

# Effect of Gonadotrophin (Pergonal®) on daily sperm output, gonadal and extragonadal sperm reserves of mature Yankasa rams in Eastern Nigeria

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**ABSTRACT:** Three groups of 6 healthy mature Yankasa rams aged 2.0 to 2.6 years weighing between 30.50 and 30.60kg were used to determine the effect of Pergonal® on daily sperm output, gonadal and extragonadal sperm reserves. The rams were assigned to either 49.50 i.u ( $T_2$ ), 99.00 i.u ( $T_3$ ) or 148.50 i.u ( $T_4$ ) Pergonal® injections (Ferring Labs, USA) each divided into 3 doses and administered for 3 consecutive days according to the manufacturer's prescription. Another group of 6 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 intra-muscular injection. The results showed significant differences ( $P>0.05$ ) among the treatment groups in daily sperm output, ( $\times 10^9$ ) daily sperm output per gram testis ( $\times 10^9$ ), gonadal sperm reserve ( $\times 10^9$ ), caput sperm reserve ( $\times 10^8$ ), corpus sperm reserve ( $\times 10^8$ ), cauda sperm reserve ( $\times 10^8$ ) and vas deferens sperm reserve ( $\times 10^8$ ). High correlations were observed between testis weight, caput weight 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 suggest that the daily sperm output, daily sperm output per gram testis, gonadal, caput, corpus, cauda and vas deferens sperm reserves would be improved when 99.00 i.u of Pergonal® is used for induction of spermatogenesis in Yankasa rams.

**Key words:** Gonadotrophin (Pergonal®), sperm output, sperm reserve, Yankasa rams.

## 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 to 70 cm at the withers and weigh 30 to 50kg with an outstanding sexual agility, hence they have been widely used for artificial insemination programs (Osinowo, 2006). Several aspects of the reproductive physiology of rams have been documented (Iheukwumere et al., 2001; Ahemen and Bitto, 2007). Measurable traits such as scrotal dimensions, sperm production rate, gonadal and extragonadal sperm reserves have been extensively

studied in some Nigerian breeds (Iliyasu et al., 2014; Kwari and Waziri, 2001; Ahemen and Bitto, 2007; Oyeyemi and Ubiogoro, 2005; Oyeyemi and Babalola, 2006; Obidike et al., 2007; Britto et al., 2002; Britto et al., 2006). Few of such reports are however, available for the Yankasa rams, the breed that is abundant in Nigeria and resistant to some local diseases (Lebbie, 2004). It has been observed that the reproductive capacity of Yankasa rams is low (Osinowo, 2006) when compared with the exotic breeds of rams in terms of offspring produced per female per year, though the occasional occurrence of triplets have been recorded.

There is cause to boost 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 Menthophin 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 (Iheukwumere et al., 2004). Follicle stimulating hormone 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 study was therefore designed to determine 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

### Location of Study

The experiment was conducted at the sheep and goat unit of the Teaching and Research Farm of the Faculty of Agriculture, Abia State University, Umudike, location near Umuahia, Nigeria. This is within the South East Agro-ecological zone of Nigeria and lies within latitude 5° 29' N and longitude 7° 33' E and at an altitude of 122 m (400 feet) above sea level. The area has an annual ambient temperature of 25 to 30°C, relative humidity between 65 to 89%, annual rainfall 2000 to 2484 mm, and the soil is sandy loamy with average pH 5.5 (Adiele et al., 2005).

### Management of Animals

Twenty-four healthy sexually matured Nigerian Yankasa rams aged 2.0 to 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 hygiene inspection was carried out. Fresh forage consisting of *Panicum maximum*, *Aspilia Africana*, *Pennisetum purpureum* (Elephant grass) were fed as basal diets and a concentrate ration of Grower Mash was used as supplement. The animals were fed twice daily (morning and evening), salt lick was also provided as mineral supplement. Water was given *ad libitum* to the animals. The study was done during the breeding season.

