclear factor  $\kappa$ B ligand (RANKL) pathway that has a clinically important role best characterized in the skeleton. In the past decade, RANKL, RANK, and OPG have also been detected in extra-skeletal tissues, including the male gonads. RANKL is expressed in the Sertoli cells and signals to RANK in the germ cells, and inhibition of testicular RANKL increases sperm production in mice models and germ cell proliferation in human testicular tissue models with none or mild testicular dysgenesis (11–13). The first pilot intervention study with denosumab showed that the response to RANKL inhibition on sperm production was either highly beneficial or detrimental (12). This implied that a single injection of denosumab may be effective, but only a sub-population of infertile men could expect a beneficial response, and that suitable biomarkers had to be identified for separating good from poor responders before treatment initiation.

A translational study that also comprised a placebo-controlled randomized controlled trial (RCT) suggested that serum antimüllerian hormone (AMH) in infertile men with severely impaired semen quality was able to distinguish good

from poor responders to denosumab treatment (12). This observation was of particular interest, as serum AMH is rarely measured during the andrological evaluation of infertile men, in contrast to women undergoing fertility evaluation, where AMH is widely used as an indicator for ovarian reserve and polycystic ovary syndrome (PCOS) (14). In men, AMH is synthesized by the Sertoli cells, and the production of AMH is regulated by follicle-stimulating hormone (FSH) and testosterone (15). In infertile men, low serum AMH is linked with poor semen quality (16, 17) and AMH may be a marker of residual Sertoli cell function. Therefore, in the present study, we randomly assigned 181 infertile men selected on the basis of a serum AMH  $\geq 38$  pmol/L to receive either a single-dose 60 mg denosumab or placebo to compare the effects on semen quality after 1 complete spermatogenesis cycle (80 days) (18).

## METHODS

### Trial design and setting

First In Treating Male Infertility (FITMI) was a single-center, sponsor-investigator-initiated, placebo-controlled, double-blinded randomized clinical phase 2 trial, performed at the Department of Growth and Reproduction, Rigshospitalet, Denmark (Clinical Trials.gov NCT05212337) between February 2022 and September 2024. Subjects were randomized in a 1:1 fashion to receive either denosumab 60 mg subcutaneously or placebo, and the primary outcome was the difference in sperm concentration after 80 days between the 2 groups. The study was approved by the regional ethical committee (H-21040145) and monitored according to good clinical practice (Good Clinical Practice Unit, Bispebjerg Hospital, Denmark). The protocol, including a detailed plan for biostatistical analysis, has previously been published (18).

### Participants

The participants were recruited through different channels, including public and private fertility clinics in the greater Copenhagen area, but mainly from the outpatient clinic at the Department of Growth and Reproduction. Inclusion criteria were male infertility, age 18–60 years, a serum AMH concentration  $\geq 38$  pmol/L, and a sperm concentration  $\leq 20$  million/mL. The inclusion criteria were selected on the basis of 2 previous intervention studies with denosumab in infertile men (11, 12). In a screening visit at the department, the trial was explained in detail, and the participant's medical history was obtained before informed consent was obtained. Also, at the screening visit, the participant produced a semen

sample and had a blood sample taken to measure serum AMH, 25(OH)D, calcium, and creatinine. Exclusion criteria were sperm concentration <2 million pr. mL, semen volume <0.9 mL, having undergone vasectomy, current/previous cancer, serious comorbidities (such as diabetes mellitus, cancer, and autoimmune diseases). Varicocele or cryptorchidism were not exclusion criteria, and the included men were therefore not idiopathic infertile men rather infertile men selected mainly on the basis of serum AMH. Furthermore, for safety reasons, men with hypocalcemia (ionized calcium of <1.18 mmol/L or total calcium <2.14 mmol/L), vitamin D deficiency (serum 25(OH)D concentrations <25 nmol/L), eGFR <60 mL/min/1.73 m<sup>2</sup>, poor dental status, hypersensitivity to denosumab or any of the excipients were excluded. The flowchart of the study population is shown in Figure 1. When a participant fulfilled the inclusion and exclusion criteria, an inclusion date was assigned. At the inclusion, an ultrasound of both testes was performed before the intervention to exclude the presence of testicular tumors or risk of having germ cell neoplasia in situ (GCNIS).

### Blinding

The blinding was prepared centrally by a local pharmacy (Glostrup Apotek). The medicine arrived in closed boxes with labels on the outside, indicating each unique randomization number, and a nurse who had no contact with the participant handled the box and transferred the contents, either a prefabricated syringe containing 1 mL denosumab 60 mg (Amgen, Prolia) or a prefabricated ampoule containing isotonic saline (B. Braun, NaCl 0.9%), to a new syringe. This

was necessary as a prefabricated matching placebo syringe could only be obtained through the pharmaceutical company Amgen, which declined to provide this. To prevent hypocalcemia, all participants received vitamin D and calcium supplementation daily (Orifarm, Cholecalciferol 10 µg + calcium 400 mg) for 180 days. To ensure that the trial was double-blinded, only the nurse preparing the injection knew whether participants had received denosumab or saline. However, this nurse had no contact with the participants as another nurse administered the injection.

