SEMEN QUALITY AND HISTOMORPHOLOGICAL ANALYSIS OF TESTICULAR TISSUES OF RABBIT BUCKS ADMINISTERED HUMAN MENOPAUSAL GONADOTROPIN

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ABSTRACT

The study investigated the effects of Menogon treatment on semen quality and histomorphology of testicular tissues in rabbit bucks. Twenty-four bucks of Chinchilla × Dutch breed, weighing 1.3 – 1.6 kg at 15 – 17 weeks of age, were divided into four groups receiving different Menogon doses (0.0 IU as control, 7.5 IU, 15.0 IU, and 22.5 IU) for 105 days in a completely randomised design (CRD). Each treatment group was replicated three times with two bucks per replicate. Semen quality and histological examination of the testes were assessed. Results showed that progressive sperm motility, sperm concentration, live sperm percentage, and libido were significantly higher (p<0.05) in the group receiving 15.0 IU of Menogon compared to other groups. The percentage of total abnormal sperm was significantly lower in the 15.0 IU group compared to other groups. Histological analysis revealed that the 15.0 IU treatment induced more hyperplasia of the germinal epithelium, which positively impacted semen production. These findings suggest that the optimal reproductive processes in rabbit bucks were observed at a Menogon dose of 15.0 IU, indicating the potential of Menogon in enhancing testicular health and spermatogenic processes.

Keywords: Menogon, Semen quality, Histopathology, Rabbit bucks

INTRODUCTION

The synthesis of gonadotrophin-releasing hormone (GnRH) in the hypothalamus is well-recognized as a crucial element in mammalian reproduction (Lee *et al*., 2008; Perrett and McArdle, 2013; Marques *et al*., 2022). According to research, GnRH is secreted from the hypothalamus, travels through the portal blood vessels to the anterior pituitary gland, and prompts the release of gonadotropins – namely luteinising hormone (LH) and follicle-stimulating hormone (FSH). These hormones circulate in the bloodstream, influencing steroid production and the maturation of reproductive cells in the gonads (Marques *et al*., 2022). FSH specifically stimulates Sertoli cells (Wang *et al*., 2022), while LH induces testosterone production in Leydig cells (Bakhtyukov and Shpakov, 2016), essential for the creation of reproductive cells. Thus, the male reproductive system's functionality hinges on the integrity of the hypothalamus-pituitary-testicular axis, with male fertility issues potentially stemming from hormonal induction deficits. Regulating the quantity and rhythm of gonadotropin stimulation to the testes can affect testosterone production and release, offering a means to manage male fertility. Historically, various hormonal treatments have been explored to enhance endogenous gonadotropins and androgen levels (Simon, 2002; Sluka *et al*., 2006; Isidori *et al*., 2017; Handelsman, 2020), aiming to boost fertility through controlled hormonal intervention. The significance of having physically robust males that produce high-quality semen is increasingly recognized, especially with

the growing reliance on fewer male animals and artificial insemination in breeding programs. Producing high-quality ejaculates allows for the semen to be diluted more extensively, yielding a higher number of insemination doses per animal.

Human menopausal gonadotropin (hMG), a hormone preparation derived from the urine of postmenopausal women, contains both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones are pivotal in regulating spermatogenesis and steroidogenesis in males. The administration of hMG in animal models, such as rabbit bucks, provides a unique opportunity to study its effects on male fertility parameters, particularly semen quality. Therefore, the current study aims to fill the gap in knowledge regarding the influence of hMG on rabbit bucks' semen quality and testicular histology. By administering hMG and conducting thorough semen analyses and histological examination of testicular tissues, this research will provide valuable data on the hormone's reproductive implications. The outcomes may have significant implications for animal breeding practices and may also offer comparative insights applicable to animal reproductive physiology.

MATERIALS AND METHODS

Location of Study: The experiment was conducted in the Rabbitry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, October 27, 2023 – February 16, 2024.

Ethics: This experiment was approved and conducted according to the provisions of the Animal Research Ethics Committee on the use of animals for biomedical research at the Nnamdi Azikiwe University, Awka, Nigeria.

