EFFECT OF HUMAN MENOPAUSAL GONADOTROPIN ON SEMEN QUALITY AND HISTOPATHOLOGICAL STUDY OF TESTICULAR TISSUES OF RABBIT BUCKS

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ABSTRACT

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Semen quality and histopathology of testicular tissues of rabbit bucks treated with Menogon were studied. Twenty-four (Chinchilla × Dutch) bucks weighing 1.3 – 1.6kg at 15 – 17weeks were randomized into four treatment Menogon doses of 0 I.U. (control), 7.5 I.U., 15.0 I.U., and 22.5 I.U in a completely randomized design for 105 days. Each treatment was replicated three times with two bucks per replicate. Semen quality as well as histological examination of the testes was evaluated. Result of seminal parameter showed that progressive sperm motility (55.3, 66.2, 78.3, and 70.3%), concentration (86.0, 110.0, 186.0, and 135.0 × 10³/mm³), live sperm (69.51, 70.92, 78.34, and 73.83%) and libido (17.30, 12.90, 11.40, and 15.50s) for control, 7.5 I.U, 15.0 I.U and 22.5 I.U treatment groups respectively, were significantly (P<0.05) higher in the 15.0 I.U group. Total abnormal sperm (%) was significantly (P<0.05) lower in 15.0 I.U (20.70) than in 22.5 I.U (26.70), 7.5 I.U (26.00) and control (28.70%) groups. Histological findings indicated that 15.0 I.U. treated group caused more hyperplasia of germinal epithelium and 7.5 I.U showed more Leydig cells hyperplasia. Different hormonal treatments have been attempted (Fevold et al., 1931; Mortimer et al., 1974; Tsuruhara et al., 1977; Shoham et al., 1992; Sluka et al., 2006) aiming to improve mainly endogenous gonadotropins and/or androgen levels. Such improvement strategies have focused on the aspect of enhancing fertility by controlling the amount of hormonal supplementation. With increasing emphasis on fewer male animals and the use of artificial insemination, the awareness of having structurally sound males in good body condition that are producing semen of high quality is paramount to achieving high reproductive performance. The production of ejaculates of high quality generally allows a greater dilution of the semen to obtain large number of insemination dosages per animal. It therefore becomes necessary to evaluate the effect of human menopausal gonadotropin on the semen quality and testicular histopathology of rabbit bucks.

INTRODUCTION

It has long been established that gonadotrophin-releasing-hormone (GnRH) synthesized in the hypothalamus is a key regulator of reproduction in mammals (Crowe, et al., 1910; Aschner, 1912). GnRH is released from the hypothalamus in a pulsatile pattern; it travels via the portal vasculature to the anterior pituitary, where it stimulates the release of gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormorne (FSH). These gonadotropins enter the circulation and regulate both steroidogenesis and gamete maturation in the gonads (Conn, 1994). More specifically, FSH exerts stimulating actions on the Sertoli cells (Means et al., 1980) and LH causes the Leydig cells of the testis to produce testosterone which is necessary for gametogenesis. Therefore, it can be deduced that the functionality of the reproductive system in the male specie is dependent on the development and functioning of the hypothalamo-pituitary-testicular axis. In this way, male-factor fertility challenges may arise following deficiencies in hormonal induction. Consequently, controlling the amount and pattern of gonadotropin stimulation to the testis influences testosterone synthesis and secretion, thereby providing a potential method of controlling fertility in the male species.

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MATERIALS AND METHODS

Location of study

The experiment was carried out at the Rabbitary Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike. Umudike is situated in Ikwuano, Abia State in South Eastern Nigeria in the west of Africa. Its geographical coordinates are 5º 28 North, 7º 33 East and at an altitude of 112m above sea level in the tropical rainforest zone. Umudike has an average rainfall of about 2177mm per annum with relative humidity of about 72% and a monthly temperature range of 22ºC to 36ºC (Meteorological station of National Root Crop Research Institute (NRCRI), Umudike).
Experimental materials and management
A total of 24 rabbit bucks (Chinchilla X Dutch) aged 15-17 weeks weighing 1.3-1.6kg were used in this study. The rabbits were managed intensively. They were quarantined for 3 weeks during which they were treated with Ivomec® injection for the control of haemoparasite, internal and external parasites. The bucks were individually kept in cages (50x55x40cm) 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 water. Commercial diet (15% crude protein and 2500kcal kg⁻¹ metabolizable energy) was supplied in the morning and supplemented with 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 1100h using 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°C and 89%, respectively.

