Effect of Gonadotrophin (Pergonal®) on body size, reproductive characteristics and Sperm reserves of mature Ouda Rams

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ABSTRACT: Two groups of 4 healthy Ouda rams aged 2.0 to 2.6 years, weighing between 38.21 kg and 38.32 kg were assigned to either 49.50i.u (T²) or 99.00i.u (T³) Pergonal injections (Ferring Labs. USA) each divided into 3 doses and given for 3 consecutive days. Another group of 4 rams was given normal saline (1 ml) during the same period to serve as control (T₁). All treatments were given to study the effect of the drug on body conformation and sperm reserves. All the treatments were given by intramuscular injections. The results showed significant differences (P<0.05) among the treatment groups in body conformation, testicular, epididymal and vas deferens weights. The results further showed that there were significant differences (P<0.05) among the treatment groups in testicular and extratesticular sperm reserves. High correlations were observed between body weight and conformation, weight of epididymal segments and testicular sperm reserves. The results of this study showed that apart from body weight, the body conformation, testes, epididymal and vas deferens weights and sperm reserves of Ouda rams would be improved at the level of 99.00i.u Pergonal and this level should be recommended for induction of spermatogenesis in Ouda rams.

Key words: Ouda rams, body size, sperm reserve, reproductive characteristics, Gonadotrophin (Pergonal®).

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 (Oni, 2002). It is commonly found in northern Nigeria, Southern Niger, central Chad, Northern Cameroon and Western Sudan. Ouda sheep is long-legged, a large breed with distinctive pie coat colour of brown or black anterior and white posterior. They are meat breed with straight and long face. Ouda rams are horned while the ewes are polled. The Ouda ram weighs slightly lower than the Balami. The weight of mature females could be 30 to 40 kg while mature rams weigh 30 to 60 kg (Oni, 2002). The reproductive performance of rams has been documented (Iheukumere et al., 2001; Ahemen and Bitto, 2007). There had been several reports on scrotal circumference, sperm production rate, gonadal and extragonadal sperm reserves in animals (Kwari and Waziri, 2001; Ahemen and Bitto, 2007; Iliyasu et al., 2014; Oyeyemi and Ubiogoro, 2005; Oyeyemi and Babalola, 2006; Obidike et al., 2007; Brito et al., 2002; Brito et al., 2006). Few of such reports are available on Ouda rams, the breed that is abundant in Nigeria and resistant to some local diseases (Lebbie, 2004). It has been reported that the reproductive capacity of Ouda rams is low (Osinowo, 2006) compared to the exotic breeds of rams. There is need to boost sperm production using inexpensive preparations with an aim to ensuring high conception rates and improved reproductive performance in both naturally and artificially inseminated ewes.

Human gonadotrophin (Pergonal®) is a fertility drug of
MATERIALS AND METHODS

Management of Animals

Twelve healthy sexually matured Ouda rams aged 2.0 to 2.6 years and weighed between 38.21 to 38.32 kg 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. Two weeks pre-experimental period was allowed to enable the animals acclimatize. The animals were dewormed and routine inspection for cleanliness was carried out. They were weighed every week and their weights recorded.

Experimental Diet

Freshly cut forage consisting of Panicum maximum, Aspilia africana, Pennisetum purpureum (Elephant grass) was fed as basal diet and 4 kilograms of Grower Mash per day for all the rams 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 to the animals.

Experimental Design and Drug Administration

The twelve rams were divided into 3 treatment groups consisting of 4 rams per group. These groups were assigned to 3 levels of Pergonal as treatments. The levels of Pergonal were 0.00i.u, 49.50i.u, 99.00i.u Pergonal® represented as T₁, T₂ and T₃ respectively. T₁ 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 1 ml 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 0.01 ml graduation. The injections were given as follows:

Group T₁: Each ram received 1.00 ml physiological saline for 3 days.

Group T₂: Each ram received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.11 ml) on the first day. Second day, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.11 ml), while on the 3rd day, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.11 ml) giving a total of 49.50 I.U of PFSH and PLH (0.33 ml) Pergonal® injections within 3 days.

