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Research Article

Effect of Gonadotrophin (Pergonal®) on Body Size, Reproductive Characteristics and Sperm Reserves of Mature Yankasa Rams

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ABSTRACT

Three groups of 6 healthy Yankasa rams aged 2.0-260 years were assigned to either 49.50i.u (T_2), 99.00i.u (T_3) or 148.50i.u (T_4) (Pergonal®) injections (of Ferring LABS, USA), each divided into 3 doses and given for 3 consecutive days. Another group of 6 rams was given normal saline (1.00ml) during the same period to serve as control (T_1). All treatments were given to study the effect of the drug on body confirmation and sperm reserves. All the treatments were given by intramuscular injections. The results showed no significant differences ($P>0.05$) among the treatment groups in body weight. However, there were significant differences ($P<0.05$) among the treatment of group in scrotal circumference, withers height, heart girth, testes, testicular parenchymal, caput, corpus, cauda and vas deferens weights. The results further showed that there were significant differences ($P<0.05$) among the treatment groups in testicular, caput, corpus, cauda and vas deferens sperm reserves. High correlations were observed between body weight, scrotal circumference, withers height, heart girt and caput, corpus, cauda and testicular sperm reserves. The results on this study indicate that apart from body weight, the body conformation, testis, epididymal, and vas deferens weights and sperm reserves of Yankasa rams may be affected when 49.50 iu or more of Pergonal are used for induction of spermatogenesis.

Key words: Yankasa rams, body size, sperm reserve, reproductive characteristics, gonadotrophin (Pergonal®)

INTRODUCTION

Yankasa is the predominant breed of sheep indigenous to the Guinea and Sudan Savannah belt of West Africa (Iheukwumere *et al.,* 2008). According to Iheukwumere *et al.* (2008), the use of Yankasa rams to upgrade the smaller village sheep in the habitat has extended this breed to southern Nigeria. The Nigerian Yankasa rams are typically tall, exceeding a height of 50-70cm at the withers and weigh 30-50kg with an outstanding sexual agility, hence they have been widely used for artificial insemination programs (Osinowo, 1990). Several aspects of the reproductive physiology of rams have been documented (Iheukwumere *et al.,* 2001; Osinowo, 1990 ;Ahemen and Bitto, 2007) measurable criteria such as scrotal dimensions, sperm production rate, gonadal and extragonadal sperm 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 for the Yankasa rams, the breed that is abundant in Nigeria and resistant to some local diseases (Iheukwumere and Okere, 1990). It has been observed that the reproductive capacity of Yankasa 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 mate and artificially inseminate ewes and ensure high conception rates in both naturally mated and artificially inseminated ewes.

Human gonadotrophin (Pergonal®), a fertility drug also known as Humagon or Mentrophin and with similar constituents as Plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of gonado-trophins consisting of follicle stimulating hormone (FSH) and Luteinizing hormone (LH) in a ratio 1:1 (Dixon and Hopkins, 1996). FSH and LH present in Pergonal play vital role in the initiation of spermatogenesis. The hormone preparation is cheap, readily available and does not require cold chain storage (Iheukwumere, 2005). There is paucity of information on the use of Pergonal in the induction of spermatogenesis in Yankasa rams. This

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study was therefore designed to investigate the effect of this fertility drug on sperm output rate, gonadal and extragonadal sperm reserves of Yankasa 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

Management of Animals

Twenty-four healthy sexually mature Nigerian Yankasa rams aged 2.0-2.60 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,africanaPennisetumpurpureum* (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 twenty-four rams were divided into 4 treatment groups consisting of 6 rams per group. These groups were assigned to 4 level of Pergonal as treatments. The levels of Pergonal were 0, 49.50 I.U, 99.00 I.U and 148.50 I.U Pergonal® represented as T_1, T_2, T_3 and T_4 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 13 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.

GroupT_1: Each ram received 1.00ml of physiological saline for 3 days.

Group T_2: Each ram received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.11ml) on the first day; 2nd 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 injection within three 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. 2nd day, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22ml). While on the 3rd, 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 injection within 3 days

Group T_4: Each ram received 24.75 I.U of PFSH and 24,75 I.U of PLH (0.33ml) on the first day. 2nd day, the group received 24.75 I.U of PFSH and 24.75 I.U of PLH (0.33ml). While on the 3rd day the group received 24.75 I.U of PFSH and 24.75 I.U of PLH (0.33ml) giving a total of 148.50 I.U of PFSH and PLH (0.99ml) Pergonal injection within 3 days. All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01ml graduation.

