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RESEARCH ARTICLE

Effect of Gonadotrophin (Pergonal®) on Sperm Output Rate, Gonadal and Extragonadal Sperm Reserves of Mature Ouda Rams

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ABSTRACT

Two groups of 4 healthy Ouda rams aged 2.0-2.60 years, weghing between 40.21kg and 40.32kg were assigned to either 49.50 i.u (T_2) or 99.00i.u (T_3) Pergonal injections (Ferring Labs. USA) each divided into 3 doses for 3 consecutive days. Another group of 4 rams was given normal saline (1ml) during the same period to serve as control (T_1). All treatments were given to study the effect of the drug on daily sperm output, gonadal and extragonadal sperm reserves. All the treatments were given by intramuscular injection. The results showed significant differences (P<0-.05) among the treatment groups in daily sperm output ($x10^9$), daily sperm output per gram testis ($x10^9$), gonadal sperm reserve ($x10^9$), caput, corpus, cauda and vas deferens sperm reserves ($x10^8$). High correlations were observed between testis weight, caput weights, and daily sperm output, gonadal sperm reserve, daily sperm output per gram testis; between caput weight, corpus weight, cauda weight and cauda sperm reserve. The results of this study indicate that the daily sperm output, daily sperm output per gram testis, gonadal, caput, corpus, cauda and vas deferens sperm reserves may be affected when 49.50i.u or more of Pergonal® are used for induction of spermatogenesis in Ouda rams.

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INTRODUCTION

The Ouda sheep is one of the hairy breeds of the Sahel type. It originated in western Asia, and entered Africa through the Isthmus of Suez and Babel Mandeb (Epstain, 1971). It is found in northern Nigeria, Southern Niger, central Chad, Northern Cameroon and Western Sudan. Ouda sheep is a meat breed, long-legged with distinctive coat colour of brown or black anterior and white posterior. They are large with straight and long face. The rams of Ouda are horned while the ewes are usually pooled. The Ouda is slightly smaller bodied than the Balami although their size ranges overlap. The weight of mature females could be 30 to 40kg while mature rams weigh 30 to 60kg (Oni, 2002). Several aspects of the physiology of reproduction of rams have been documented (Iheukwumere *et al*., 2001; Osinowo, 1990; Ahemen and Bitto, 2007). Measurable criteria such as scrotal circumference, sperm production rate, gonadal and extragonadal seperm reserves have been extensively studied in some Nigerian breeds (Osinowo *et al*., 1992; Kwari and Waziri, 2001; Ahemen and Bitto, 2007). Few of such reports are however available on Ouda rams, the breed that is abundant in Nigeria and resistant to some local diseases (Charray *et al.,* 1992). It has been observed that the reproductive capacity of Ouda rams is low (Osinowo, 2006) when compared with the exotic breeds of rams. There is cause to stimulate sperm production using inexpensive preparations with an aim to ensuring high conception rates in both naturally and artificially in seminated ewes.

Human gonadotrophin (Pergonal®), a fertility drug of Ferring labs. USA also known as Humagon or Mentrophin and with similar constituents as Plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of gonadotrophins consisting of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH present in Pergonal play vital role in the initiation of spermatogenesis (Abu *et al.,* 2006). There is paucity of information on the use of Pergonal in the induction of spermatogenesis in Ouda rams. This study was therefore designed to determine the effect of this fertility drug on sperm output rate, gonadal and extragonadal sperm reserves of Ouda rams. The information is essential in the determination of

male/female ratio during natural mating and artificial insemination programmes (Ahemen and Bitto, 2007) and also in evaluating male reproductive efficiency of a breed.

MATERIALS AND METHODS

Experimental Animals and Their Management

Twelve healthy sexually matured Ouda rams aged 2-2.6 years were used for this study. The animals were purchased from the local markets and housed in clean pens constructed in such a way that the rams could come outside during the day for access to sunlight and forage. The animals were dewormed and routine inspection for cleanliness was carried out. Freshly cut forage consisting of *Panicum maximum, Aspilia africana, Pennisetum purpureum* (Elephant grass) was fed as basal diet and a concentrate ration of Grower Mash was used as supplement. The animals were fed twice daily, in the morning and evening, salt lick was provided as mineral supplement. Water was given *ad libitum* the animals.