Group T₃: Each ram received 33.00 I.U of PFSH and 33.00 I.U of PLH (0.22 ml) on the first day. Second day, the group received 33.00 I.U of PFSH and 33.00 I.U of PLH (0.22 ml), while on the 3rd day, the group received 33.00 I.U of PFSH and 33.00 I.U of PLH (0.22 ml) giving a total of 99.00 I.U of PFSH and PLH (0.66 ml) 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 100 ml formal buffer saline. One gram of caput, corpus and cauda epididymis were also minced separately in 100 ml of formal buffer saline with a scalpel 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 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 Egbanike, 2006). Daily sperm output per gram testis (DSOG) was determined by dividing the DSO by the weight of testicular parenchyma (Bitto and Egbanike, 2006).

Body Size and Testicular Measurement

Scrotal circumference (SC) was measured in cm using a measuring tape at the broadest part of the scrotum. Testicular, epididymal and vas deferens weights were measured in grams using a sensitive weighing balance. Body weight was measured in kg using a hanging scale and withers height was measured in cm using a measuring tape.

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 (2006). Significant treatment means were separated using Duncan’s New Multiple Range Test as described by Obi (2002).

RESULTS AND DISCUSSION

The results of gonadotrophin (Pergonal®) administration on body size, testicular and epididymal measurements of mature Ouda rams are presented in Table 1. There were no significant differences (P>0.05) among the treatment group in body weight. This suggests that Pergonal treatment is safe for the rams. Rams on T3 recorded the highest value in body weight of 40.32 kg. The lowest body weight of 40.21 kg was observed in rams on T2 and T1. The body weight values obtained in this study were within the normal range 30 to 50 kg reported by Oni (2002) in Ouda rams. This suggests that Pergonal was safe for the rams.

There were significant differences (P<0.05) among the treatment groups in scrotal circumference. Rams on T3 recorded the highest value in scrotal circumference 24.00 cm and this differed significantly (P<0.05) from rams on T1 and T2 which were also significantly different (P<0.05) from each other. The highest value in scrotal circumference obtained in this study (24.00 cm) was lower than the mean value of 29.50 cm reported by Muhammed et al. (2016) in Ouda rams, but higher than the mean value of 22.40 ± 0.54 reported by Iheukwumere et al. (2008) in Yankasa rams of similar ages. This disparity may not be unconnected to the differences in environment and nutritional status of the Ouda rams.

There were significant differences (P<0.05) among the treatment groups in withers height. Rams on T3 recorded the highest value in height at the withers 76.00 cm and this differed significantly (P<0.05) from rams on T2 (64.00 cm) and T1 (74.00 cm) which were also significantly different (P<0.05) from each other. The values of height at the withers obtained in this study were lower than the value 83.9 ± 0.21 cm reported by Yakubu and Ibrahim (2011) in Ouda rams, and lower than 84.54 ± 0.24 cm reported by Yakubu and Akinyemi (2010) and 84.00 ± 0.16 cm reported by Yakubu (2012) in Ouda rams, but higher than the range of 50 to 70 cm reported by Iheukwumere et al. (2001) in Yankasa rams of similar ages. This could be attributed to environmental, age, nutritional status of the Ouda rams, genotype and drug administration.

There were significant differences (P<0.05) among the treatment groups in heart girth. Rams on T3 recorded the highest value in heart girth 29.50 cm and this differed significantly (P<0.05) from rams on T1 which had 21.50 cm. However, there was no significant difference

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### Table 1. Body Size and Testicular Measurement of Mature Ouda Rams Treated with Gonadotrophin (Pergonal®)

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</tr>
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<td>T2</td>
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<tr>
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<td>40.21</td>
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<td>22.00&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>64.00&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Heart girth (cm)</td>
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<tr>
<td>Testes weight (g)</td>
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<td>109.88&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Caput weight (g)</td>
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<td>5.65&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Corpus weight (g)</td>
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<td>2.63&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Cauda weight (g)</td>
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<td>8.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Vas deferens Weight (g)</td>
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<td>1.10&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>
(P>0.05) between rams on T$_3$ and T$_2$ in heart girth values. The lowest value in heart girth was observed in rams on T$_1$. The heart girth values obtained in this study were much lower than the mean value of 91.8 ± 9.26 cm in chest circumference reported by Yakubu and Ibrahim (2011) in Ouda rams. This disparity may not be unconnected to the differences in environment, age and nutritional status of the Ouda rams.