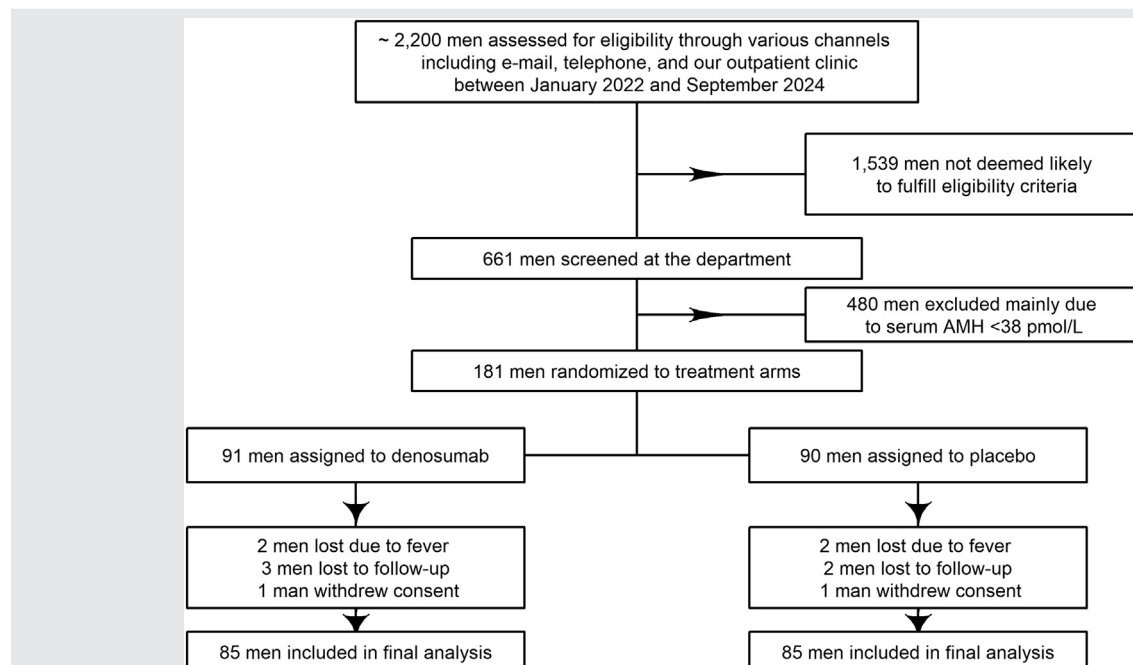
### Randomization

To ensure a completely randomized distribution according to the eligibility criteria, participants were divided into 4 groups on the basis of their sperm concentration and serum AMH. Separation thresholds were sperm concentration at 9 million pr. mL and serum AMH at 50 pmol/L. In this way, the 4 groups were created (lower or higher sperm concentration paired with lower or higher AMH concentration). The thresholds used for sperm concentration and serum AMH were estimated on the basis of previously conducted experiments (11, 12).

### Outcomes and sample size

The primary outcome of the study was defined as the difference in sperm concentration between the denosumab and placebo arms, evaluated 80 days after injection. For this purpose, the average sperm concentration of 2 semen samples delivered on day 80 and day 83 after inclusion was used. Secondary endpoints included changes in other semen parameters

**FIGURE 1**



Flow diagram of participants in the FITMI study. Flowchart of selection of study population for the FITMI trial, including exclusions.

Yahyavi. Effect of denosumab on semen quality. *Fertil Steril* 2025.

(total sperm count, total number of motile spermatozoa, percentage of motile spermatozoa, total number of progressively motile spermatozoa, percentage of progressively motile spermatozoa, total number of morphologically normal spermatozoa, and percentage of morphologically normal spermatozoa) between the denosumab and placebo arm. In the study protocol (18) we had prespecified subgroup analyses according to baseline variables that were potential contributors to the treatment effect. With the power to avoid a type II error set to 80% ( $1-\beta$ ) at a two-sided 5% significance level, a sample size of 141 men in each of the investigation arms was needed to detect a difference in sperm concentration of 45% between the intervention and placebo group. It was expected that the placebo group would have a posttrial sperm concentration of 11 million pr. mL whereas the denosumab group would have 16 million pr. mL with a maximum SD of 15. An interim analysis of safety and efficacy would be conducted when 170 patients had delivered their final semen samples on day 83.

### Biochemical analysis, testicular parameters, and semen analysis

All blood and semen samples were analyzed at Rigshospitalet. Serum AMH was measured by a sensitive immunoassay (Access, Beckman Coulter, Inc. Brea, CA) with an inter-assay coefficient of variation (CV) <5%. Measurements of serum 25(OH)D concentrations were conducted using isotope-dilution liquid chromatography–tandem mass spectrometry with inter-assay CVs <10%, or Cobas 8000 (Roche, also with CV <10%). Total calcium with a CV of 2.5%, PTH (CV <4%), and creatinine (CV <5%) were measured using Cobas 8000 (Roche), whereas serum ionized calcium (CV <3%) was measured on ABL837. Testis size was assessed using an orchidometer, and the average of both testes is presented in the tables. Testis echo scores were evaluated by ultrasound, and in cases with a side difference, the highest score was presented. Cryptorchidism was defined as unilateral or bilateral undescended testicles in childhood, as reported by the men at the clinical visit. All semen samples were produced by masturbation, with duration of abstinence, fever, and spillage being self-reported. Analysis of semen samples was performed as described previously (12) and included semen volume, which was determined by weighing, and assessment of sperm concentration by a Nucleocounter image cytometer NC-3000 (ChemoMetec, Denmark). In cases with sperm concentration below 3.0 million/mL concentration was re-assessed by manual counting in a Bürker-Türk hemocytometer. Sperm motility was classified as motile spermatozoa (World Health Organization (WHO) class ABC%) and progressively motile spermatozoa (class AB%). Sperm morphology was evaluated according to “strict” criteria (19).

### Interim analysis and end of study

An interim analysis of data was performed when 170 patients had delivered their final semen samples on day 83. This assessment was made by a biostatistician (J.H.P.) focusing on the primary outcome (18). It showed that the ratio of the median levels of the concentration was a factor of 1.13 times

higher among individuals treated with placebo relative to those treated with denosumab. The difference was not significant at the prespecified level ( $\alpha = 0.01$ ). On the basis of this, a safety committee comprising P.S., J-E.B.J., F.N.B., and A.J. evaluated the data analyzed by the biostatistician and recommended to end the trial as there would be no expected difference in the outcome after the inclusion of an additional 100 infertile men. At the time of the trial's end, 181 participants had been enrolled, with 2 participants withdrawing consent, leaving 179 in the final analysis.