Menogon® bearing batch No: CE0310B was purchased from Grace and Mercy Pharmacy, Umuahia. A pack of Menogon® contained 5 ampoules of dry substance and an accompanying 5 ampoules of diluents. One ampoule of Menogon® contains menotrophin (human menopausal gonadotrophin [HMG]) corresponding to 75 I.U. FSH and 75 I.U. LH. One ampoule of diluents contained isotonic sodium chloride solution. Packs of Menogon® used in the study were stored in the refrigerator (below 25°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 Randomized Design (CRD). The treatment groups were as follows:
Group A: This served as the control (No Menogon® treatment).
Group B: 0.1ml of Menogon® (equivalent to 7.5 I.U. of FSH and LH) was administered to each rabbit buck.
Group C: 0.2ml of Menogon® (equivalent to 15.0 I.U. of FSH and LH) was administered to each rabbit buck.
Group D: 0.3ml of Menogon® (equivalent to 22.5 I.U. of FSH and LH) was administered to each rabbit buck.
A vial containing 75 I.U. FSH and 75 I.U. LH was reconstituted in 1ml 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 prior to semen collection. This preliminary period was adopted in order 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 menotrophin 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 hour (local time) on Mondays and Thursdays for five consecutive weeks. Rabbit doe was taken to the buck’s 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 40–42°C at the time of semen collection. Lubrication of the inner sleeve was performed using glycerine.

Estimation of semen characteristics
Semen evaluation involved 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 a low power magnification (×400) using a warm stage adjusted at 37°C, according to the procedure outlined by Arrebola and Fernandez (2011). Ejaculate volume was read-off directly in millimeters from a calibrated glass collection tube attached to the AV.

Percentage of sperm abnormalities was determined in the laboratory in the same smears prepared for live/dead ratio using a low magnification (×400). 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°C for 15 minutes. The slides were washed in running tap water for 15 to 20 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 a low magnification (×400) to estimate the percentage of spermatozoa with acrosome abnormality.

Sperm cell concentration (×10⁹/mm³) was determined in the laboratory using haemacytometer after a dilution of 1 in 200 in a solution of 45ml normal saline and 5ml formalin. Total sperm (×10⁹ per ejaculate) was determined by multiplying the semen ejaculate volume by the sperm cell concentration.

Libido was estimated by observing the reaction time (seconds) which elapsed between exposure of a buck to a doe and the first copulation (serving the AV).
Histological Studies: At the end of the semen collection period, 3 bucks from each treatment group were randomly selected from the experimental bucks for histological analysis. The animals were euthanized 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 Buain’s fluid 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 5-7μ thickness. Staining was done with Haemotoxylin and Eosin (H and E). The slides were covered with DPX (Distyrene, Plasticizer, and Xylene) mountant to increase refractive index of the stained preparation and covered with slides to prevent scratches. All sections were examined under light microscope using ×400 magnification. Photographs of tissues were taken with 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 Duncan Multiple Range Test (DMRT). The statistical model for this experiment is:

\[ Y_{ij} = \mu + T_i + e_{ij} \]

Where:
- \( Y_{ij} \) = Individual observation
- \( \mu \) = Overall mean
- \( T_i \) = Effect of treatment
- \( e_{ij} \) = Error term

All statistical analyses were in accordance with Steel and Torrie (1980).

RESULTS AND DISCUSSION

Semen evaluation

The results of the semen evaluation of rabbit bucks treated with different levels of Menogon are presented in Table 1. The result of this study showed marked variations (\( P < 0.05 \)) in progressive sperm motility. With the exception of bent tail which varied significantly (\( P < 0.05 \)) as shown in Table 1, other detailed incidence of morphological aberrations evaluated in this study revealed no statistically significant (\( P > 0.05 \)) differences among the treatment groups. Other parameters such as volume, libido and semen viability were also not significantly affected (\( P > 0.05 \)). The values of the analyzed semen parameters increased progressively with increasing levels of Menogon up to 15.0 I.U. and declined at higher dose (22.5 I.U.). The observed ejaculate trend in response to the treatment doses suggested that a high dose rate of menotrophin could produce suppressive effect on the hypothalamus. A negative feedback action could have been established at a higher dose of Menogon which led to the 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 ×10⁶/mm³ reported by Brackett (2004) and also similar to what was obtained by Hafez (1970) and Herbert and Adejumo (1993) 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 Means et al. (1980). Sertoli cells are known to be responsible for nurturing the developing spermatids and on Leydig cells to produce androgenic hormones which enhance male reproductive traits. The results herein further confirmed the report of Davies (1981) that the administration of gonadotropin to immature rats and mice increases the number of spermatagonia by reducing the proportion that degenerate. This may be due to a stimulatory effect of the hormone on DNA synthesis of the sperm cells. Furthermore, gonadotropin also increases the proportion of cells passing through meiosis and spermatogenesis (Davies, 1981; Haywood et al., 2003; Matthiesson et al., 2006) thus increasing semen concentration. Similarly, the total sperm per ejaculate conforms to the values recorded by Herbert and Acha (1995) but much higher than the findings of Gregoire et al. (1958).

