Dear Editor,

During the last decade, there has been an increase in the usage of dietary protein supplements for bodybuilding, especially among young men who work out in commercial gyms. Recently, it was shown that 23 of 24 dietary supplements available in fitness equipment and online shops contained anabolic steroids. However, these supposedly pro-androgenic supplements presumably differ from the more commonly used whey and soy protein supplements. Notwithstanding, both products are listed as dietary supplements rather than medical drugs; thus, they are not rigorously tested, do not necessarily list all contents, and consequently might contain known and unknown active components.

In fact, it was shown that 14.8% of 634 nonhormonal nutritional supplements contained undeclared anabolic androgenic steroids. The impact of dietary protein supplements among young men remains poorly explored concerning their potential adverse effects on reproductive health. Although the present findings should be considered preliminary, we observed that abstinence from protein supplements was associated with improved sperm concentration but had no significant effect on total sperm number in a group of 20 subfertile men.

This prospective observational study reports a cohort of 20 men who attended the Fertility Clinic Skive Regional Hospital due to subfertility between 2014 and 2016. The inclusion criteria for the study were (i) infertile men with a sperm concentration below 15 × 10^6 ml^-1 at the time of enrollment, (ii) use of dietary protein supplements for bodybuilding on a regular basis (i.e., above two times per week for >3 months), (iii) nonsmokers, (iv) alcohol intake below the maximum intake of 14 units per week recommended by the Danish Health Authority; (v) no use of medication with potential gonadotoxic effect for at least 6 months prior to enrollment, (vi) absence of clinical varicocele.

At the initial fertility consultation, patients provided a semen specimen on site after 2–5 days of ejaculatory abstinence. Specimens were analyzed in the laboratory of the Fertility Clinic following, in part, the recommendations of the 2010 World Health Organization (WHO) criteria for the examination of human semen. Specifically, we strictly followed the WHO recommendations concerning sample collection, macroscopic evaluation, and sperm motility. However, we used a low-volume chamber (Makler, Sefi-Medical Instruments Ltd., Haifa, Israel) for determining sperm number. In addition, the patients answered a questionnaire to acquire data on their protein supplement intake and other relevant factors, including potential genetic and work-related factors. The questionnaires were thoroughly examined by the treating physician and, if needed, elaborated during consultation. Moreover, the men were retrospectively called by phone to provide further knowledge about their intake of protein supplementation, i.e., which product was used, the duration of intake (days, months or years), the frequency of intake (number of portions per day or week), and the concentration of protein supplement intake per portion. Finally, all men underwent a physical genital examination. All 20 men agreed to terminate their intake of dietary protein supplements, leading to an abstinence period of 2–16 (median 4.5 [interquartile range 2.0–8.5]) months before follow-up either if pregnant or when starting fertility treatment (Table 1). Following this washout period, a new semen sample was delivered and the sperm concentration and total sperm number were used as primary follow-up values (Table 1).

Data were analyzed as two sample paired data based on paired t-test as the assumption of normality (QQ-plot) and variance (Bland–Altman plot) were confirmed after log-transformation of the data. The mean difference on the log-scale was transformed into a median ratio with a 95% confidence interval (CI). Patient number 9 (azooospermic) was excluded from the analysis as data could not be log-transformed. All calculations were made in Stata version 12.1 (Statacorp, Texas, USA). The study was approved by the Ethical Committee of Central Denmark Region.

Patients had a mean age of 32.2 (standard deviation [s.d.] = 5.1) years, and mean body mass index (BMI) of 25.8 kg m^-2 (s.d. = 9.2) with no relevant change during the follow-up period. Furthermore,
Table 1: Sperm concentration and total sperm number in neat semen (pre- and postintervention) in a group of 20 infertile men subjected to cessation of protein supplementation for bodybuilding and resistance training