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 epipidymal samples were determined using an improved Neubauer 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 gonadtrophin (Pergonal®) administration on body size, testicular and epididymal measurements of mature Yankasa rams are presented in Table 1. There were no significant differences ($P>0.05$) among the treatment groups in body weight. This suggests that Pergonal treatement is safe for the ram. Rams on T_1 and T_4 recorded the highest body weight of 30.60kg. The lowest body weight of 30.50kg was observed in rams on T_3. The body weight values obtained in this study were within the normal range of 30-50kg reported by Iheukwumere *et al*. (2008) in Yankasa rams.

There were significant differences ($P<0.05$) among the treatment groups in scrotal circumference, height at withers, heart girth, testes and testicular parenchymal weights.

Table 1: Body Size and Testicular Measurements of Matured Yankasa Rams Treated with Gonadotrophin (Pergonal®).

Parameters	Treatment (Pergonal(R) i.u)				SEM
	T_1 0.00	T_2 48.50	T_3 99.00	T_4 148.50	
Body weight (kg)	30.60	30.55	30.50	30.60	0.02
scrotal circumference (cm)	27.50^{a}	26.50^{b}	27.50^{a}	26.50^{b}	0.29
Height at withers (cm)	63.50^{b}	65.00^{bc}	70.00^{a}	69.00^{ac}	1.56
Heart girth (cm)	24.00^{a}	22.50^{b}	23.00^{c}	23.00^{c}	0.31
Testes weight (g)	110.20^{c}	112.40^{b}	115.50^{a}	109.10^{c}	1.41
Weight of testicular parenchyma (g)	90.10^{b}	92.20^{ab}	95.40^{a}	89.00^{b}	1.41
Caput weight (g)	19.40^{a}	13.95^{b}	12.55^{b}	12.40^{a}	1.67
Corpus weight (g)	2.70^{b}	3.70^{b}	3.50^{a}	3.40^{a}	0.22
Cauda weight (g)	8.65^{a}	9.45^{a}	9.00^{a}	5.80^{b}	0.82
Vas deferens weight (g)	1.15^{b}	1.40^{b}	2.50^{a}	2.10^{ab}	0.31

[abc:] Means in the same row with different superscript are significantly (P<0.05) different. SEM = standard error of mean.

Rams on T_1 and T_3, recorded the highest scrotal circumference value of 27.50cm and these differed significantly (P<0.05) from ram on T_2 and T_4 which were similar (P>0.05) to each other in scrotal circumference values. The lowest value of 26.50cm in scrotal circumference was observed in rams on T_2 and T_4. The scrotal circumference values obtained in this study were higher than the mean value of 22.40±0.54cm reported by Iheukwumere *et al.* (2008) in Yankasa rams. This disparity may not be unconnected to the differences in environment and nutritional status of the Yankasa rams.

Rams on T_3 recorded the highest value in height at the withers (70.00cm) 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 height at the withers. Rams on T_2 was similar (P>0.05) to the rams on T_4 in height at the withers. However, there was no significant difference (P>0.05) between the rams on T_3 and T_4 in height at the withers. The lowest value of 63.50cm in height at the withers was observed in rams on T_1. The values of height at the withers obtained in this study were within the normal range 50-70cm reported by Iheukwumere *et al.* (2001) in Yankasa rams.

Rams on T_1 recorded the highest value in heart girth (24.00cm) and this differed significantly (P<0.05) from rams on T_2, T_3 and T_4. Rams on T_3 and T_4 were similar (P>0.05) to each other, but were significantly different (P<0.05) from rams on T_2 in heart girth value. The lowest value in heart girth was observed in rams on T_2 (22.50cm).

Rams on T_3 recorded the highest value of 115.50g in testes weight and this differed significantly (P<0.05) from rams on T_1, T_2 and T_4. The lowest value of 109.10(g) in testes weight was observed in rams on T_4. Rams on T_4 were similar (P>0.05) to rams on T_1 (110.20g), but they differed significantly (P<0.05) from rams on T_2 which had (112.40g). The testes weights values obtained in this study were lower than the value 154.25±2.48 reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams. This disparity may not be unconnected to the nutritional status of the Yankasa rams and drug administration.