### Statistics

A comprehensive description of the statistical principles can be found in the supplementary material of the study protocol (18). The primary analysis was performed with a covariance analysis (ANCOVA) on day 80, regressing follow-up sperm concentrations on baseline measurements and treatment. Baseline characteristics of the participants are presented as means with SD, or medians with interquartile range (IQR) for the semen parameters, whereas categorical variables are presented as numbers with percentages (Tables 1 and 2). Crude comparisons of means (t-test) or distributions (Mann-Whitney test) for semen parameters were made (Supplemental Fig. 1, available online). Baseline is defined as the average sperm concentration of all semen samples made within 2 years, whereas the day 80 sample is defined as the average of the 2 samples provided at the end of the trial period. Categorical variables were analyzed using the Chi-square test. We conducted an interaction analysis using quantile regression to evaluate whether the effect of denosumab treatment on sperm concentration varied across prespecified factors. These included body mass index (BMI), testis size, presence of varicocele or cryptorchidism (yes/no), serum AMH, and parathyroid hormone (PTH) concentrations at baseline. Confidence intervals for the coefficients were calculated to assess the precision of the estimates, identifying significant effects and interactions, both for crude values and adjusted for baseline sperm concentration (Fig. 2A). Evaluation of changes from baseline to day 80 was made with the Wilcoxon Signed-Rank test (Fig. 2B). For all tests, a *P* value below .05 was considered statistically significant. In general, all statistical calculations were conducted using IBM SPSS Statistics version 28 and R version 3.4.1 (R Core Team 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>).

## RESULTS

### Flow of trial

More than 2,200 men were initially evaluated between January 2022 and September 2024 (Fig. 1). Most of these contacted us after seeing information pamphlets at various private or public fertility clinics, or through news reports. Furthermore, some were also referred to our department with male infertility and were informed about the study at the site and given our contact information. Of these contacts, 661 infertile men were deemed likely to be eligible for the study and were therefore invited to a screening visit. The

TABLE 1

## Baseline characteristics of the study population.

|   | Total             | Denosumab         | Placebo           |
|---|-------------------|-------------------|-------------------|
| Included men (n)  | 179               | 90                | 89                |
| Age (years)   | 34.0 (4.8)        | 34.2 (4.8)        | 33.7 (4.9)        |
| Height (cm)   | 183 (6)           | 183 (6)           | 182 (6)           |
| Weight (kg)   | 86 (15)           | 87 (17)           | 84 (12)           |
| BMI (kg/m <sup>2</sup> )                                  | 25.7 (4.1)        | 26.2 (4.8)        | 25.1 (3.2)        |
| Total calcium (mmol/L)                                    | 2.43 (0.08)       | 2.42 (0.07)       | 2.43 (0.08)       |
| Ionized calcium (mmol/L)                                  | 1.24 (0.04)       | 1.24 (0.04)       | 1.24 (0.04)       |
| 25 (OH)D (nmol/L)   | 68 (22)           | 68 (23)           | 67 (22)           |
| AMH (pmol/L)  | 63 (24)           | 63 (25)           | 63 (24)           |
| PTH (pmol/L)  | 3.9 (1.3)         | 4.0 (1.3)         | 3.8 (1.3)         |
| Albumin (g/L)   | 42 (2)            | 42 (2)            | 42 (3)            |
| Creatinine (μmol/L)                                       | 83 (11)           | 84 (11)           | 82 (10)           |
| Infertility history (months)                              | 20 (14, 30)       | 20 (12, 28)       | 20 (16, 30)       |
| Avg. testis size, palpitations (mL)                       | 17.5 (15.0, 20.0) | 18.5 (15.0, 22.5) | 17.5 (15.0, 20.0) |
| Varicocele, no, (%)                                       | 41 (23)           | 16 (18)           | 25 (28)           |
| Cryptorchidism, no, (%)                                   | 13 (7)            | 7 (8)             | 6 (7)             |
| Ultrasound echo score (1–5)                               | 2 (2, 3)          | 2 (2, 3)          | 2 (2, 3)          |
| Duration of abstinence (days)                             | 3 (3, 4)          | 3 (3, 4)          | 3 (3, 4)          |
| Semen volume (mL)   | 4.0 (3.1, 5.2)    | 3.8 (3.0, 5.1)    | 4.2 (3.1, 5.3)    |
| Sperm concentration (10 <sup>6</sup> /mL)                 | 10.3 (6.9, 15.6)  | 10.7 (7.0, 15.3)  | 10.0 (6.9, 15.8)  |
| Total sperm count (10 <sup>6</sup> )                      | 41.6 (23.3, 68.2) | 41.5 (25.2, 67.6) | 41.6 (21.9, 68.6) |
| Motile spermatozoa (ABC%)                                 | 50 (38, 62)       | 51 (41, 60)       | 49 (37, 63)       |
| Total no. of motile spermatozoa (10 <sup>6</sup> )        | 20.3 (10.0, 32.7) | 21.8 (10.0, 31.1) | 18.1 (10.3, 34.5) |
| Progressively motile spermatozoa (AB%)                    | 37 (24, 51)       | 38 (25, 46)       | 35 (22, 52)       |
| Total no. of prog. motile spermatozoa (10 <sup>6</sup> )  | 14.1 (6.5, 24.4)  | 14.5 (6.6, 25.8)  | 13.3 (6.4, 23.6)  |
| Normal sperm morphology (%)                               | 3.0 (1.6, 5.0)    | 3.0 (1.5, 5.0)    | 3.3 (1.8, 5.3)    |
| Total no. of morph. normal spermatozoa (10 <sup>6</sup> ) | 1.0 (0.5, 2.4)    | 1.0 (0.6, 2.2)    | 0.9 (0.4, 2.7)    |

Note: Data are presented as mean with (SD) or median with (25th, 75th) percentiles. Significance levels;  $P < .05$  after crude comparison of means ( $t$ -test) or medians (Mann-Whitney  $U$  test). 25(OH)D = 25-hydroxyvitamin D; AMH = antimüllerian hormone; BMI = body mass index; PTH = parathyroid hormone.