There were significant differences (P<0.05) among the treatment groups in testes weight. Rams on T$_3$ recorded the highest value in testes weight 160.54 g and this differed significantly (P<0.05) from rams on T$_2$ 109.88 g and T$_1$ 99.19 g which were also significantly different (P<0.05) from each other in testes weight. The highest value in testes weight obtained in this study was lower than the mean value of 2.431 x 10$^3$ g reported by Muhammed et al. (2016) in Ouda rams, but higher than the mean value of 154.25 ± 2.48 g reported by Iheukwumere et al. (2008) in Yankasa rams of similar ages. This disparity may not be unconnected to environment, age and nutritional status of the Ouda rams. This suggested that Pergonal administration enhanced testicular development in the treated animals.

There were significant differences (P<0.05) among the treatment groups in testicular parenchymal weight. Rams on T$_3$ recorded the highest value in testicular parenchymal weight 143.52 g and this differed significantly (P<0.05) from rams on T$_2$ (94.83 g) and T$_1$ (86.32 g) which were also significantly different (P<0.05) from each other. The lowest value in testicular parenchymal weight was observed in rams on T$_1$ 86.52 g. This suggested that Pergonal administration enhanced testicular growth in the treated animals and hence increased testicular mass for sperm production.

There were significant differences (P<0.05) among the treatment groups in caput weight. Rams on T$_3$ recorded the highest value in caput weight 9.57 g and this differed significantly (P<0.05) from rams on T$_1$ which had 5.14 g. There was no significant difference (P>0.05) between rams on T$_3$ and T$_2$ 5.66 g in caput weight. The lowest value in caput weight was observed in rams on T$_1$ 5.14 g. The highest caput weight obtained in this study was higher than the mean value of 8.54 ± 0.2 g reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams of similar ages. This disparity may not be unconnected to the nutritional status of the Ouda rams and drug administration. This suggested that the Pergonal administration enhanced the development of the head of the epididymis in the treated animals.

There were significant differences (P<0.05) among the treatment groups in corpus weight. Rams on T$_1$ recorded the highest value in corpus weight 4.98 g and this differed significantly (P<0.05) from rams on T$_3$ which had 1.96 g. There was no significant difference (P>0.05) between rams on T$_1$ and T$_2$ in corpus weight. The lowest value in corpus weight was observed in ram on T$_3$. The highest corpus weight value 4.98 g obtained in this study was higher than the mean value of 4.2 ± 0.3 g reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams. This suggested that Pergonal administration enhanced the development of the head of the epididymis in the treated animals.

There were significant differences (P<0.05) among the treatment groups in cauda weight. Rams on T$_3$ recorded the highest value in cauda weight 9.02 g and this differed significantly (P<0.05) from rams on T$_1$ which had 8.36 g. There was no significant difference (P>0.05) between T$_3$ and T$_2$ (8.49 g) in cauda weight. The lowest value in cauda weight 8.36 g was observed in rams on T$_1$. The cauda weight values obtained in this study were higher than the mean value of 8.00 ± 0.07 g reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams. This suggested that Pergonal administration enhanced the development of the body of the epididymis in the treated animals.

There were significant differences (P<0.05) among the treatment groups in vas deferens weight. Rams on T$_3$ recorded the highest value in vas deferens weight 3.92 g and this differed significantly (P<0.05) from rams on T$_1$ which had 0.79 g. There was no significant difference (P>0.05) between rams on T$_3$ and T$_2$ which had 1.10 g in vas deferens weight. The lowest value of 0.7 g in vas deferens weight was observed in rams on T$_1$. The highest vas deferens weight 3.92 g obtained in this study was higher than the mean value of 2.35 ± 0.16 g reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams of similar ages. This suggested that Pergonal administration enhanced the development of the vas deferens in the treated animals.

The results of gonadotrophin (Pergonal®) on sperm reserves of Ouda rams are shown in Table 2. There were significant differences (P<0.05) among the treatment groups in testicular sperm reserves. Rams on T$_3$ recorded the highest value of 19.25 x 10$^6$ in testicular sperm reserve and this differed significantly (P<0.05) from rams on T$_1$ (14.20 x 10$^6$) and T$_2$ (15.13 x 10$^6$) which were also significantly different (P<0.05) from each other in testicular sperm reserve. The lowest value was observed in rams on T$_1$. The highest testicular sperm reserve value obtained in this study (19.25 x 10$^6$) was higher than the range 12.15 ± 1.50 to 17.45± 1.64 (x10$^6$) 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).