| Patient number | Baseline sperm concentration in neat semen (×10^6 ml⁻¹) | Follow-up sperm concentration in neat semen (×10^6 ml⁻¹) | Baseline total sperm number in neat semen (×10^9) | Follow-up total sperm number in neat semen (×10^9) | Follow-up period (month) |
|----------------|--------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------|
| 1              | 0.05ᵃ                                                | 3.5                                                    | 0.2                                             | 19.3                                             | 7                       |
| 2              | 2.4                                                  | 4.4                                                    | 5.3                                             | 8.4                                             | 14                      |
| 3              | 0.05ᵃ                                                | 0.2                                                    | 0.3                                             | 0.8                                             | 16                      |
| 4              | 14.0                                                 | 9.0                                                    | 61.6                                            | 27.0                                            | 8                       |
| 5              | 0.3                                                  | 0.05ᵇ                                                | 1.7                                             | 0.1                                             | 2                       |
| 6              | 5.2                                                  | 9.0                                                    | 20.8                                            | 18.9                                            | 6                       |
| 7              | 0.6                                                  | 2.2                                                    | 0.6                                             | 2.0                                             | 3                       |
| 8              | 8.6                                                  | 23.0                                                   | 25.8                                            | 46.0                                            | 7                       |
| 9              | 0.0                                                  | 0.0                                                    | 0.0                                             | 0.0                                             | 2                       |
| 10             | 0.05ᵃ                                                | 6.4                                                    | 0.2                                             | 17.9                                            | 3                       |
| 11             | 1.9                                                  | 30.0                                                   | 2.5                                             | 120.0                                           | 2                       |
| 12             | 1.7                                                  | 10.0                                                   | 3.1                                             | 19.0                                            | 2                       |
| 13             | 14.0                                                 | 33.0                                                   | 29.4                                            | 92.4                                            | 3                       |
| 14             | 0.6                                                  | 1.1                                                    | 1.1                                             | 1.1                                             | 10                      |
| 15             | 14.0                                                 | 26.0                                                   | 49.0                                            | 46.8                                            | 9                       |
| 16             | 4.8                                                  | 1.8                                                    | 17.8                                            | 3.8                                             | 2                       |
| 17             | 1.3                                                  | 0.4                                                    | 2.1                                             | 0.6                                             | 7                       |
| 18             | 1.8                                                  | 1.2                                                    | 11.3                                            | 3.0                                             | 12                      |
| 19             | 0.8                                                  | 0.8                                                    | 3.0                                             | 2.2                                             | 2                       |
| 20             | 1.9                                                  | 16.0                                                   | 3.4                                             | 57.6                                            | 3                       |

Median (IQR): 1.8 (0.5–5.0) | 8.9 (1.0–13.0) | 3.1 (1.0–18.5) | 13.1 (1.8–31.8) | 4.5 (2.0–8.5)

ᵃWe estimated the concentration to be 0.05×10⁶ ml⁻¹ based on visualization of only a few spermatozoa seen within or outside the Makler chamber. IQR: interquartile range

all but two patients were Caucasians. Three patients reported a history of urogenital problems (cryptorchidism [n = 1], uncomplicated treated chlamydia infection [n = 1], and testis trauma [n = 1]). On physical examination, one patient presented with testis hypotrophy. The remaining sixteen patients were classified as having idiopathic oligozoospermia.

In the present cohort of subfertile men, abstinence from protein supplementation resulted in a significant increase in median sperm concentration, which was 2.6 (95% CI: 1.1–5.8) times higher than the baseline median sperm concentration (paired t-test, P = 0.03). This effect was also significant using nonparametric statistics (Wilcoxon signed rank test, P = 0.02). In contrast, total sperm number was not significantly different between baseline and follow-up, albeit we observed an estimated effect of 2.1 (95% CI: 0.8–9.1) times increase in median total sperm number at follow-up compared to baseline median total sperm number (paired t-test, P = 0.14). This effect was also not significant using nonparametric statistics (Wilcoxon signed rank test, P = 0.27). Individual patient information regarding primary outcomes, i.e., sperm concentration and total sperm number are presented in Table 1. Furthermore, secondary outcomes in terms of percentage of progressive motile sperm, total motile count, and fertility outcomes from all cycles after follow-up are presented in Table 2.

After completion of the initial workup, 14 patients (case 1, 2, 3, 5, 7, 8, 9, 10, 12, 14, 17, 18, 19, and 20; Table 1) were offered intracytoplasmic sperm injection (ICSI) according to the policy of our clinic as they were either cryptozoospermic or their total motile count was below 1 million after seminal preparation by gradient centrifugation (rotate speed for seminal preparation: 300 g Eppendorf model 5702, AH Diagnostics, Aarhus, Denmark). Among them, one patient (case 10) improved his sperm concentration significantly achieving natural conception after a follow-up period of 3 months.

Furthermore, eight patients (case 1, 4, 6, 14, 15, 16, 17, and 20; Table 1) obtained live births and four patients (case 5, 12, 18, and 19; Table 1) obtained clinical pregnancies (i.e., fetal heartbeat at gestational week 7–9) through different assisted reproductive treatments after the abstinence period. Patient 11 improved his sperm concentration from 1.9 to 30 million ml⁻¹ and his total motile count after preparation was 6.7 million; however, no further information regarding fertility outcome could be obtained, as the couple withdrew from the study. Patient 3 had a significant increase in sperm concentration from only 0.05×10⁶ ml⁻¹ at baseline to 0.2×10⁶ ml⁻¹ after follow-up. Interestingly, this case refers to the patient with hypotrophic testes mentioned above (12 ml and 10 ml, respectively; normal value 18–20 ml), but unfortunately the couple faced a missed abortion after ICSI. One azoospermic patient (case 9) remained azoospermic after the washout period and the couple obtained an ongoing pregnancy with donor sperm.