Yahyavi. Effect of denosumab on semen quality. Fertil Steril 2025.

most common reasons for not being invited were either a sperm concentration from previous examinations below 2 million/mL or above 20 million/mL. Furthermore, many of the men interested in the study had comorbidities such as diabetes mellitus, inflammatory bowel disease, or other chronic diseases that made them ineligible, and they were therefore not invited for a screening visit. Some men also declined

participation despite being eligible because of various reasons. Of the 661 screened participants, a total of 181 were randomized to receive either denosumab ( $n = 91$ ) or placebo ( $n = 90$ ). Two participants withdrew their consent, leaving a total of 179 for the baseline analysis. During the study, 3 participants in the denosumab group and 2 participants in the placebo group were lost to follow-up. At day

TABLE 2

## Outcomes 80 days after treatment with either denosumab or placebo.

|   | Denosumab         | Placebo           | Difference (95% CI)  | $P$ value |
|---|-------------------|-------------------|----------------------|-----------|
| Total calcium (mmol/L)                                    | 2.35 (2.34, 2.37) | 2.42 (2.41, 2.44) | -0.06 (-0.09, -0.04) | <.001     |
| Ionized calcium (mmol/L)                                  | 1.22 (1.21, 1.23) | 1.24 (1.23, 1.25) | -0.02 (-0.03, -0.01) | <.001     |
| 25 (OH)D (nmol/L)   | 73 (69, 76)       | 72 (68, 76)       | 1 (-5, 6)            | .56       |
| PTH (pmol/L)  | 5.6 (5.0, 6.1)    | 4.1 (3.8, 4.4)    | 1.5 (0.9, 2.1)       | <.001     |
| Albumin (g/L)   | 42 (41, 42)       | 42 (42, 43)       | 0 (-1, 1)            | .67       |
| Creatinine (μmol/L)                                       | 81 (79, 83)       | 81 (79, 83)       | 0 (-3, 3)            | .94       |
| Duration of abstinence (days)                             | 3 (3, 4)          | 3 (3, 4)          | 0 (-1, 0)            | .39       |
| Semen volume (mL)   | 3.8 (2.7, 4.7)    | 4.0 (3.1, 5.2)    | -0.2 (-0.6, 0.4)     | .59       |
| Sperm concentration (10 <sup>6</sup> /mL)                 | 11.0 (6.7, 18.0)  | 12.0 (6.8, 20.3)  | -1.0 (-2.7, 1.1)     | .36       |
| Total sperm count (10 <sup>6</sup> )                      | 44.3 (19.4, 73.4) | 49.8 (23.0, 85.4) | -7.6 (-19.3, 3.2)    | .16       |
| Motile spermatozoa (ABC%)                                 | 50 (37, 61)       | 53 (36, 65)       | -2 (-7, 4)           | .63       |
| Total no. of motile spermatozoa (10 <sup>6</sup> )        | 19.3 (8.2, 37.5)  | 24.1 (8.9, 50.9)  | -3.8 (-10.7, 2.1)    | .20       |
| Progressively motile spermatozoa (AB%)                    | 38 (24, 49)       | 39 (24, 54)       | -1 (-7, 5)           | .79       |
| Total no. of prog. motile spermatozoa (10 <sup>6</sup> )  | 14.4 (5.8, 30.1)  | 17.0 (6.4, 42.6)  | -2.6 (-7.8, 2.0)     | .29       |
| Normal sperm morphology (%)                               | 3.0 (1.8, 6.0)    | 3.5 (2.0, 4.8)    | -0.5 (-0.8, 0.8)     | .97       |
| Total no. of morph. normal spermatozoa (10 <sup>6</sup> ) | 1.1 (0.5, 2.8)    | 1.6 (0.5, 3.4)    | -0.5 (-0.7, 0.2)     | .33       |