Experimental Materials and Management: A total of 24 rabbit bucks (Chinchilla X Dutch) aged 15 – 17 weeks weighing 1.3 – 1.6 kg were used in this study. The rabbits were managed intensively. They were quarantined for 3 weeks during which they were treated with Ivomec for the control of haemoparasite, endo and ectoparasites. The bucks were individually kept in cages (50 × 55 × 40 cm^3) of a three-tier hutch and each cage was provided with a feeder and a drinker. The experimental animals were given *ad libitum* access to feed and water Top feed grower (15% crude protein and 2500 kcal kg^{-1} metabolizable energy) was fed in the morning and supplemented with unrestricted access to *Tridax procumbens, Centrosema pubescens, Calopogonium mucunoides* and *Panicum maximum* in the evening.

Ambient temperature (°C) and relative humidity (%) inside the rabbit building were measured daily throughout the experimental period between 0900 and 1100 hours using a mercury thermometer (to the nearest 0.1°C) and wet and dry bulb hygrometer (to the nearest 1 %). The ambient temperature and relative humidity were averagely recorded as 24.00 ± 6.0°C and 89.0 ± 11.0%, respectively.

Menogon bearing batch No: CE0310B was purchased from Ferring Pharmaceuticals, Saint-Prex, Switzerland. A pack of Menogon contained 5 ampoules of dry substance and an accompanying 5 ampoules of diluents. One ampoule of Menogon contains menotropin (human menopausal gonadotropin) corresponding to 75 IU FSH and 75 IU LH. One ampoule of diluents contained isotonic sodium chloride solution. Packs of Menogon used in the study were stored in the refrigerator (below 4°C) and protected from light.

Experimental Design: The rabbit bucks were randomly assigned to four treatment groups. Each treatment was replicated three times with two bucks constituting a replicate in a completely randomised design (CRD). The treatment groups were as follows: Group A: This served as the control (No Menogon treatment). Group B: 0.1 ml of Menogon (equivalent to 7.5 IU of FSH and LH) was administered to each rabbit buck. Group C: 0.2 mL of Menogon (equivalent to 15.0 IU of FSH and LH) was administered to each rabbit buck. Group D: 0.3 mL of Menogon (equivalent to 22.5 IU of FSH and LH) was administered to each rabbit buck. A vial containing 75 IU FSH and 75 IU LH was reconstituted in 1 mL of physiological saline solution and injected intramuscularly. Thus, different doses of Menogon were administered after every 72 hours for 56 days.

Any unused reconstituted material was discarded.

Semen Collection: The rabbit bucks were trained to serve an Artificial Vagina (AV) using a teaser rabbit doe two weeks before semen collection. This preliminary period was adopted to ensure that the rabbits were reproductively normal as judged by their libido. It also helped to evacuate old spermatozoa from within the epididymis.

On the 57th day following the administration of the menotropin injection, the 24 bucks used in this study were placed on a semen collection schedule of two times per week. One ejaculate was collected from each rabbit buck once between 0800 to 1100 hours on Mondays and Thursdays for five consecutive weeks. The rabbit doe was taken to the bucks' cage and the doe was held in position for service. When the male attempted to mount, the AV was strategically placed below the belly of the doe in such a way that the penis of the male was introduced into the AV. The temperature of the inner liner rubber sleeve of the AV was adjusted to 37 ± 2^{o}C at the time of semen collection. Lubrication of the inner sleeve was performed using glycerin.

Estimation of Semen Characteristics: Semen evaluation involves the estimation of both microscopic and macroscopic indices. Progressive sperm motility percentage score was subjectively assessed in a drop of fresh semen on a warm glass slide covered with a warm cover slip and examined under ×400 magnification using a warm stage adjusted at 37^{o}C, according to the procedure outlined by Agarwal *et al.* (2022). Ejaculated volume was read off directly in millimetres from a calibrated glass collection tube attached to the AV.

The percentage of sperm abnormalities was determined using the same smears prepared for live/dead ratio using ×400 magnification. The percentage of acrosomal abnormalities was estimated by a drop of fresh extended semen smeared on a pre-warmed slide and dried in a current of warm air. The smears were fixed by immersion in buffered normal saline in a water bath at 37^{o}C for 15 minutes. The slides were washed in running tap water for 15 minutes. The smears were dried and immersed in the buffered Giemsa stain for 90 minutes after which it was rinsed in distilled water and dried. One hundred stained spermatozoa were examined for each sample under low magnification (×400) to estimate the percentage of spermatozoa with acrosome abnormality (Boitrelle *et al.*, 2021).