## 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 levels of Pergonal® as treatments. The levels of Pergonal® were 0, 49.50 I.U, 99.00 I.U and 148.50 I.U represented as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. T<sub>1</sub> which contained no Pergonal® served as the control. 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. The rams were treated by intramuscular injection. The injections were as follows:

**Group T<sub>1</sub>:** Each ram received 1.00ml of physiological saline for 3 days.

**Group T<sub>2</sub>:** Each ram received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.11 ml) on the first day; 2nd day the group received 8.25 I.U of PFSH and 8.25 I.U of PLH (0.11 ml). 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<sub>3</sub>:** Each ram received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22 ml) on the first day. 2nd day, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22 ml). While on the 3rd, the group received 16.50 I.U of PFSH and 16.50 I.U of PLH (0.22 ml) giving a total of 99.00 I.U of PFSH and PLH (0.66 ml) Pergonal injection within 3 days.

**Group T<sub>4</sub>:** 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 (0.33 ml) giving a total of 148.50 I.U of PFSH and PLH (0.99 ml) Pergonal injection within 3 days.

All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01 ml 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 100 ml 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 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 (Egu et al., 2016).

### 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). Means separation were done using Duncan's New Multiple Range Test as described by Obi (2002).

## RESULTS

The results of gonadotrophin (Pergonal®) administration on scrotal circumference, paired testes, paired epididymal, epididymal segments and vas deferens weights of Yankasa rams are shown in Table 1. The results of gonadotrophin (Pergonal®) administration on sperm output rate, gonadal and extragonadal sperm reserves of Yankasa rams are shown in Table 2.

There were significant differences ( $P < 0.05$ ) among the treatment groups in daily sperm output. Rams on  $T_3$  recorded the highest value of  $4.12 \times 10^9$  in daily sperm output and this differed significantly ( $P < 0.05$ ) from rams on  $T_1$  (control treatment) and  $T_2$  which were similar ( $P > 0.05$ ) to each other. However, there was no significant difference ( $P > 0.05$ ) between rams on  $T_3$  and  $T_4$  in daily sperm output. Rams on  $T_4$  differed significantly ( $P < 0.05$ ) from rams on  $T_1$  which were similar ( $P > 0.05$ ) to rams on  $T_2$  in daily sperm output. The lowest value of  $2.60 \times 10^9$  in daily sperm output was observed in rams on  $T_1$  (Table 2).

**Table 1.** Scrotal Circumference, Testicular, Epididymal and Vas Deferens Weights in Mature Yankasa Rams.

| Parameters                    | Mean $\pm$   | SEM  |
|-------------------------------|--------------|------|
| Scrotal circumference (cm)    | 26.26 $\pm$  | 0.14 |
| Paired testes weight (g)      | 109.95 $\pm$ | 0.75 |
| Paired epididymal weight (g)  | 30.10 $\pm$  | 0.42 |
| Weight of epididymal segments |              |      |
| Caput (g)                     | 17.50 $\pm$  | 0.29 |
| Corpus (g)                    | 2.65 $\pm$   | 0.05 |
| Cauda (g)                     | 6.77 $\pm$   | 0.15 |
| Vas deferens (g)              | 1.20 $\pm$   | 0.02 |

There were significant differences ( $P < 0.05$ ) among the treatment groups in daily sperm output/gram/testis. Rams on  $T_3$  recorded the highest daily sperm output/gram/testis ( $0.42 \times 10^9$ ) and this differed significantly ( $P < 0.05$ ) from rams on  $T_1$  which was similar ( $P > 0.05$ ) to rams on  $T_2$ . However, there were no significant differences ( $P > 0.05$ ) among rams on  $T_3$ ,  $T_4$  and  $T_2$ . The lowest daily sperm output/gram/testis ( $0.22 \times 10^9$ ) was observed in rams on  $T_1$ . There were significant differences ( $P < 0.05$ ) among the treatment groups in gonadal sperm reserve. Rams on  $T_3$  recorded the highest gonadal sperm reserve of  $14.42 \times 10^9$  and this differed significantly ( $P < 0.05$ ) from rams on  $T_1$  which were similar ( $P > 0.05$ ) to rams on  $T_2$ . However there was no significant difference ( $P > 0.05$ ) between rams on  $T_3$  and  $T_2$  in gonadal sperm reserve. The lowest gonadal sperm reserve ( $2.30 \times 10^9$ ) was observed in rams on  $T_1$  (Table 2).