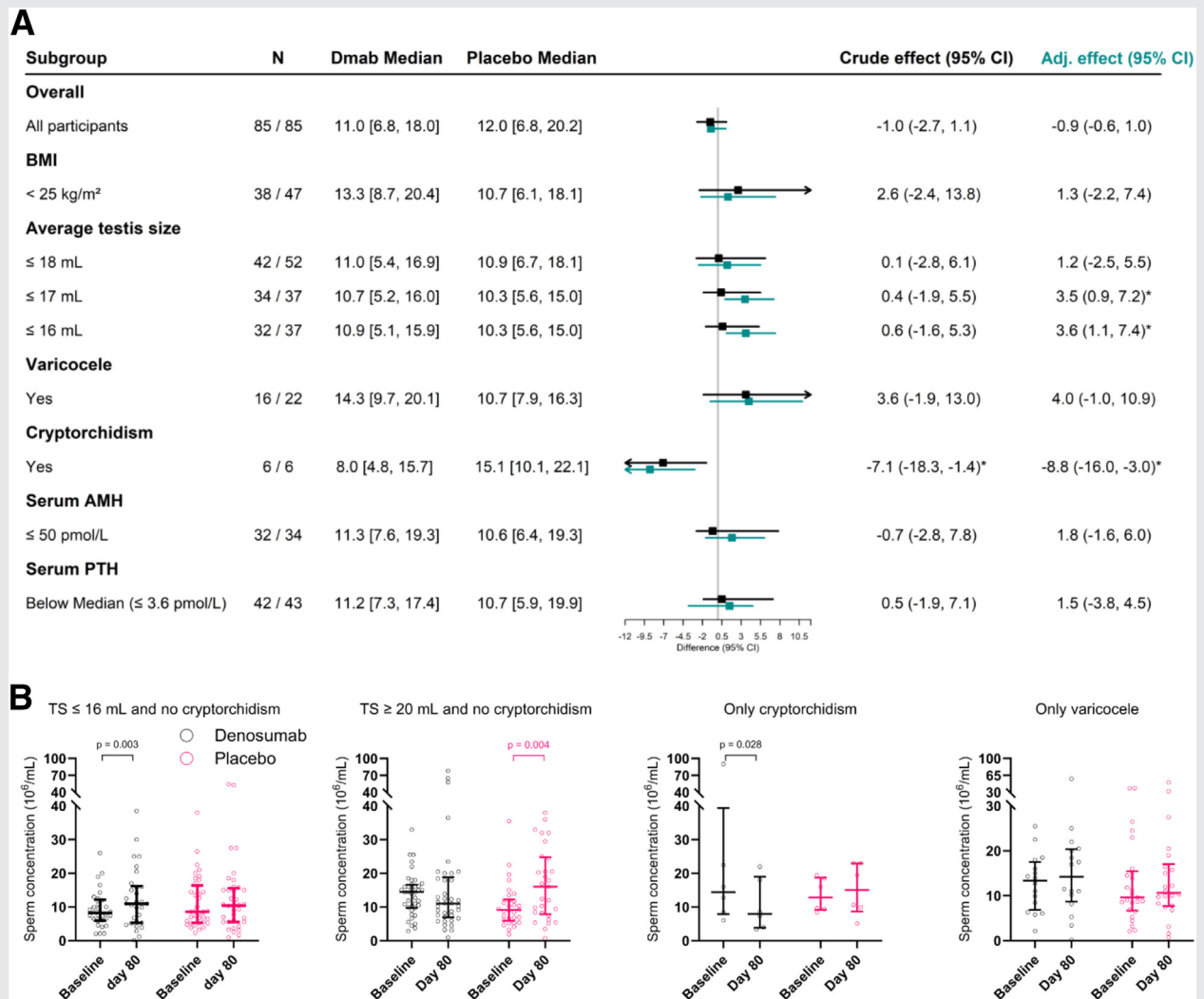
Note: Data are presented as mean with (95% confidence interval) or median with (25th, 75th) percentiles. Differences are presented with 95% confidence interval. Significance levels;  $P < .05$  after crude comparison of means ( $t$ -test) or medians (Mann-Whitney  $U$  test).

25(OH)D = 25-hydroxycholecalciferol; CI = confidence interval; no. = number; PTH = parathyroid hormone.

Yahyavi. Effect of denosumab on semen quality. Fertil Steril 2025.



FIGURE 2



Interaction analysis of the RCT and relevant subgroups showing sperm concentration after 80 days. (A) Forest plot showing the primary outcome (sperm concentration at day 80) in an interaction analysis, both crude (black) and adjusted (green) for baseline sperm concentration. Dmab = denosumab. N refers to number of participants in each subgroup (Denosumab/Placebo), medians refer to the median sperm concentration in million/mL, and treatment effect is the effect of denosumab treatment compared with placebo. \* indicates statistically significant finding (significance level of 0.05). (B) Changes in sperm concentration from baseline to day 80 in 4 subgroups of infertile men. A) Subgroup of infertile men with testis size ≤ 16 mL and no cryptorchidism, B) Subgroup of infertile men with testis size > 20 mL and no cryptorchidism, C) Subgroup of infertile men with cryptorchidism, and D) Subgroup of infertile men with varicocele. Data given as median with 25th and 75th quartiles shown. P-values indicate comparison from baseline to day 80 in the respective groups with a Wilcoxon Signed-Rank test.

Yahyavi. Effect of denosumab on semen quality. *Fertil Steril* 2025.

80, 4 men (2 from placebo group and 2 from treatment group) had experienced fever (defined as a temperature above 38.5°C for >24 hours) during the trial period, and their semen samples were therefore excluded, leaving a total of 85 participants in each group for the final analysis.

### Baseline characteristics

The trial consisted of 179 men at baseline, with 90 assigned to the denosumab group and 89 to the placebo group (Table 1). There were no differences between the groups for age ( $P=.51$ ), BMI ( $P=.075$ ), serum ionized calcium ( $P=.97$ ),

PTH ( $P=.46$ ), 25(OH)D ( $P=.71$ ), or AMH ( $P=.81$ ). All baseline characteristics are summarized in Table 1. The duration of infertility was comparable between the denosumab and placebo groups, with a median of 20 months [IQR 14, 30] for the overall cohort with 23% having varicocele and 7% cryptorchidism, with no difference between the groups ( $P>.05$  for all). The mean testis size was not significantly higher in the denosumab group (18.5 mL [IQR 15.0, 22.5]) compared with the placebo group (17.5 mL [IQR 15.0, 20.0],  $P=.15$ ). Semen analysis revealed no differences between the 2 groups in duration of abstinence, semen volume, or semen parameters (Table 1).

## Safety and incidents

A total of 29 adverse events (AEs) were reported during the trial, with 15 occurring in the active treatment arm and 14 in the placebo arm. Among these, 6 events were classified as adverse reactions (ARs), equally distributed with 3 in each arm. Two serious adverse events (SAEs) were reported, both in the active treatment arm. No serious adverse reactions (SARs) or suspected unexpected serious adverse reactions (SUSARs) were observed during the trial. Both men with SAEs were admitted to the hospital with abdominal pain that was resolved without treatment. No cases of self-reported paresthesia or hypocalcemia at day 80 were reported. Overall, the safety profile of the investigational drug was consistent with expectations on the basis of previous trials (11, 12).