Sperm cell concentration ($\times 10^7/mm^3$) was determined using a haemocytometer after a dilution of 1 in 200 in a solution of 45 mL normal saline and 5 mL formalin. Total sperm ($\times 10^9$ per ejaculate) was determined by multiplying the semen ejaculate volume by the sperm cell concentration (Björndahl *et al.*, 2003).

Libido was estimated by observing the reaction time (seconds) which elapsed between the exposure of a buck to a doe and the first copulation (serving the AV) (Korkmaz *et al.*, 2023).

Histological Studies: At the end of the semen collection period, three bucks from each treatment group were randomLy selected from the experimental bucks for histological analysis. The rabbits were humanely euthanized under anaesthesia (pentobarbitone sodium IP at 180 mg/kg) and the testes were harvested. Testes were carefully separated and freed of tunica albuginea and all adhering connective tissues. The recovered testes were fixed in Bouin's solution for 24 hours. The tissues were washed in ascending grades of ethanol (50, 75 and 100%) and cleared with xylene. They were embedded in paraffin wax and then sectioned using microtome at 15 µ thickness. Staining was done with Haematoxylin and Eosin (H and E). The slides were covered with DPX (Distyrene, Plasticizer, and Xylene) mountant to increase the refractive index of the stained preparation and covered with slides to prevent scratches. All sections were examined under a light microscope using ×400 magnification. Photographs of tissues were taken with an Olympus photomicroscope for observation and documentation of histopathology (Drury and Wallington, 1976).

Statistical Analysis: The data generated were analysed using Analysis of Variance (ANOVA).

Significant means were separated using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Semen Evaluation: The results of the semen evaluation of rabbit bucks treated with different levels of Menogon showed marked variations ($p<0.05$) in progressive sperm motility (Table 1). Except for bent tail which varied significantly ($p<0.05$), other detailed incidences of morphological aberrations evaluated in this study revealed no statistically significant differences ($p>0.05$) among the treatment groups (Table 1). Other parameters such as volume, libido and semen viability were also not significantly affected ($p>0.05$) (Table 1).

The values of the analysed semen parameters increased progressively with increasing levels of Menogon up to 15.0 IU and declined at higher doses (22.5 IU). The observed ejaculate trend in response to the treatment doses suggested that a high dose rate of menotropin produced a suppressive effect on the hypothalamus. A negative feedback action may have been established at a higher dose of Menogon which led to a decrease in testosterone levels, which in turn reduced the process of spermatogenesis.

Observations made on semen concentration in this study are consistent with the range of 50 to 350 $\times 10^6/mm^3$ reported by Brackett (2004) and also similar to what was obtained by Herbert and Adejumo (1993) and Ansa and Imasuen (2015) for rabbit bucks. The increase in sperm count following Menogon treatment in this experiment indicated that human gonadotrophin is effective in exerting stimulatory actions on Sertoli cells as reported by Shah *et al*. (2021). Sertoli cells are recognized for their role in supporting the growth of developing spermatids, while Leydig cells are relied upon for the production of androgenic hormones that enhance male reproductive characteristics. The results herein further confirmed the report of Akhondi *et al.* (2010) that the administration of gonadotropin to immature rats and mice increases the number of spermatogonia by reducing the proportion that degenerate. This may be due to a stimulatory effect of the hormone on the DNA synthesis of the sperm cells. Furthermore, gonadotropin also increases the proportion of cells passing through meiosis and spermatogenesis (Haywood *et al*., 2003; Matthiesson *et al*., 2006; Akhondi *et al.,* 2010) thus increasing semen concentration.

Percent abnormal spermatozoa obtained in this study is consistent with an earlier report (Brackett, 2004). However, the total abnormal sperm traits in these findings revealed a significant decrease ($p<0.05$) at 15.0 IU dose of Menogon treatment. This significantly lower value in abnormal sperm cells of the 15.0 IU group compared to the control group indicates that more viable sperm cells will be available for fertilization. Undoubtedly, this is an indication that the dose of 15.0 IU encouraged high functional integrity of the epididymis (Awojobi and Oyeyemi, 2001) which consequently caused a slow release of immature spermatozoa and enhanced maturation of the sperm cell. This factor may be responsible for the corresponding significant increase ($p<0.05$) in sperm concentration and progressive sperm motility (Brackett, 2004). It is also documented by Aitken and Baker (2013) that gonadotropin reverses apoptotic changes in sperm structure occurring because of microbial infections and increases fertilizing ability. Nevertheless, abnormal sperm characteristics can either be passed down genetically or developed due to injuries and exposure to increased temperatures caused by various diseases or toxins (Brackett, 2004) and improper semen handling (Boitrelle *et al*., 2021).