Rams on T_3 recorded the highest value of 95.40g in testicular parenchymal weight and this differed significantly (P<0.05) from rams on T_1 and T_4 which were similar (P>0.05) to each other. However, there was no significant difference (P>0.05) between rams on T_3 and T_2 in testicular parenchymal weight. The lowest value of 89.00 (g) in testicular parenchymal weight was observed in rams on T_4.

There were significant differences (P<0.05) among the treatment groups in caput, corpus, cauda and vas deferens weights. Rams on T_1 recorded the highest value in caput weight (19.40g) and this differed significantly (P<0.05) from rams on T_3 and T_2 which were similar (P>0.05) to each other in caput weight. There was no significant difference (P>0.05) between rams on T_1 and T_4 in caput weight. The lowest value of 12.20g in caput weight was observed in rams on T_4. The caput weight values obtained in this study were higher than the mean value of 8.54±0.21g reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams. This could be attributed to the environment and the nutritional status of the Yankasa rams.

Rams on T_2 recorded the highest value of 3.70g in corpus weight and this differed significantly (P<0.05) from rams on T_1. There were no significant differences (P>0.05) among rams on T_2, T_3 and T_4 in corpus weight. The lowest value of 2.70g in corpus weight was observed in rams on T_1. The corpus weight values obtained in this study were lower than the mean value 4.21±0.32g reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams.

Rams on T_2, recorded the highest cauda weight (9.45g) and this differed significantly (P<0.05) from rams on T_4. There were no significant differences (P>0.05) among rams on T_2, T_1 and T_3 in cauda weight. The lowest value of 5.80g in cauda weight was observed in rams on T_4. The highest value of 9.45g in cauda weight obtained in this study was higher than the mean value of 8.00±0.07g reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams.

Rams on T_3 recorded the highest value of 2.50g in vas deferens weight and this differed significantly (P<0.05) from rams on T_1 and T_2 which were similar (P>0.05) to each other and also similar (P>0.05) to the rams on T_4 in vas deferens weight. There was no significant difference (P>0.05) between rams on T_3 and T_4 in vas deferens weight. The lowest value of 1.15g in vas deferens weight was observed in rams on T_1. The highest vas deferens weight (2.50g) obtained in this study was slightly higher than the mean value 2.35±0.16g reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams.

The results of gonadotrophin (Pergonal®) administration on sperm reserves of Yankasa rams are shown in Table 2. There were significant differences

(P<0.05) among the treatment groups in testicular, caput, corpus, cauda and vas deferens sperm reserves. Rams on T_3 recorded the highest testicular sperm reserve of 14.42 (x 10^9) and this differed significantly (P<0.05) from rams on T_1which weresimilar (P>0.05) to rams on T_2 in testicular sperm reserve value. There were no significant differences (P>0.05) among rams on T_3, T_2 and T_4 in testicular sperm reserve values. The lowest value in testicular sperm reserve (2.30 x 10^9) was observed in rams on the control treatment (T_1). The testicular sperm reserve values obtained in this study were within the range of 12.15±1.50 to 17.45±1.64 (x10^9) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams of similar ages. However, the testicular sperm reserve values obtained in this study were lower than the mean value of 18.80±1.0 (x10^9) for testicular sperm reserve reported by Kwari and Waziri (2001) in Balami rams. 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 caput sperm reserve value of 16.40 (x10^8) and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_2 in caput sperm reserve. There were no significant differences (P>0.05) among rams on T_3, T_2 and T_4 in caput sperm reserve. The lowest value in caput sperm reserve was observed in rams on T_1. The highest value of 16.40 (x10^8) in caput sperm reserve obtained in this study was higher than the highest value of 4.10±0.06 (x10^9) reported by Iheukwumere *et al*. (2008) in Nigerian Yankasa rams. Rams on T_3 recorded the highest value of 15.14 (x10^8) in corpus sperm reserve and this differed significantly (P<0.05) from rams on T_1. There were no significant differences (P>0.05) among rams on T_3, T_2 and T_4 in corpus sperm reserve. The lowest value in corpus sperm reserve was observed in rams on T_1 (6.74×10^8). The corpus sperm reserve values obtained in this study were 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 cauda sperm reserve value of 46.44 (x10^8) and this was similar (P>0.05) to rams on T_2 but differed significantly (P<0.05) from rams on T_1 and T_4 which were similar (P>0.05) to each other and similar to rams on T_2 in cauda sperm reserve. The lowest value of 10.64 (x10^8) in cauda sperm reserve was observed in rams on the control treatment (T_1). Cauda sperm reserve values obtained in this study were higher than the highest cauda sperm reserve value of 6.25± 0.54 (x10^8) reported by Iheukwumere *et al.* (2008) in Nigerian Yankasa rams.