## Primary endpoint

No differences in sperm concentration were detected at day 80 between the treatment and the placebo groups (Table 2). Sperm concentration in the denosumab group was 8% lower compared with the placebo group (difference of  $-1.0$  million/mL [95% CI  $-2.7, 1.1$ ]) with no significant difference ( $P = .36$ ). Denosumab treatment had a positive response rate (PRR) of 51% (43/85), whereas placebo treatment had a PRR of 56% (48/85) ( $P = .42$ ) when defining a PRR as an increase of sperm concentration of  $\geq 5\%$  at day 80 compared with baseline. The participants had similar duration of abstinence (3 days [IQR 3, 4],  $P = .39$ ), and the semen volume was also comparable between groups, with the denosumab group having a median volume of 3.8 mL [IQR 2.7, 4.7] and the placebo group 4.0 mL [IQR 3.1, 5.2] ( $P = .59$ ).

## Secondary endpoints

Medians on day 80 and relative changes of the different semen variables from baseline are shown in Table 2 and Supplemental Figure 1. On day 80, total sperm count was not different between the denosumab and placebo group (44.3 million [IQR 19.4, 73.4] vs. 49.8 million [IQR 23.0, 85.4],  $P = .16$ ). The percentage of motile, progressively motile and morphologically normal spermatozoa was not significantly different in the denosumab group, respectively ( $P = .63$ ,  $P = .79$  and  $P = .97$ ). The total serum calcium concentrations in the denosumab group were 3% lower compared with placebo (2.35 mmol/L vs. 2.42 mmol/L,  $P < .001$ ). Similarly, ionized calcium concentrations were 2% lower in the denosumab group (1.22 mmol/L vs. 1.24 mmol/L,  $P < .001$ ). In accordance, PTH concentration was 36% higher in the denosumab group than in the placebo group (5.6 pmol/L vs. 4.1 pmol/L,  $P < .001$ ). There was no difference in serum 25(OH)D, albumin, or creatinine concentrations (Table 2).

## Interaction analysis of prespecified subgroups of interest

The predefined subgroups included serum AMH  $\geq / < 50$  pmol/L, average testis size  $\geq / < 20$  mL, serum PTH (split by median concentration), BMI  $< 25$  and  $> 30$  kg/m<sup>2</sup>, and presence of varicocele or cryptorchidism. Figure 2A shows the

interaction analysis performed on the basis of the above variables for the effect on sperm concentration, but with adjustments to fit the dataset (i.e., as too few had BMI  $> 30$  kg/m<sup>2</sup>). The overall effect (denosumab vs. placebo) was not significant, with a difference of  $-1.0$  million/mL (95% CI:  $-2.7, 1.1$ ) in sperm concentration. There was an improved treatment effect of denosumab in men with average testis size  $\leq 17$  and  $\leq 16$  mL, where denosumab-treated men had a 0.4 and 0.6 million/mL higher sperm concentration compared with placebo. After adjusting for baseline sperm concentration, the trend became more pronounced, with differences of 3.5 and 3.6 million/mL, respectively, both with 95% confidence intervals exceeding zero. Similarly, in the subgroup with the presence of cryptorchidism, the treatment effect was negative, with a median difference of  $-7.1$  million/mL (95% CI:  $-18.3, -1.4$ ). No differences were found for the other subgroups (Fig. 2A). In an explorative subgroup of men with testis size  $\leq 16$  mL and no cryptorchidism denosumab-treated men, there was a 7% higher sperm concentration (11.0 million/mL [IQR 5.4, 16.0] vs. 10.3 million/mL [5.6, 15.0]) (Fig. 2B), 27% higher total sperm count (45 million [IQR 18, 68] vs. 35 million [20, 67]), 10% higher percentage of motile spermatozoa (57 % [IQR 43, 65] vs. 52 % [33, 64]), and 21% higher percentage of progressively motile spermatozoa (53 % [IQR 22, 55] vs. 43 % [30, 55]) compared with placebo treated. Furthermore, sperm concentration increased from baseline to day 80 within the group of men treated with denosumab ( $P = .003$ ) (Fig. 2B). In contrast, in a subgroup of men with testis size  $\geq 20$  mL and no cryptorchidism (Fig. 2B), there was no effect of denosumab treatment. In a subgroup of men with cryptorchidism (Fig. 2B), there was a negative effect of denosumab treatment ( $P = .028$ ), although no significant effect on sperm concentration was found in men with varicocele (Fig. 2B).

## DISCUSSION

This randomized clinical trial shows that a single dose of denosumab 60 mg sc. did not affect sperm concentration or semen parameters in infertile men with a sperm concentration between 2 and 20 million/mL that were preselected on the basis of serum AMH concentration  $\geq 38$  pmol/L. Previous translational studies suggested that serum AMH could serve as a marker for preserved Sertoli cell function and be a reliable serum biomarker for selecting infertile men, who are more likely to benefit from stimulatory treatments such as denosumab (12, 16). Unfortunately, this RCT did not verify this hypothesis. The 2 best explanations for the lack of effect are as follows: (1) denosumab may not be an effective inducer of sperm production, or (2) serum AMH is unable to identify patients with a beneficial response to denosumab treatment.

