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EFFECT OF HUMAN MENOPAUSAL GONADOTROPHIN ON TESTICULAR MORPHOMETRY, GONADAL AND EXTRAGONADAL SPERM RESERVES OF RABBIT BUCKS

ANSA A. A.* AND IMASUEN J. A.†

*Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike. Abia State, Nigeria.
†Department of Animal Science, University of Benin, Benin City. Nigeria.

Abstract: Testicular morphometry, gonadal and extragonadal sperm reserves of rabbit bucks treated with Human Menopausal Gonadotrophin (Menogon®) were studied. Twenty-four crossbred rabbit bucks weighing 1.3-1.6 kg at 15-17 wk were randomised into 4 treatment doses of 0 (control), 7.5, 15.0, and 22.5 I.U. in a completely randomised design with 6 bucks per group. A vial of Menogon® was reconstituted in 1 mL of physiological saline solution and administered intramuscularly at 72 h intervals for 56 d prior to commencement of sample collection. Parameters evaluated were ejaculate concentration, testicular morphometry, gonadal and extragonadal sperm concentrations. Results for ejaculate concentration (86, 110, 186, and 135×10^6/mm^3) revealed a significantly (P<0.05) higher difference in the 15.0 I.U. group. Gonadal and extragonadal sperm reserve indices were not significantly (P>0.05) affected by the treatment. Testicular morphometry results showed that paired testis volume (cm^3), testis circumference (cm), ductus deferens length (cm) and epididymal length (cm) were significantly (P<0.05) different, with a progressive increase that corresponds to increased Menogon® administration. High and positive correlations (P<0.01) were observed between testis weight and gonadal sperm reserves (r=0.99) and body weight and gonadal sperm reserves (r=0.99). Similarly, correlations between epididymal weight and epididymal sperm reserves (r=0.85), testis weight and ejaculate concentration (r=0.97), body weight and ejaculate concentration (r=0.96), body weight and testis weight (r=0.97), testis volume and ejaculate concentration (r=0.97), testis volume and gonadal sperm reserve (r=0.91), testis volume and testis weight (r=0.96), and testis volume and body weight (r=0.90) were positive and significant (P<0.05). Taking all the reported results into consideration, controlling the amount of Menogon® administration at 15.0 I.U. for rabbit bucks could be a promising approach to regulating testosterone synthesis and secretion, thereby affording a potential method of enhancing fertility.

Key Words: rabbit bucks, Menogon®, testicular morphometry, gonadal and extragonadal sperm reserves.

INTRODUCTION

Controlling the amount and pattern of gonadotrophin stimulation to the testis influences testosterone synthesis and secretion, thereby providing a potential method of controlling fertility in the male of the species. Following this, 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 gonadotrophins and/or androgen levels. Often, Human Menopausal Gonadotrophin (HMG) is used. HMG is a purified preparation of gonadotrophins, usually luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are extracted from the urine of post-menopausal women (Van De Weijer *et al.*, 2003). HMG medications include Menopur, Menogon, Repronex, Pergonal and HMG Massone (Fuller and Sajatovic, 2003). For the purpose of this research, Menogon® is adopted and each ampoule contains menotropin corresponding to 75 I.U. FSH and 75 I.U. LH as well as lactose and sodium hydroxide for pH-adjustment as other ingredients.

Correspondence: A. Ansa, anniansa@gmail.com. Received *February 2015* - Accepted *April 2015.*
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Menogon® is an active substance for the treatment of fertility disturbances achieved by triggering FSH and LH production in the body (Liu *et al.*, 2008). HMG therapy is now taken for granted and abused as an essential component in routine management for enhancing reproductive capability. Therefore, a great deal of research is necessary in order to develop a safety margin that is effective for its clinical use in rabbit bucks. To achieve this, as semen characteristics are not the only criteria on which to base an evaluation of the reproductive capacity of the male (Osman and El Azab, 1974; Gage and Freckleton, 2003), morphometric, gonadal and extragonadal analysis is here considered crucial in the assessment and prediction of not only sperm production but also of the storage potential and fertilising ability of rabbit bucks.

MATERIALS AND METHODS

Study location

The experiment was carried out at the Rabbitry 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.