Percent abnormal spermatozoa obtained in this study are consistent with earlier reports in the literature (Brackett, 2004; Bearden and John, 1992). However, the total abnormal sperm traits in this findings revealed significant decrease (\( P < 0.05 \)) at 15.0 I.U dose of Menogon treatment. This significantly lower value in abnormal sperm cells of 15.0 I.U. 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 I.U encouraged a 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 (\( P < 0.05 \)) increase in sperm concentration and progressive sperm motility (Brackett, 2004). It is also documented by Baccetti et al. (1997) that gonadotropin reverses apoptotic changes in sperm structure occurring because of microbial infections and increases fertilizing ability. Nevertheless, abnormal semen parameters may be inherited or acquired through trauma and exposure to increased temperature accompanying various disease, to
toxins (Brackett, 2004) and improper semen handling (Donadeu, 2006). Herbert and Acha (1995) further elucidated that abnormalities may also occur as artifact caused by the staining process. Concerning the motility of the spermatozoa, 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 spermatozoal motility levels 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 (P < 0.05) increase observed for progressive motility in Menogon treated groups in this study points to the beneficial effect of Menogen on fertility as earlier confirmed by Abu et al. (2006). The mean semen volume (Table 1) compares favourably with the findings of Herbert and Acha (1995) and Brackett (2004) who reported that rabbit semen volume varied between 0.4-0.6ml. Although the semen volume recorded in this study showed no significant (P > 0.05) differences among the treatment groups, the slight numerical increase in the Menogon treated groups is indicative of an enhanced semen production following Menogon administration. Furthermore, although the mean percentage of live spermatozoa among the treatment groups were not significantly (P>0.05) different, 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 Bamba (1998) and Brun et al. (2002b) for good quality sperm cells. These authors outlined that more than 70-80% viability of sperm cells are graded as very good, 70% as good, 60-69% as regular and below 60% as poor. Table 1 showed non-significant (P > 0.05) differences for 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 gonadotrophin in the development and maintenance of libido and general body features that are associated with the male (Pineda, 2003; Frandson, 2003; Guyton and Hall, 2004; Brackett, 2004). Consequent on the treatment of rabbit bucks with Menogon, the presented results demonstrate the enhancement of such parameters like progressive sperm motility, semen concentration, live sperm cells, and morphology. This result is supported by Abu et al. (2006), Davies (1981), Haywood et al. (2003) 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 Menogen are presented on plates 1-4. Histopathological study showed the cycle of spermatogenesis was regular in all experimental and control group. However, in all animals exposed to 15.0 IU of menogen, accumulations of sperm, in the lumen of seminiferous tubules were seen (Plate 3). This has clearly revealed the effect of Menogen on the testicular cells. The normal morphology appeared in the control and all the Menogen treated groups. The normal testicular morphology of Plate 1, 2, 3 and 4 were seen in the hyperplasia of germinal epithelium, Sertoli cells proliferated with different stages of sperm cell formation, and an accumulation of spermatozoa at the lumen of the seminiferous tubules. However, plate 3 representing 15.0 IU of Menogen treated group exhibited more hyperplasia of testicular cells than other experimental groups herein which conforms to the significant (P < 0.05) differences observed in the sperm concentration and total sperm per ejaculate observed in 15.0 IU. Menogen treated group the result of semen analysis (Table 1).

It is known that Sertoli cells have the role to keep and to protect the sperm cells during its development and to nurture the spermatocytes, spermatids, and the spermatozoa. Accordingly, testicular degeneration can lead to Sertoli cell atrophy, thus, affecting the role of Sertoli cells in spermatogenesis (Brackett, 2004). However, the result of histology placed side by side with the observed ejaculate trend in Table 1 implied that a high dose rate of menotrophin could produce suppressive effect on the hypothalamus. Therefore, a negative feedback action could have been established at a higher dose (22.5 I.U.) of Menogen that may have led to a decrease in testosterone levels, which in turn reduced the process of spermatogenesis. This suggests that the qualitative traits of ejaculated sperm in Menogen induced rabbits is dose dependent.

**CONCLUSION**

Results revealed that administration of 15.0 I.U. of Menogen significantly increased sperm percentage, normal sperm cells, and sperm motility. This suggested that human menopausal gonadotropin (Menogen$^3$) may be promising in enhancing sperm healthy parameters.

**REFERENCE**

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

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

\(^a,b\)Means bearing different letters of superscript within the same row differ significantly (P < 0.05)


Donadeu, M. 2006. All you ever wanted to know about Boar semen. Plc Western Europe.