Male infertility varies in pathogenesis and pathophysiology, and the present study cohort of infertile men is also highly heterogeneous. Although we excluded some of those men with a known etiology to their infertility disease (e.g., hypogonadotropic hypogonadism and obstructive azoospermia), the cohort still included men with varicocele, cryptorchidism, Y-chromosome microdeletions, different testicular size, reduced sperm motility and morphology, and

therefore not just idiopathic infertility. Previous studies using other stimulatory treatments such as FSH, clomiphene citrate, and aromatase inhibitors have shown that some of these treatments may have a beneficial effect in subgroups of infertile men (20–22). We had prespecified some factors that could be of importance and conducted an interaction analysis on the basis of them, which showed that men with smaller testis size ( $\leq 16$  mL) without cryptorchidism had a beneficial response to denosumab compared with placebo. This finding is intriguing as it suggests that we cannot treat all infertile men with the same drug, and testicular size may, in addition to serum AMH be used to identify infertile men who are more likely to benefit from denosumab treatment and maybe also other stimulatory agents, such as gonadotropins. In adult men, testis size is largely determined by the number of germ cells and related to markers of spermatogenesis such as inhibin B and s-FSH, but also serum AMH (16, 17). This cohort was prescreened for serum AMH and impaired sperm production, but a high testicular volume could harbor an increased risk of spermatogenic arrest that often has genetic causes and can be difficult to circumvent with denosumab. In the adult testis, germ cells are the most prevalent cell type and account for the largest volume, in contrast to the prepubertal testis, which predominantly comprised Sertoli cells (23).

This double-blinded, placebo-controlled trial had some limitations. Ideally, the primary endpoint should have been a robust and clinically relevant measure, such as pregnancy or livebirth rates, instead of the highly variable sperm concentration. There was the same number of pregnancies in each of the intervention arms, which indicates that the treatment administered to the male participants did not improve the chances of achieving a live birth, although most infants were born after ART. Reproductive hormones, including FSH, testosterone, LH, and AMH were not measured routinely at baseline or during the study, except for AMH during screening. Also, we did not routinely assess Y-chromosome microdeletions or perform routine karyotype analysis, and thus cannot comment on their presence within the cohort. Denosumab induced a decrease in serum calcium, which demonstrates that the intervention was efficient and without safety issues, because no men were admitted due to hypocalcemia, and no SAR or SUSAR was registered. Although 2 men in the denosumab arm were admitted with abdominal pain with an undetectable explanation besides being a common side effect of denosumab. Despite the encouraging potential of denosumab in improving semen quality in infertile men, the FITMI trial underlines that it is important to recognize that this treatment is not a “one-size-fits-all” solution for male infertility. The response to denosumab may likely vary among individuals, influenced by factors such as underlying health conditions, hormonal concentrations of FSH, testosterone, AMH, and inhibin B, and genetic predispositions. Moreover, the ideal dosing and dosing interval are not known, and the testicles likely require a different dosing regimen than the skeleton. One of the challenges in using denosumab for male infertility is its typical use in repeated doses for conditions like osteoporosis or to prevent skeletal-related events

in patients with bone metastasis. Repeated injections can lead to adverse effects, such as accelerated bone loss after treatment. Therefore, when considering denosumab for improving semen quality, it should be regarded as a short-term treatment, limited to a duration of 6 months.

The main strengths of this study are the single-center design, with serum AMH measured on sensitive immunoassay with the use of only a single laboratory. However, in studies with semen quality as a main outcome, there are challenges concerning a high variation in sperm concentration and total sperm count (24). The use of at least 2 semen samples, both for the analysis before and after intervention helped reduce these variations. The main limitation of our study is the use of only serum AMH as a predictive biomarker. Other reproductive hormones, such as serum FSH, inhibin B, LH, testosterone, and insulin-like peptide 3 (INSL3), may have been helpful in distinguishing good from poor responders. Another limitation of this study is the high number of men excluded because they had comorbidities, which limits generalizability. Finally, the discrepancy between systemic and ex vivo direct testicular effects of denosumab (12) suggests that concentrations of the drug inside the seminiferous tubules may be inadequate to stimulate spermatogenesis, or the systemic changes induced by denosumab, for instance, in mineral homeostasis (25) may counteract the presumed testicular effects of denosumab.

## CONCLUSION

In conclusion, this study showed that for infertile men selected on the basis of a moderate/high serum AMH concentration, treatment with denosumab did not improve sperm concentration, which implies that selecting infertile men based exclusively on serum AMH to predict the treatment effect of denosumab is not feasible.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The trial was approved by the Danish Medicines Agency (approval no. 2010-024588-42) and the Danish National Committee on Health Research Ethics (permit nr. H-KF-012006-3472 and H-KF-289428). Informed consent was obtained from all participants.

## Acknowledgments

The authors thank the assistance and help of all people who contributed to the clinical trial. In particular, the staff at the Department of Growth and Reproduction, including Maiken Probst and Anne-Mette Kjøge.

## CRedit Authorship Contribution Statement

**Sam Kafai Yahyavi:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rune Holt:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mads Joon Jorsal:** Writing – review & editing, Formal analysis, Data curation. **Gustav Wall-Gremstrup:** Data curation. **Fredrikke Bay Toft:** Data curation. **Li Juel Mortensen:** Writing – review & editing. **Bugge Nøhr:** Writing – review & editing.