Concerning the motility of the spermatozoa, a significant increase ($p<0.05$) was obtained and the mean values were in agreement with 70% and above reported by Brackett (2004) for good-quality fresh sperm. Pineda (2003) reported a low score of 30% as a minimally acceptable spermatozoa motility level for fresh ejaculates advocated by some breeding organizations. Sperm motility is an important index in reproductive examination because it demonstrates the viability and vigour with which sperm cells are propelled during the process of fertilization. Therefore, the significant increase ($p<0.05$) observed for progressive motility in Menogon-treated groups in this study points to the beneficial effect of Menogon on fertility as

Table 1: Mean values of semen characteristics and libido of rabbit bucks treated with Menogon

Parameters	0.0 IU	7.5 IU	15.0 IU	22.5 IU
Volume (mL)	0.42 ± 0.07	0.56 ± 0.07	0.56 ± 0.07	0.51 ± 0.07
Motility (%)	55.3 ± 6.85^{a}	66.2 ± 6.85^{ab}	78.3 ± 6.85^{b}	70.3 ± 6.85^{ab}
Ejaculate Conc. (×10^{6}/mm^{3})	86.0 ± 22.6^{a}	110.0 ± 22.6^{ab}	186.0 ± 22.6^{b}	135.0 ± 22.6^{ab}
Total Sperm (×10^{9}/mm^{3})	33.6 ± 16.99^{a}	62.1 ± 16.99^{ab}	106.2 ± 16.99^{b}	69.0 ± 16.99^{ab}
Acrosomal Changes (%)	0.00 ± 0.29	0.33 ± 0.29	0.33 ± 0.29	0.33 ± 0.29
Twin Head (%)	0.00 ± 0.17	0.33 ± 0.17	0.00 ± 0.17	0.00 ± 0.17
Giant Head (%)	0.33 ± 0.29	0.00 ± 0.29	0.33 ± 0.29	0.67 ± 0.29
Pyriform Head (%)	0.33 ± 0.29	0.33 ± 0.29	0.33 ± 0.29	0.00 ± 0.29
Narrow Head (%)	2.00 ± 0.60	2.00 ± 0.60	1.00 ± 0.60	1.33 ± 0.60
Detached Head	8.33 ± 1.34	8.00 ± 1.34	8.33 ± 1.34	8.00 ± 1.34
Double Tail (%)	0.00 ± 0.24	0.00 ± 0.24	0.33 ± 0.24	0.67 ± 0.24
Bent Tail (%)	11.67 ± 1.35^{b}	10.67 ± 1.35^{ab}	7.00 ± 1.35^{a}	12.33 ± 1.35^{b}
Shoehook Tail (%)	2.33 ± 1.10	1.67 ± 1.10	1.33 ± 1.10	1.33 ± 1.10
Coil Tail (%)	1.67 ± 0.94	2.00 ± 0.94	1.00 ± 0.94	1.33 ± 0.94
Cytoplasmic Droplet (%)	2.00 ± 0.50	0.67 ± 0.50	0.67 ± 0.50	0.67 ± 0.50
Total Abnormal Sperm (%)	28.70 ± 2.22^{b}	26.00 ± 2.22^{ab}	20.70 ± 2.22^{a}	26.70 ± 2.22^{ab}
Live sperm (%)	69.51 ± 2.80	70.92 ± 2.80	78.34 ± 2.80	73.83 ± 2.80
Libido (seconds)	17.30 ± 2.23	12.90 ± 2.23	11.40 ± 2.23	15.50 ± 2.23

^{a,b}Means bearing different letters of superscript within the same row differ significantly ($p<0.05$)

earlier confirmed by Abu *et al*. (2006). The mean semen volume (Table 1) compares favourably with the findings of Brackett (2004) who reported that rabbit semen volume varied between 0.4 – 0.6 mL. Although the semen volume recorded in this study showed no significant differences ($p>0.05$) among the treatment groups, the slight numerical increase in the Menogon-treated groups is indicative of enhanced semen production following Menogon administration.