Experimental Design and Drug Administration

The twelve rams were divided into 3 treatment groups consisting of 6 rams per group. These groups were assigned to 3 levels of Pergonal as treatments. The levels of Pergonal were 0, 49.50 I.U, 99.00 I.U Pergonal® represented as T_1, T_2 and T_3 respectively. T_1 which contained no Pergonal served as the control. The rams were treated by intramuscular injection. The injections were as follows:

Pergonal was supplied in 5 vials, each vial containing FSH 75 I.U and LH 75 I.U. The content of the first vial was dissolved in 1ml of physiological saline solution immediately prior to use, resulting in a solution of PFSH 75 I.U plus PLH 75 I.U per ml. All treatments were administered intramuscularly on the leg (thigh) of each ram using a one ml syringe with 0.01ml graduation. The injections were given as follows:

Group T_1: Each ram received 1.00ml physiological saline for 3 days.

Group T_2: Each ram received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.1ml) on the first day. Second day, the group received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.11ml), while on the 3rd day, the group received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.11ml) giving a total of 49.50 I.U of PFSH and PLH (0.33ml) Pergonal® injections within 3 days.

Group T_3: Each ram received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22ml) on the first day. Second day, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22ml), while on the 3rd day the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22ml) giving a total of 99.00 I.U of PFSH and PLH (0.66ml) Pergonal® injections within 3 days.

Sperm collection and evaluation

Sixty five 65 days after Pergonal injection 6 rams in each group were castrated and gonadal and extragonadal sperm reserves were estimated following the homogenized count using a haemocytometer and a microscope (Bitto and Egbunike, 2006). The testes and the three parts of the epididymis (caput, corpus and cauda) were weighed. Before the weighing, the connective tissue that adhered to each part was separated. One gram of testicular parenchyma of each testis was sectioned and homogenized in 100ml formal buffer saline. One gram of caput, corpus and cauda epididymis were also minced separately in 100ml of formal buffer saline with a scapel blade for 5 minutes. The spermatozoa in the testicular and epididymal homogenates were then aspirated with a pipette for evaluation.

The number of spermatozoa and spermatids in the testicular and epididymal samples were determined using an improved Neubaer chamber. Two counts per sample were performed, and the mean used in the analysis to obtain the sperm reserves.

Daily sperm output (DSO) was estimated for testicular homogenates by dividing the gonadal sperm reserves by a time divisor of 3.66 corresponding to the time in days of the duration of the seminiferous epithelium cycle (Bitto and Egbunike, 2006). Daily sperm output per gram testis (DSOG) was determined by dividing the DSO by the weight of testicular parenchyma (Bitto and Egbunike, 2006).

Testicular Measurement

Scrotal circumference (SC) was measured using a measuring tape at the broadest part of the scrotum. Testicular, epididymal and vas deferens weights were measured using a sensitive weighing balance.

Data analysis

Data collected on testicular measurements and sperm reserves were subjected to one-way analysis of variance (ANOVA) using the technique of Steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (1990).

RESULTS AND DISCUSSION

The results of gonadotrophin (Pergonal®) administration on scrotal circumference, paired testes, paired epididymal, epididymal segments and vas deferens weighs are shown in Table 1. Mean values of scrotal circumference, paired testes and paired epididymal weights, epididymal segments; caput, corpus and cauda, and vas deferens weights obtained in this study are higher than the values reported for other Nigerian breeds of similar ages by Ahemen and Bitto (2007) in West African dwarf rams; Iheukwumere *et al.* (2008) in Yankasa rams. This could be attributed to the size, and nutritional status of the Ouda rams. The Ouda rams had been described as one of the largest breeds of sheep indigenous to Nigeria (Osinowo, 1990).

The results of gonadotrophin (Pergonal®) administration on sperm output rate, gonadal and extragonadal sperm reserves of Ouda rams are shown in Table 2. There were significant difference ($P<0.05$) among the treatment groups in daily sperm output. Rams on T_3 recorded the highest value in daily sperm output 3.46 ($x10^9$) and this differed significantly ($P<0.05$) from rams on T_1 (2.09 $x10^9$) and T_2 ($2.37x10^9$) which were also significantly different ($P<0.05$) from each other. The lowest value in daily sperm output was observed in rams on the control treatment (T_1). The values for daily sperm output obtained in this study were lower than the highest

value 5.16±0.12 ($x10^9$) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams. This could be attributed to environment, breed differences and nutritional status of these rams.

There were significant differences (P<0.05) among the treatment groups in daily sperm production per gram testis. Rams on T_3 recorded the highest value in daily sperm output/gram/testis 0.26 ($x10^9$) and this differed significantly (P<0.05) from rams on T_1 and T_2 which were similar (P>0.05) to each other in daily sperm output/gram/testis values. The lowest value was observed in rams on T_1 ($0.12x10^9$). the daily sperm output/gram/testis values obtained in this study were much lower than the highest value of 0.98± 0.05 ($x10^9$) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams of similar ages.