Of the 20 patients investigated, five patients (case 4, 5, 16, 17, and 18) had a slight reduction in sperm concentration at follow-up ranging from 0.3 to 5.0×10⁶ ml⁻¹. In these five patients, ICSI was chosen and all five patients achieved either live birth or ongoing pregnancies.

In the present pilot study, we observed that abstinence from protein supplements was associated with a 2.6 (95% CI: 1.1–5.8) times increase in sperm concentration compared to baseline sperm concentration (median follow-up time was 4.5 [interquartile range: 2.0–8.5] months) in a group of 20 subfertile men attending fertility treatment. However, no statistically significant effect was observed on total sperm number, albeit the effect estimate was much similar to the observed effect on sperm concentration, of 2.1 (95% CI: 0.8–9.1) times increase compared to baseline total sperm number. We emphasize that the present findings are preliminary and need to be corroborated in larger prospective studies. As 25.0% (5/20) had a slight decrease in sperm concentration, the limited power of this study, to
determine natural and seasonal variation within sperm concentration from one ejaculate to the next in some men, and the profound effect of abstinence from protein supplements seen in other men, is noticeable.

Approximately 60% of the population of reproductive age has a high intake of protein supplements. Unfortunately, young male users are not aware of the possible adverse effects of dietary supplements on semen quality compared to older more experienced users. Therefore, this new dietary trend should be investigated more closely. To the best of our knowledge, our study is the first to report a possible negative association between protein supplementation for bodybuilding/resistance training and sperm concentration in infertile men. We attempted to identify similar studies using literature search in electronic databases (PubMed, EMBASE, MedlinePlus, Ovid MEDLINE, and Scopus) from their inception to October 2017, albeit we failed to identify any previous publications on this matter.

Based on the known product declaration of the protein supplements used by the men in the present pilot study, it predominantly consisted of whey, which is one of the two generally used protein dietary supplements – the other being soy. Previous studies investigated the potential impact of soy supplements on sex hormone levels whereas the evidence for whey products are scarce. In a cross-over trial by Kraemer et al., it was seen that the acute testosterone response after heavy resistance training in men was significantly lower when the dietary supplement intake consisted of soy protein supplements compared to whey and placebo supplements. However, after 1 h, the decreasing effect of soy started to disappear. In contrast, another study did not find any differences in long-term testosterone levels among 20 participants who underwent a heavy training program while consuming either a soy or whey dietary supplement or a mixture of both. Therefore, it might still be discussed whether soy, only, has an inhibitory effect on the acute testosterone response to physical performance in men. More importantly, the studies did not find any significant inhibitory effect of whey supplements compared to placebo supplement which raises doubts regarding the plausible component of the protein supplements that might induce the negative effect on semen.

Interestingly, a broad-based study performed by Geyer et al. found that 14.8% of 634 nonhormonal nutritional supplements contained undeclared anabolic androgenic steroids, which will be phrased as “positive products.” They were purchased in 13 different countries from 215 different suppliers. The study found that positive products predominantly came from prohormone selling companies as a result of cross-contamination, were produced in companies located in the USA, the Netherlands, the UK, Italy and Germany, and that most positive products were found in capsules (19.6%) compared to tablets (11.7%) and powders (6.9%). The percentages indicate the proportion within a specific product type that tested positive in the study. Supporting the above-mentioned findings, another study analyzed 75 supplements used in sports, of which 7 out of 17 prohormone supplements contained undeclared hormonal substances. Notwithstanding, both studies were conducted several years back; a 2015 review by Outram and Stewart stated that 10%–15% of nutritional supplements might contain prohibited substances. However, due to inadequate amount of research within this field, it cannot be firmly determined which active component causes the decreased sperm quality among infertile men.

It is not ideal to take the results of single semen analyses for comparison as regression toward the mean may impact the results. This is the event of which extreme measurements within the same individual tend to approach average values when analyzing repetitive semen samples. Furthermore, we have used a low-volume chamber for counting sperm, which is shown to be suboptimal as it can influence the accuracy of sperm concentration estimates. However, all analyses were conducted in the same laboratory by experienced donors.