Rams on T_3 recorded the highest vas deferens sperm reserve of 12.60 (x10^8) and this differed significantly (P<0.05) from rams on T_1, T_2 and T_4 which were similar (P>0.05) to each other in vas deferens sperm reserve. The lowest value of 9.20 (x10^8) in vas deferens sperm reserve was observed in rams on T_4. 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 injection.

The sperm reserve of the caput epididymis represented 13.92% of the total sperm reserve of the organ, while the corpus and cauda accounted for 17.39% and 68.70% respectively. The distribution of epididymal sperm reserves observed in this study is similar to what has been reported for other breeds (Kwari and Waziri, 2001 in Balami rams; Osinowo, 2006; Ahemen and Bitto, 2007 in WAD rams; Iheukwumere *et al.,* 2008 in Nigerian Yankasa rams). 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).

Table 2: Sperm Reserves of Mature Yankasa Rams Treated with Gonadotrophin (Pergonal®)

Parameters	Treatment (Pergonal® i.u)				
	T_1 0.00	T_2 49.50	T_3 99.00	T_4 148.50	SEM
Testicular					
Sperm reserve (x10^9)	12.30^b	13.13^{ab}	14.42^a	14.10^a	0.47
Caput sperm reserve (x10^8)	2.10^b	9.22^{ab}	16.40^a	11.16^a	2.96
Corpus sperm reserve (x10^8)	6.74^b	12.81^a	15.14^a	13.88^a	1.86
Cauda sperm reserve (x10^8)	10.64^b	22.75^{ab}	46.44^a	12.10^b	8.27
Vas deferens sperm reserve (x10^8)	9.74^b	9.29^b	12.60^a	9.20^b	0.81

Relative epididymal sperm distribution; Caput 13.92; Corpus 17.39; Cauda 68.70

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

Table 3: Correlation (r) between Body Conformation and Sperm Reserves in Mature Yankasa Rams

	Cauda sperm reserve	Corpus sperm reserve	Caput sperm reserve	Testicular sperm reserve	Heart girth	Scrotal circum-ference	Withers height	Body weight
Body weight	0.35^{ns}	0.67*	0.62**	0.45^{ns}	0.41^{ns}	0.50*	0.51*	-
Withers height	0.36^{ns}	0.20^{ns}	0.95**	0.98**	0.12ns	0.03^{ns}	-	
Scrotal circumference	0.58*	0.33^{ns}	0.86**	0.95**	0.09^{ns}	-		
Heart girth	0.28^{ns}	0.25^{ns}	0.88**	0.86**	-			
Testicular sperm reserve	0.37^{ns}	0.62*	0.09^{ns}	-				
Caput sperm reserve	0.28^{ns}	0.53*	-					
Corpus sperm reserve	0.25^{ns}	-						
Cauda sperm reserve	-							

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

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).

Table 3 shows correlation (r) between body conformation and sperm reserves in mature Yankasa rams. High correlations were observed between body weight and corpus sperm reserve ($r = 67$, $P<0.05$), withers height and caput sperm reserve ($r = 0.95$, $P<0.01$); withers height and testicular sperm reserve ($r = 0.98$, $P<0.01$). Scrotal circumference and caput sperm reserve ($r = 0.86$, $P<0.01$); scrotal circumference and testicular sperm reserve ($r = 0.95$, $P<0.01$). Heart girth and caput sperm reserve ($r = 0.88$, $P<0.01$); heart girth and testicular sperm reserve ($r = 0.86$, $P<0.01$).

These high and positive correlations observed are suggestive of the relationship between the above mentioned parameters and testicular and epididymal sperm reserves.

Conclusion

The values obtained for testicular and epididymal measurements and sperm reserves fall within the range reported in literature (Osinowo, 2006; Ahemen and Bitto, 2007). From the results of this study, it can be concluded that Pergonal® improved sperm production and sperm reserves in Yankasa rams at the level of 49.50i.u, without any deleterious effects on testicular and epididymal characteristics of the rams.

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