Furthermore, although the mean percentage of live spermatozoa among the treatment groups was not significantly different ($p>0.05$), there was a progressive increase in the trend of semen viability up to 15.0 IU dose with a subsequent decline at a higher dose of 22.5 IU. Comparatively, the values obtained for percentage viability in this study are consistent with the report of Brun *et al*. (2002) for good-quality sperm cells. These authors outlined that more than 70 – 80% viability of sperm cells is graded as very good, 70% as good, 60 – 69% as regular and below 60% as poor.

Table 1 showed non-significant differences ($p>0.05$) in libido as measured by the reaction time of the rabbit bucks to mounting and subsequent ejaculation. However, the numerical increase in libido across the treatment groups supports the role of gonadotropin in the development and maintenance of libido and general body features that are associated with the male (Pineda, 2003; Brackett, 2004; Fails and Magee, 2018).

The treatment of rabbit bucks with Menogon leads to the enhancement of such parameters as progressive sperm motility, semen concentration, live sperm cells, and morphology. This result agreed with the report of Haywood *et al*. (2003), Abu *et al*. (2006) and Matthiesson *et al*. (2006) which further buttressed the efficacy of human gonadotropin in semen production.

Histopathological Study: The findings of testicular histology of rabbit bucks treated at various levels of Menogon are presented in Figures 1 – 4. The histopathological study indicated that the process of sperm development was consistent across both the experimental and control groups. This is evident from the observed hyperplasia of germinal epithelium, proliferation of Sertoli cells, and various stages of sperm cell development, along with sperm accumulation. However, the sample from the group treated with 15.0 IU of Menogon displayed a greater degree of testicular cell hyperplasia compared to other groups.

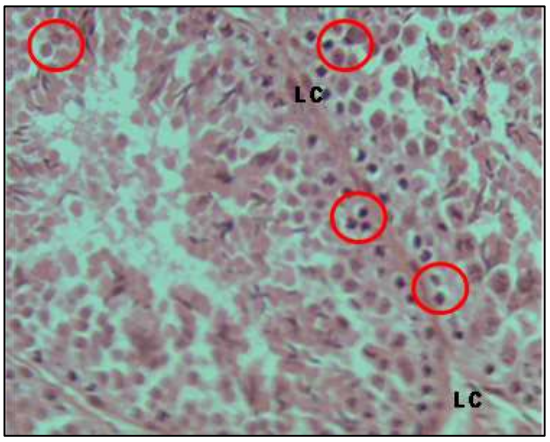

Figure 1: Photomicrograph of testicular tissues of rabbit bucks administered 0.00 IU of human menopausal gonadotropin (control) showing normal testicular structure. ***Key:*** *LC – Leydig cells; Red circles – Spermatogenic cells, H&E Mag. X400*

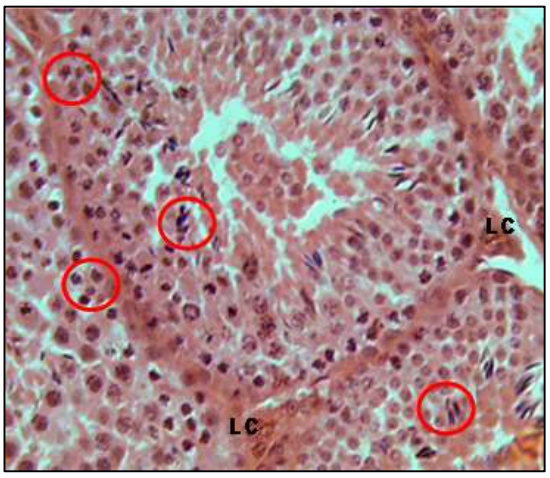

Figure 2: Photomicrograph of testicular tissues of rabbit bucks administered 7.50 IU of human menopausal gonadotropin showing normal testes with hyperplasia of germinal epithelium. ***Key:*** *LC – Leydig cells; Red circles – Spermatogenic cells, H&E Mag. X400*

This correlates with the significant differences ($p<0.05$) in sperm concentration and total sperm count per ejaculation shown in the semen analysis of the 15.0 IU Menogon-treated groups (Table 1). Similarly, Glage *et al*. (2013) conducted a comparative analysis between the testicular morphology of gonadotropin-treated male mice and untreated counterparts. Their findings revealed no significant testicular architectural changes between the two groups, with all stages of spermatogenesis observed in both the control and treated groups.