There were significant differences (P<0.05) among the treatment groups in caput sperm reserve. Rams on T$_3$ recorded the highest value in caput sperm reserve 16.12 x10$^6$ and this differed significantly (P<0.05) from rams on T$_1$ (6.10x10$^6$) and T$_2$ (14.26x10$^6$) which were also significantly (P<0.05) different 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 Ihekwumere et al. (2008) in Nigerian Yankasa rams of similar ages. This suggested high capacity for induction of spermatogenesis by Pergonal(R) injection.

There were significant differences (P<0.05) among the treatment groups in corpus sperm reserve. Rams on T3 recorded the highest value in corpus sperm reserve 19.33 (x10^8) and this differed significantly (P<0.05) from rams on T1 which had 13.30 (x10^8). However, there was no significant difference (P>0.05) between rams on T3 and T2 in corpus sperm reserve. The lowest value in corpus sperm reserve was observed in rams on T1 (13.30 x10^8). The corpus sperm reserve values obtained in this study were much higher than the range of 5.48 ± 0.63 (x10^8) reported by Ihekwumere et al. (2008) in Nigerian Yankasa rams. This be attributed to high capacity for induction of spermatogenesis by Pergonal(R) injection.

There were significant differences (P<0.05) among the treatment groups in cauda sperm reserve. Rams on T3 recorded the highest value of 50.25 (x10^8) in cauda sperm reserve and this differed significantly (P<0.05) from rams on T1 (38.26x10^8) and T2 (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 T1. The cauda sperm reserve values obtained in this study were much higher than the value 6.25 ± 0.54 (x10^8) reported by Ihekwumere et al. (2008) in Nigeria Yankasa rams of similar ages. This suggested a high capacity for induction of spermatogenesis by Pergonal(R) injection.

There were significant differences (P<0.05) among the treatment groups in vas deferens sperm reserve. Rams on T3 recorded the highest value in vas deferens sperm reserve 13.50 (x10^8) and this differed significantly (P<0.05) from rams on T1. However, there was no significant difference (P>0.05) between rams on T3 and T2 in vas deferens sperm reserve. The lowest value in vas deferens sperm reserve was observed in rams on T1 (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 to 0.65 ± 0.04 (x10^8) reported by Ihekwumere 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 23.32% 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 (Ihekwumere 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; Dyce et al., 2002; Olukole and Obayemi, 2010).

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(R) 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).

The level of follicle stimulating hormone (FSH) released by the Anterior pituitary could be a factor as FSH reacts

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<th>Relative epididymal sperm distribution</th>
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<td>Cauda</td>
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abc, means in the same row with different superscript are significantly (P<0.05) different. SEM, Standard error of means.
with receptors on the sertoli cells to cause production of androgen-binding protein (ABP), conversion of testosterone to dihydrotestosterone and estrogen, stimulation of spermatogenesis, completion of sperm release (spermiation) and secretion of inhibin (Egu and Ukpabi, 2016). 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 (Egu, 2015; Egu, 2016; Herbert et al., 2005).

Table 3 shows correlation (r) between body conformation and sperm reserves in mature Ouda rams. High correlations were observed between body weight and corpus sperm reserve (r = 0.69* P<0.05), withers height and caput sperm reserve (r = 0.97 P<0.01); withers height and testicular sperm reserve (r = 0.99, P<0.01); scrotal circumference and corpus sperm reserve (r = 0.37 P<0.01); heart girth and caput sperm reserve (r = 0.97 P<0.01); heart girth and testicular sperm reserve (r = 0.88 P<0.01); heart girth and testicular sperm reserve (r = 0.84 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. The correlation model is linear regression equation:

\[ Y = a + bx + e_i \]

Where Y is the dependent variable, x is the independent variable, a and b are constants, a is equal to intercept, b is equal to slope of the equation and that is the rate of change of Y per unit change in X, e_i is random error.

**CONCLUSION**

From the results of this study, it can be concluded that Pergonal® improved sperm production and sperm reserves in Ouda rams at the level of 99.00i.u without any deleterious effects on the testicular and epididymal characteristics of the rams. This level of Pergonal is therefore recommended for improvement of spermatogenesis and sperm reserves and invariably reproductive performance of Ouda rams.

**REFERENCES**


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**Table 3. Correlation (r) between Body Conformation and Sperm Reserves of Mature Ouda Rams.**

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<tr>
<th>Parameters</th>
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<td>0.66**</td>
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</table>

*Significant (P<0.05). **Highly significant (P<0.01). ns, not significant (P>0.05).