Experimental materials and management

A total of 24 crossbred rabbit bucks aged 15-17 wk weighing 1.3-1.6 kg were used in this study. The rabbits were managed intensively. They were quarantined for 3 wk, during which they were treated with Ivomec® injection for the control of haemoparasites and internal and external parasites. The bucks were individually kept in cages (50×55×40 cm) of a 3 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 2500 kcal/kg^{-1} metabolisable 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 09:00 and 11:00 h 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 a Pharmacy. Packs of Menogon® used in the study were stored in the refrigerator (below 25°C) and protected from light.

Experimental design

Twenty-four rabbit bucks were randomly assigned to four treatment groups. Each treatment group constituted six bucks in a completely randomised design.

Menogon® administration protocol

A vial containing Menogon® was reconstituted in 1 mL of physiological saline solution and injected intramuscularly. Different doses of Menogon® were administered after every 72 h for 56 d prior to starting sample collection. The treatment groups were as follows, 0, 7.5, 15.0 and 22.5 I.U. (equivalent to 0, 0.1, 0.2 and 0.3 mL of Menogon®, respectively).

Data collection and evaluation

Semen

Two weeks prior to semen collection, the rabbit bucks were trained to serve an Artificial Vagina (AV) using a teaser rabbit doe. 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 d after administration of the Menogon® injection, the 24 bucks used in this study were placed on a semen collection schedule of twice per week. One ejaculate was collected from each rabbit buck once between 08:00 to 13:00 h (local time) on Mondays and Thursdays for 5 consecutive weeks. The 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.

Sperm cell concentration ($\times 10^6/mm^3$) was determined in the laboratory using a haemocytometer after a dilution of 1 in 200 in a solution of 45 mL normal saline and 5 mL formalin.

Testicular morphometry

At the end of the semen collection period, 2 wk was allowed for the rabbit bucks to rebuild their sperm reserves. Thereafter, 3 bucks from each treatment group were randomly selected from the experimental bucks for morphometric analysis and estimation of the gonadal and extragonadal sperm reserves. The animals were sacrificed and the reproductive organs harvested. Testes, epididymides and vas deferens were carefully separated and freed of tunica albuginea and all adhering connective tissues. The length and width of each testis were measured, using a vernier calliper. Testis circumference, length of epididymides and vas deferens were measured with a flexible metal tape. The testes and epididymal weight were measured on an electronic scale. Testis volume was measured volumetrically using the Archimedes principle of water displacement in a measuring cylinder. The testes density was calculated as: Testes density=Testes weight (g)/Testes volume (cc).

Gonadal and extragonadal sperm reserves

Known weight of the left and right testes were homogenised separately in physiological saline at 200 mg/mL according to Igboeli and Rakha (1971) and Rekwot *et al.* (1994). The homogenate was stored overnight at 4˚C to allow sperm cells to ooze out of the tissues. The suspensions were mixed and strained through a double layer of cheesecloth into graduated tubes and dilution of 1:25 v/v was made for enumeration in a Neubauer haemocytometer (Egbunike *et al.,* 2007). For extragonadal sperm reserves, the epididymides were divided into 3 portions (caput, corpus and cauda) and labelled for proper identification. The segmented portions of the epididymides and vas deferens were separately and completely macerated in normal saline at 100 mg/mL and diluted at 1:25 v/v for counting (Egbunike *et al.,* 2007). All sperm reserves were expressed in millions.

Statistical analysis

The data generated were analysed using analysis of variance. Significant means were separated using Duncan multiple range test. All statistical analyses were in accordance with Steel and Torrie (1980).

RESULTS AND DISCUSSION

The mean testicular morphometric parameters of rabbit bucks are summarised in Table 1. Paired testes volume, testis circumference and ductus deferens length of rabbit bucks in 22.5 I.U. group were significantly ($P<0.05$) higher than those in the control group, but similar to the 7.5 and 15.0 I.U. treatment groups. Testicular circumference revealed significant ($P<0.05$) differences in 15.0 and 22.5 I.U. group compared to the control. Epididymal length of 15.0 and 22.5 I.U. bucks were significantly ($P<0.05$) higher than those of 7.5 I.U. treated bucks, which in turn significantly ($P<0.05$) differed from the control group. Although data obtained on testis weight, testis length, testis width, epididymal weight and testis density did not indicate statistically significant ($P>0.05$) differences among the treatment means, it can be noted that except for epididymal weight which favoured bucks in 15.0 I.U. group, means of bucks in 22.5 I.U. group appeared numerically superior. The mean testis weight recorded in this study was higher than 3.1 g reported by Franca *et al.* (2002) but lower than 6.7 g reported by Herbert *et al.*, (2005). However, other measured testicular parameters were consistent with the report of Oyeyemi and Okediran (2007) but higher than the mean values reported by Oguike and Archibong (2011). These differences in testicular parameters could be due to breed differences, or variation in age of bucks (Oyeyemi and Okediran, 2007).