| Patient number | Baseline percentage of progressive motile sperm in neat semen (%) | Follow-up percentage of progressive motile sperm in neat semen (%) | Baseline total motile count (x10⁶) | Follow-up total motile count (x10⁶) | Fertility outcome |
|----------------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------|-----------------------------------|------------------|
| 1              | 50.0                                                         | 55.0                                                         | 0.1                               | 10.6                              | Live birth       |
| 2              | 58.0                                                         | 57.0                                                         | 3.1                               | 4.8                               | Under treatment  |
| 3              | 50.0                                                         | 50.0                                                         | 0.1                               | 0.4                               | Missed abortion  |
| 4              | 82.0                                                         | 77.0                                                         | 50.5                              | 20.8                              | Live birth       |
| 5              | 75.0                                                         | 75.0                                                         | 1.2                               | 0.1                               | Clinical pregnancy |
| 6              | 38.0                                                         | 67.0                                                         | 7.9                               | 12.7                              | Live birth       |
| 7              | 15.0                                                         | 41.0                                                         | 0.1                               | 0.8                               | Under treatment  |
| 8              | 34.0                                                         | 53.0                                                         | 8.8                               | 24.4                              | Under treatment  |
| 9              | 0.0                                                          | 0.0                                                          | 0.0                               | 0.0                               | Azoospermic, clinical pregnancy with donor sperm |
| 10             | 50.0                                                         | 59.0                                                         | 0.1                               | 10.6                              | Natural conception, clinical pregnancy |
| 11             | 68.0                                                         | 69.0                                                         | 1.7                               | 83.8                              | Withdrew         |
| 12             | 13.0                                                         | 59.0                                                         | 0.4                               | 11.2                              | Clinical pregnancy |
| 13             | 66.0                                                         | 88.0                                                         | 19.4                              | 81.3                              | Under treatment  |
| 14             | 17.0                                                         | 64.0                                                         | 0.2                               | 0.7                               | Live birth       |
| 15             | 45.0                                                         | 69.0                                                         | 22.1                              | 32.3                              | Live birth       |
| 16             | 65.0                                                         | 60.0                                                         | 11.5                              | 2.3                               | Live birth       |
| 17             | 31.0                                                         | 25.0                                                         | 0.7                               | 0.2                               | Live birth       |
| 18             | 64.0                                                         | 68.0                                                         | 7.3                               | 2.0                               | Clinical pregnancy |
| 19             | 50.0                                                         | 12.0                                                         | 1.5                               | 0.3                               | Clinical pregnancy |
| 20             | 68.0                                                         | 76.0                                                         | 2.3                               | 43.8                              | Live birth       |

Median (IQR) 50.0 (33.3–65.3) 60.0 (52.3–69.0) 1.6 (0.3–8.1) 7.7 (0.6–21.7)

IQR: interquartile range

Table 2: Percentage of progressive motile sperm and total motile count in neat semen (pre- and postintervention), and fertility outcomes from all cycles after follow-up in a group of 20 infertile men subjected to cessation of protein supplementation for bodybuilding and resistance training.
technicians following strict standards. The magnitude of effect size after protein supplementation cessation was large and the decrease in sperm concentration that was observed in five patients after the washout period could be physiological. As the participants could not fully recall details regarding their protein supplement consumption, only overall information was available regarding their intake. Therefore, the present study was not able to control for the type of dietary supplemental product, the active component, and the dosage. Although all men stated that they did not smoke, did not ever consume anabolic steroids, and had a low alcohol intake, the information was based on questionnaire/clinical interview data. In continuation hereof, although not mentioned by the men, it is possible that some implemented other lifestyle changes during the follow-up period, enabling unknown confounders to influence the results. Moreover, the sample size of the current study contributes to the limitations. Finally, although couples received relevant fertility treatment, we did not report on female factors which could obviously bias the reproductive outcome reported, thus emphasizing the need for further studies in this field.

Future research examining the role of temporarily resumption of protein supplementation after the washout period could help determine whether the results were due to biological variations in sperm concentration and to evaluate if a predicted decrease in sperm concentration would occur. In addition, a control group of fertile men could help determine whether the results are caused by any natural or seasonal variation in sperm concentration and to assess other potential confounders. Importantly, serum measurements of androgens and gonadotropins should be performed in future studies to help identify nonreported anabolic steroid intake and the possible effect of protein supplementation on the endocrine profile. In a recent case–control study comparing a group of former anabolic androgenic steroid (ASS) abusers to a group of current AAS users and a control group, former ASS abusers had significantly lower plasma testosterone levels and higher frequencies of symptoms of hypogonadism, i.e., erectile dysfunction, decreased libido, and increased depression than the control group.15

This pilot study opens the question whether termination of protein supplementation might be associated with increased sperm parameters in oligozoospermic men. As protein supplementation has no evident health effects, in otherwise healthy young men, it could further be questioned whether protein supplements should generally be discouraged in oligozoospermic men attending fertility treatment as this seems to represent an easily applied intervention adding potential benefits and no risk.

**AUTHOR CONTRIBUTIONS**

SK, TH, BP, RL, BA, HE, SCE and PH were part of study design and execution. SK performed data collection and manuscript drafting and contributed to data analysis. TH performed data analysis. TH, BP, RL, BA, HE, SCE, and PH contributed to data collection and manuscript drafting. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

The authors declared no competing interests.

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