There were significant differences (P<0.05) among the treatment groups in gonadal sperm reserve, caput, corpus, cauda and vas deferens sperm reserves.

Rams on T_3 recorded the highest value in gonadal sperm reserve 19.25 ($x10^9$) and this differed significantly (P<0.05) from rams on T_1 (14.20 $x10^9$) and T_2 (15.13 $x10^9$) which were also significantly different (P<0.05) from each other in gonadal sperm reserve. The lowest value was observed in rams on T_1. The highest gonadal sperm reserve value obtained in this study 19.25 ($x10^9$) was higher than the range 12.15±1.50 to 17.45±1.64 ($x10^9$) reported by Iheukwumere *et al* .(2008) in Nigerian Yankasa rams of similar ages. This could be attributed to genotype, testicular size and technique of estimation (Ahemen and Bitto, 2007) and drug administration (Herbert *et al.,* 2002).

Rams on T_3 recorded the highest value in caput sperm reserve 16.12 ($x10^8$) and this differed significantly (P<0.05) from rams on T_1 ($6.10x10^8$) and T_2 ($14.26x10^8$) which were also significantly different (P<0.05) from each other in caput sperm reserve. The lowest value in caput sperm reserve was observed in rams on T_1. The caput sperm reserve values obtained in this study were higher than the highest value of 4.10±0.06 ($x10^8$) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams of similar ages.

Rams on T_3 recorded the highest value in corpus sperm reserve 19.33 ($x10^8$) and this differed significantly (P<0.05) from rams on T_1 which had 13.30 ($x10^8$). However, there was no significant difference (P>0.05) between rams on T_3 and T_2 in corpus sperm reserve. The lowest value in corpus sperm reserve was observed in rams on T_1 (13.30 $x10^8$). The corpus sperm reserve values obtained in this study were much higher than the highest corpus sperm reserve value of 5.48 ±0.63 ($x10^8$) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams.

Rams on T_3 recorded the highest value of 50.25 ($x10^8$) in cauda sperm reserve and this differed significantly (P<0.05) from rams on T_1 ($38.26x10^8$) and T_2 ($42.50x10^8$) which were also significantly different (P<0.05) from each other in cauda sperm reserve. The lowest value in cauda sperm reserve was observed in rams on T_1. The cauda sperm reserve values obtained in this study were much higher than the value 6.25±0.54 ($x10^8$) reported by Iheukwumere *et al.* (2008) in Nigeria Yankasa rams of similar ages.

Table 1: Scrotal Circumference, Testicular, Epidiymal and Vas Deferens Weights in Mature Ouda Rams

Parameters	Mean	SEM
Scrotal circumference (cm)	27.31	0.50
Paired testes weight (g)	158.30	1.18
Paired epididymal weight (g)	35.20	2.25
Weight of epididymal segments		
Caput (g)	20.14	0.31
Corpus (g)	5.43	0.22
Cauda (g)	9.00	0.27
Vas deferens (g)	3.05	0.14

Rams on T_3 recorded the highest value in vas deferens sperm reserve 13.50 ($x10^8$) and this differed significantly (P<0.05) from rams on T_1. However, there was no significant difference (P>0.05) between rams on T_3 and T_2 in vas deferens sperm reserve. The lowest value in vas deferens sperm reserve was observed in rams on T_1 (9.23 $x10^8$). The vas deferens sperm reserve values obtained in this study were much higher than the range of 0.45±0.02 – 0.65±0.04 ($x10^8$) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams of similar ages. This could be attributed to high capacity for induction of spermatogenesis by Pergonal$^{(R)}$ injection.

The sperm reserve of the caput epididymis represented 16.55% of the total sperm reserve of the organ, while the corpus and cauda accounted for 23.22% and 60.23% respectively. The distribution of epididymal sperm reserves obtained in this study is similar to what has been reported for Balami rams (Kwari and Waziri, 2001), WAD rams (Osinowo, 2006; Ahemen and Bitto, 2007) and Yankasa rams (Iheukwumere *et al.,* 2008). It is generally agreed that the cauda epididymis contains most of the epididymal sperm reserves and hence, it is the major site for sperm storage (Kwari and Waziri, 2001).

In this study, it was observed that Pergonal induced spermatogenesis in the treated groups. It is common knowledge that LH as interstitial cell stimulating hormone (ICSH) stimulates the interstitial cell of leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert *et al*., 2002). However, in a similar study, Herbert *et al.* (2002) had indicated differences in the serum testosterone levels that showed slightly higher values for the Clomid® treated group than the control group but were not significantly different (P>0.05). This implies that it may not be through increased production of testosterone under the influence of ICSH alone that may be responsible for improve sperm production rates in treated animals (Herbert *et al.,* 2002). Herbert *et al.* (2002) also reported that FSH mediates in the maturation of sperm cells prior to ejaculation. It has also been reported that exogenous administration of testosterone itself leads to a suppressive effect on the hypothalamus thus reducing the sperm production process (Adamopoulous *et al*., 1990; Jayakumar *et al.,* 1997).