Following the trend of this result for testicular morphometry and placing it side by side with the result of gonadal and extragonadal sperm reserves (Table 2), it would be agreed with Paufler *et al.* (1969) and Oyeyemi *et al.* (2002) that the higher the testicular value (without any abnormality), the higher the capacity of cells during spermatogenesis. Skinner

Table 1: Mean values of testicular morphometry of rabbit bucks treated with different dosis of Menogon®.

	0.0 I.U	7.5 I.U	15.0 I.U	22.5 I.U	SEM
Paired testis weight (g)	3.50	3.50	4.00	5.00	0.61
Paired testis volume (cm^3)	3.10^a	3.35^{ab}	3.40^{ab}	4.00^b	0.22
Paired testis circum. (cm)	5.90^a	6.55^{ab}	7.45^b	7.25^b	0.23
Paired testis length (cm)	5.75	5.25	5.65	5.75	0.42
Paired testis width (cm)	1.81	2.35	2.25	2.45	0.19
Paired epididymal weight (g)	1.64	1.89	2.01	1.85	0.10
Paired epididymal length (cm)	17.90^a	21.60^b	27.05^c	27.30^c	0.80
Paired ductus deferens length (cm)	15.70^a	16.55^{ab}	17.60^{ab}	19.80^b	0.84
Paired testis density (g/cc)	1.13	1.05	1.19	1.24	0.13

SEM: standard error of the mean.
a,b Means bearing different letters of superscript within the same row differ significantly (P<0.05).

(1975) in his findings also postulated that increase in testicular parameters is followed by a corresponding increase in the sperm production of related animal. Table 3, which presents the correlation between testicular morphometry, ejaculate concentration and sperm reserves, further strengthens this assertion.

Table 2 shows the results of ejaculate concentration, gonadal and extragonadal sperm reserves and relative epididymal sperm distribution. The values of the analysed ejaculate concentration were significant and 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 (22.5 I.U) of Menogon® administration posed a counterproductive effect on sperm production. This may have been as a result of the already saturated Leydig cell receptors by exogenous LH (from Menogon®) which was probably sufficient for the production of testosterone for the development and maintenance of spermatogenesis at 15.0 I.U. dose. Observations made on semen concentration in this study are consistent with the range of 50 to $350\times10^6/mm^3$ reported by Brackett (2004) and also similar to what was obtained by Hafez (1970) 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 gonadotrophin 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 DNA synthesis of the sperm cells. Furthermore, gonadotrophin 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. The mean values of gonadal and extragonadal sperm reserves were not significantly (P>0.05) different among the treatments. There were no discrepancies between the results of sperm reserves obtained in this study and the mean value of $4.01\pm0.25\times10^9/mm^3$, $1.25\pm0.10\times10^8/mm^3$, $0.58\pm0.07\times10^8/mm^3$,

Table 2: Mean values of ejaculate concentration, gonadal and extragonadal sperm reserves of rabbit bucks treated with different dosis of Menogon®.

	0.0 I.U	7.5 I.U	15.0 I.U	22.5 I.U	SEM
Ejaculate Conc. ($\times10^6/mm^3$)	86.0^a	110.0^{ab}	186.0^b	135.0^{ab}	22.6
GSR ($\times10^9$)	3.72	3.65	3.99	4.36	0.28
Caput ($\times10^8$)	1.20	1.45	1.32	1.26	0.26
Corpus ($\times10^8$)	0.50	0.56	0.79	0.62	0.08
Caudal ($\times10^9$)	6.34	6.21	6.44	7.02	0.67
TESR ($\times10^9$)	8.04	8.22	9.13	8.32	0.71
DDSR ($\times10^8$)	0.42	0.45	0.47	0.57	0.09

Relative epididymal sperm distribution (%): Caput: 15.55; Corpus: 7.27; Caudal: 77.18. SEM: standard error of the mean.
GSR: gonadal sperm reserve; TESR: total epididymal sperm reserve; DDSR: ductus deferens sperm reserve.
a,b Means bearing different letters of superscript within the same row differ significantly (P<0.05).