Project administration, Data curation. **Linda Magnusson Melsen:** Writing – review & editing, Data curation. **Lisbeth Prætorius:** Writing – review & editing. **Henriette Svarre Nielsen:** Writing – review & editing, Project administration, Data curation. **Anja Pinborg:** Writing – review & editing, Project administration, Data curation. **Jens-Erik Beck Jensen:** Writing – review & editing. **Peter Schwarz:** Writing – review & editing. **Finn Noe Bennedbæk:** Writing – review & editing. **Lise Aksglaede:** Writing – review & editing. **Anne Jørgensen:** Writing – review & editing, Validation. **Niels Jørgensen:** Writing – review & editing, Validation. **Jørgen Holm Petersen:** Writing – review & editing, Formal analysis. **Anders Juul:** Writing – review & editing, Validation, Supervision. **Martin Blomberg Jensen:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

### Declaration of Interests

S.K.Y. has nothing to disclose. R.H. has nothing to disclose. M.J.J. has nothing to disclose. G.W.-G. has nothing to disclose. F.B.T. has nothing to disclose. L.J.M. has nothing to disclose. B.N. has nothing to disclose. L.M.M. has nothing to disclose. L.P. has nothing to disclose. H.S.N. has nothing to disclose. A.P. has nothing to disclose. J.-E.B.J. has nothing to disclose. P.S. has nothing to disclose. F.N.B. has nothing to disclose. L.A. has nothing to disclose. A.J. has nothing to disclose. N.J. has nothing to disclose. J.H.P. has nothing to disclose. A.J. has nothing to disclose. M.B.J. holds 2 patents on the use of RANKL inhibitors to treat male infertility and is the CEO and founder of the Region Hovedstaden spin-out “XY Therapeutics,” which received some of the grants for the study.

### SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at <https://doi.org/10.1016/j.fertnstert.2025.05.151>.

### REFERENCES

- Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015;13:37.
- Xavier MJ, Salas-Huetos A, Oud MS, Aston KI, Veltman JA. Disease gene discovery in male infertility: past, present and future. *Hum Genet* 2021;140:7–19.
- Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson A-M, Eisenberg ML, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol Rev* 2016;96:55–97.
- Turner KA, Rambhatla A, Schon S, Agarwal A, Krawetz SA, Dupree JM, et al. Male infertility is a women’s health issue—research and clinical evaluation of male infertility is needed. *Cells* 2020;9:1–14.
- Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. *Lancet* 2021;397:319–33.
- Krausz C. Male infertility: pathogenesis and clinical diagnosis. *Clin Endocrinol Metab* 2011;25:271–85.
- Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. EAU guidelines on male infertility. *Eur Urol* 2019;48:703–11.
- Andersen AN, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, De Mouzon J, et al. Assisted reproductive technology and intrauterine inseminations in Europe, 2005: Results generated from European registers by ESHRE. *Hum Reprod* 2009;24:1267–87.
- Cummings SR. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009;361.
- Orwoll E, Teglbjærg CS, Langdahl BL, Chapurlat R, Czerwinski E, Kendler DL, et al. A randomized, placebo-controlled study of the effects of denosumab for the treatment of men with low bone mineral density. *J Clin Endocrinol Metab* 2012;97:3161–9.
- Blomberg Jensen M, Andreassen CH, Jørgensen A, Nielsen JE, Juel Mortensen L, Boisen IM, et al. RANKL regulates male reproductive function. *Nat Commun* 2021;12:2450.
- Andreassen C, Holt R, Mortensen LJ, Knudsen N, Nielsen J, Poulsen N, et al. Denosumab stimulates spermatogenesis in infertile men with preserved Sertoli cell capacity. *Cell Rep Med* 2024;5:101783.
- Andreassen CH, Lorenzen M, Nielsen JE, Kafai Yahyavi S, Toft BG, Ingerslev LR, et al. RANKL regulates testicular cancer growth and Denosumab treatment has suppressive effects on GCNIS and advanced seminoma. *Br J Cancer* 2022;127:408–21.
- Cedars MI. Evaluation of female fertility—AMH and ovarian reserve testing. *J Clin Endocrinol Metab* 2022;107:1510–9.
- Edelsztein NY, Valeri C, Lovaia MM, Schteingart HF, Rey RA. AMH regulation by steroids in the mammalian testis: underlying mechanisms and clinical implications. *Front Endocrinol (Lausanne)* 2022;13:906381.
- Holt R, Yahyavi SK, Kooij J, Andreassen CH, Andersson A-M, Juul A, et al. Low serum anti-Müllerian hormone is associated with semen quality in infertile men and not influenced by vitamin D supplementation. *BMC Med* 2023;21:79.
- Holt R, Yahyavi SK, Wall-Gremstrup G, Jorsal MJ, Toft FB, Jørgensen N, et al. Low-serum antimüllerian hormone is linked with poor semen quality in infertile men screened for participation in a randomized controlled trial. *Fertil Steril* 2024;122:278–87.
- Yahyavi SK, Holt R, Juel Mortensen L, Petersen JH, Jørgensen N, Juul A, et al. Effect of a single-dose denosumab on semen quality in infertile men (the FITMI study): study protocol for a randomized controlled trial. *Trials* 2022;23:525.
- Menkveld R, Stander FS, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Hum Reprod* 1990;5:586–92.
- Santi D, Granata ARM, Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. *Endocr Connect* 2015;4:R46.
- Puia D, Pricop C. Effectiveness of clomiphene citrate for improving sperm concentration: a literature review and meta-analysis. *Cureus* 2022;14:e25093.
- Pajai S, Potdar J, Gopal U, Banait T. A review on the use of letrozole in female and male infertility. *Cureus* 2022;14:e31291.
- Edelsztein NY, Grinspon RP, Schteingart HF, Rey RA. Anti-Müllerian hormone as a marker of steroid and gonadotropin action in the testis of children and adolescents with disorders of the gonadal axis. *Int J Pediatr Endocrinol* 2016;2016:20.
- Carlsen E, Swan SH, Petersen JH, Skakkebaek NE. Longitudinal changes in semen parameters in young Danish men from the Copenhagen area. *Hum Reprod* 2005;20:942–9.
- Yahyavi SK, Holt R, Juel Mortensen L, Boisen IM, Árting LB, Jørgensen A, et al. Effect of a single-dose denosumab on mineral homeostasis in infertile men: insights from a pilot intervention study and a randomized controlled trial. *BMC Med* 2025;23:145.