Table 3, shows correlation (r) between testicular morphometry, sperm output rate, gonadal and extragonadal sperm reserves. High correlations were observed between testicular weight and daily sperm output per gram testis (r = 0.78, P<0.01); testicular weight and daily sperm output (r = 0.99, P<0.01); testicular weight and caput sperm reserve (r = 0.95, P<0.01); testicular weight and gonadal sperm reserve(r = 0.99, P<0.01 caput weight and cauda sperm reserve (r = 0.89,

Table 2: Sperm Output Rate, Gonadal and Extragonal Sperm Reserves of Mature Ouda Rams Treated with Gonadotrophin (Pergonal®)

Variables	Treatment (Pergonal®)			SEM
	T_1 0.00 i.u	T_2 49.50 i.u	T_3 99.00 i.u	
Daily sperm output ($x10^9$)	2.09^c	2.37^b	3.46^a	0.21
Daily sperm output/ Gram/testis ($x10^9$)	0.12^c	0.15^b	0.26^a	0.02
Gonadal sperm reserve ($x10^9$)	14.20^c	15.13^b	19.25^a	0.78
Caput sperm reserve ($x10^8$)	6.10^c	14.26^b	16.12^a	1.55
Corpus sperm reserve ($x10^8$)	13.30^b	18.39^a	19.33^a	0.95
Cauda sperm reserve ($x10^8$)	38.26^c	42.50^b	50.25^a	1.78
Vas deferens sperm reserve ($x10^8$)	9.23^b	13.15^a	13.50^a	1.74
Relative epididymal sperm distribution				
Caput	16.55			
Corpus	23.22			
Cauda	60.23			

$^{abc:}$ Means in the same row with different superscript are significantly (P<0.05) different. SEM = Standard error of mean.

Table 3: Correlation (r) between Testicular Morphometry, Sperm Output Rrate, Gonadal and Extragonadal Sperm Reserves in Mature Ouda Rams

	Daily sperm/ output per gram testis	Daily sperm output rate	Cauda sperm reserve	Corpus sperm reserve	Caput sperm reserve	Gonadal sperm reserve	Cauda weight	Corpus weight	Testis weight
Testis weight	0.78**	0.99**	0.36^{ns}	0.35^{ns}	0.95^{**}	0.99^{**}	0.47^{ns}	0.86^{**}	0.35^{ns}
Caput weight	0.87^{**}	0.80^{**}	0.89^{**}	0.66*	0.69^{x}	0.82^{**}	0.28^{ns}	0.88^{**}	-
Corpus weight	0.45ns	0.40ns	0.95^{xx}	0.41^{ns}	0.48^{ns}	0.24^{ns}	0.45ns	-	
Cauda weight	0.87**	0.43^{ns}	0.98**	0.25^{ns}	0.23^{ns}	0.46^{ns}	-		
Gonadal sperm reserve	0.58*	0.21^{ns}	0.39^{ns}	0.66*	0.15ns	-			
Caput sperm reserve	0.54^{ns}	0.19^{ns}	0.35^{ns}	0.57^{*}	-				
Corpus sperm reserve	0.25^{ns}	0.59*	0.30^{ns}	-					
Cauda sperm reserve	0.24^{ns}	0.40^{ns}	-						
Daily sperm out put	0.60*	-							
Daily sperm output per gram testis	-								

** = significant (P<0.05); ** = highly significant (P<0.01); ns = not significant (P>0.05)

P<0.01); caput weight and gonadal sperm reserve (r= 0.82; P<0.01). Cauda weight and daily sperm output per gram testis (r = 0.87, P<<0.01); cauda weight and cauda sperm reserve (r = 0.98, P<0.01). These high and positive correlations observed are suggestive of the relationship between the above mentioned parameters and sperm output rate, gonadal and extragonadal sperm reserves.

Conclusion

The values obtained for gonadal and extragonadal sperm reserves fall within the range reported in the literature (Osinowo, 2006; Ahemen and Bitto, 2007; Kwari and Waziri, 2001; Iheukwumere *et al.,* 2008). From the results of this study, it can be concluded that Pergonal improved spermatogenesis and sperm output in Ouda rams at the level of 99.00i.u without any deleterious effects on gonadal and extragonadal sperm reserves.

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