Table 3: Correlation coefficients of testicular morphometry, ejaculate concentration, gonadal and extragonadal sperm reserve.

	EC	GSR	ESR	TW	TC	EW	BW	TV
EC	1							
GSR	0.95*	1						
ESR	0.18	0.14	1					
TW	0.97*	0.99**	0.03	1				
TC	0.81	0.72	0.72	0.68	1			
EW	0.47	0.29	0.85*	0.26	0.87	1		
BW	0.96*	0.99**	0.27	0.97*	0.82	0.42	1	
TV	0.98*	0.91*	-0.01	0.96*	0.69	0.35	0.90*	1

*Correlation is significant at $P<0.05$ (1-tailed).

** Correlation is significant at $P<0.01$ (1-tailed).

TW: testis weight; TC: testis circumference; EC: ejaculate concentration; GSR: gonadal sperm reserve; BW: body weight, EW-epididymal weight; ESR: epididymal sperm reserve; TV: testis volume.

$6.94\pm0.72\times10^9/mm^3$, and $0.55\pm0.15\times10^8/mm^3$ for gonadal, caput, corpus, caudal and ductus deferens sperm reserves, respectively, reported by Orgebin-Crist (1968). The sperm reserve of the caput epididymis represented 15.55% of the total reserve of the organ while the corpus and caudal accounted for 7.27 and 77.18%, respectively. Although the works of Orgebin-Crist (1968) and Kirton *et al.* (1967) focused on the determination of sperm reserves contained only in the caudal epididymides, and independently documented 73 and 79% distribution respectively, the result obtained in this study for caudal epididymis distribution (77.18%) compares with earlier studies by these two authors. Therefore, following the reports of other researchers and as further affirmed by the results of this study, the cauda epididymis contains most epididymal sperm reserves and hence is the major site of sperm storage (Kwari and Waziri, 2001), followed by the caput and corpus epididymis.

Table 3 shows the correlation between ejaculate concentrations, testicular morphometry, gonadal and extragonadal sperm reserves. High correlations were found between testis weight and gonadal sperm reserves ($r=0.99$, $P<0.01$); epididymal weight and epididymal sperm reserves ($r=0.85$, $P<0.05$). These high and positive correlations observed are suggestive of high spermatozoa reserves per unit mass of the testis and epididymis. A similar observation was made by Orgebin-Crist (1968), where he reported that epididymal reserves are significantly correlated with epididymal weight and to a lesser extent with the body weight. Significant correlations were also observed between testis weight and ejaculate concentration ($r=0.97$, $P<0.05$); body weight and ejaculate concentration ($r=0.96$, $P<0.05$); body weight and gonadal sperm reserve ($r=0.99$, $P<0.01$); and body weight and testis weight ($r=0.97$, $P<0.05$). From these results, it could be deduced that since testis weight is significantly ($P<0.05$) related to body weight and also a significant ($P<0.05$) and positive correlations exist between testis weight, gonadal sperm reserves and ejaculate concentration as presented in Table 3, the possibility abounds that daily sperm production and gonadal sperm reserves could be accurately estimated from body weight or testis weight. This deduction is supported by Adeyemo *et al.* (2007) and Gbore and Egbunike (2008), who observed high correlations between testis weight, body weight, sperm production and testis sperm reserve potential. In line with this, Orlu and Egbunike (2010) also propounded that because testis weight is highly significantly ($P<0.01$) related to gonadal and extragonadal sperm reserves, daily sperm production, gonadal and extragonadal sperm reserves could be calculated from testicular weight. On the contrary, Orgebin-Crist (1968), Weisgold and Almquist (1979), and Wildeus and Entwistle (1982) reported no correlation between gonadal sperm reserves and body weight in rabbits. Other correlated parameters in this study were between testis volume and ejaculate concentration ($r=0.97$, $P<0.05$); testis volume and gonadal sperm reserve ($r=0.91$, $P<0.05$); testis volume and testis weight ($r=0.96$, $P<0.05$) and testis volume and body weight ($r=0.90$, $P<0.05$).

CONCLUSION

Given the results of this study, it would be illogical to expect a superior outcome at higher concentrations in animals with hormones already present at a normal level except in hormonal deficient cases. Hence, controlling the amount of exogenous gonadotrophin administration at 15.0 I.U. for rabbit bucks could be a promising approach to regulating testosterone synthesis and secretion, thereby affording a potential method of enhancing fertility.

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