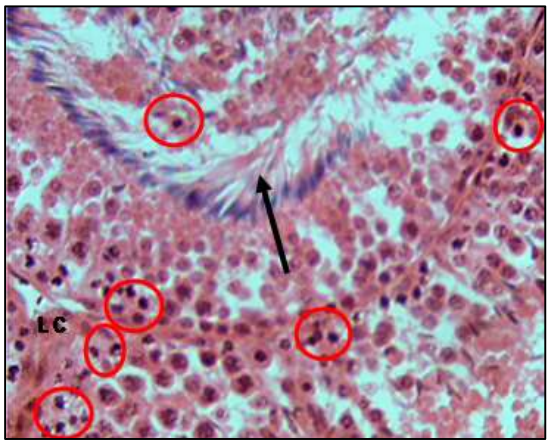

Figure 3: Photomicrograph of testicular tissues of rabbit bucks administered 15.00 IU of human menopausal gonadotropin showing normal testes with hyperplasia of germinal epithelium and accumulation of sperm in the lumen (arrow) of seminiferous tubules. ***Key:*** *LC – Leydig cells; Red circles – Spermatogenic cells, H&E Mag. X400*

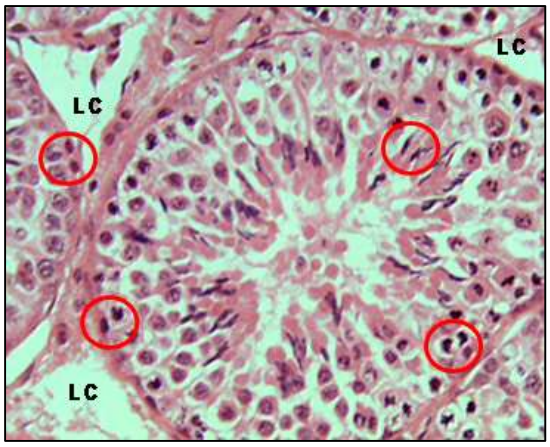

Figure 4: Photomicrograph of testicular tissues of rabbit bucks administered 22.50 IU of human menopausal gonadotropin showing normal testicular structure with hyperplasia of germinal epithelium. ***Key:*** *LC – Leydig cells; Red circles – Spermatogenic cells, H&E Mag. X400*

Also, the numbers of spermatogonial stages were similar across all groups, indicating consistency in spermatogenic processes regardless of gonadotropin treatment. Additionally, the histological findings, when compared with the ejaculated semen, suggest that high doses of menotropin may exert a suppressive effect on the hypothalamus, establishing a negative feedback loop at 22.5 IU of Menogon. This likely led to reduced testosterone levels and a subsequent decrease in spermatogenesis. This observation underscores

the impact of Menogon on testicular cells. Despite this, the overall structure of the testicular cells remained normal in both the control and Menogon-treated groups. Thus, qualitative sperm traits in Menogon-induced rabbits appear to be dose-dependent. Studies by Kaya *et al*. (2006) and Pirinççi *et al*. (2021) reported that human chorionic gonadotropin administration in male rats led to the deterioration in testicular histology and histological changes. Interestingly, Karaman *et al*. (2006) reported that these histological changes caused by hCG in the testicles are dose-dependent and reversible. Zhao *et al.* (2020) observed that elevated gonadotropin levels could damage the structure of testicles and lead to reduced sperm counts, although their findings were not statistically significant. Earlier research by Beastall *et al.* (1987) suggested that high LH or FSH levels could indicate a reduced ability of the testicles to produce sperm normally. Kumanov *et al.* (2006) also reported an inverse relationship between gonadotropin levels and sperm quality, including count, motility, and morphology. Meeker *et al.* (2007) found similar negative associations between these hormones and sperm characteristics. In summary, there is a consistent observation across studies that higher gonadotropin stimulation may impair testicular tissues and be linked to poorer sperm quality, but the statistical significance of these findings varies.

Conclusion: Results revealed that administration of 15.0 IU of Menogon significantly increased sperm percentage, normal sperm cells, and sperm motility. This suggested that human menopausal gonadotropin (Menogon) may be promising in enhancing sperm health parameters but at a dose-dependent rate.

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