There were significant differences ( $P < 0.05$ ) among the treatment groups in caput sperm reserve. Rams on  $T_3$  recorded the highest caput sperm reserve of  $16.40 \times 10^8$  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  $T_2$ ,  $T_3$  and  $T_4$  in caput sperm reserve. The lowest value in caput sperm reserve was observed in rams on  $T_1$  (Table 2).

There were significant differences ( $P < 0.05$ ) among the treatment groups in corpus sperm reserve. Rams on  $T_3$  recorded the highest value of  $15.14 \times 10^8$  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  $T_1$  ( $6.74 \times 10^8$ ). There were significant differences ( $P < 0.05$ ) among the treatment groups in cauda sperm reserve. Rams on  $T_3$  recorded the highest cauda sperm reserve value of  $46.44 \times 10^8$  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

**Table 2.** Sperm Output Rate, Gonadal and Extragonadal Sperm Reserves of Mature Yankasa Rams Treated with Gonadotrophin (Pergonal®).

| Variables  | Treatment (Pergonal® i.u) |                         |                         |                          | SEM  |
|--|---------------------------|-------------------------|-------------------------|--------------------------|------|
|  | T <sub>1</sub><br>0.00    | T <sub>2</sub><br>49.50 | T <sub>3</sub><br>99.00 | T <sub>4</sub><br>148.50 |      |
| Daily sperm output (x10 <sup>9</sup> )                 | 2.60 <sup>c</sup>         | 2.90 <sup>bc</sup>      | 4.21 <sup>a</sup>       | 4.03                     | 0.40 |
| Daily sperm output/<br>Gram/testis (x10 <sup>9</sup> ) | 0.22 <sup>b</sup>         | 0.33 <sup>ab</sup>      | 0.42 <sup>a</sup>       | 0.38 <sup>a</sup>        | 0.04 |
| Gonadal sperm reserve (x10 <sup>9</sup> )              | 12.30 <sup>b</sup>        | 13.33 <sup>ab</sup>     | 14.42 <sup>a</sup>      | 14.10 <sup>a</sup>       | 0.47 |
| Caput sperm reserve (x10 <sup>8</sup> )                | 2.10 <sup>b</sup>         | 9.22 <sup>ab</sup>      | 16.40 <sup>a</sup>      | 11.16 <sup>a</sup>       | 2.96 |
| Corpus sperm reserve (x10 <sup>8</sup> )               | 6.74 <sup>a</sup>         | 12.81 <sup>a</sup>      | 15.14 <sup>a</sup>      | 13.88 <sup>a</sup>       | 1.86 |
| Cauda sperm reserve (x10 <sup>8</sup> )                | 10.64 <sup>b</sup>        | 22.75 <sup>ab</sup>     | 46.44 <sup>a</sup>      | 12.10 <sup>b</sup>       | 8.27 |
| Vas deferens sperm reserve (x10 <sup>8</sup> )         | 9.74 <sup>b</sup>         | 9.29 <sup>b</sup>       | 12.60 <sup>a</sup>      | 9.20 <sup>b</sup>        | 0.81 |
| Relative epididymal sperm distribution                 |                           |                         |                         |                          |      |
| Caput  | 13.32                     |                         |                         |                          |      |
| Corpus   | 17.39                     |                         |                         |                          |      |
| Cauda  | 68.70                     |                         |                         |                          |      |

<sup>abc</sup>Means in the same row with different superscript are significantly ( $P < 0.05$ ) different. **SEM**, Standard error of mean.

sperm reserve. The lowest value of 10.64 (x10<sup>8</sup>) in cauda sperm reserve was observed in rams on the control treatment (T<sub>1</sub>) (Table 2).

There were significant differences ( $P < 0.05$ ) among the treatment groups in vas deferens sperm reserve. Rams on T<sub>3</sub> recorded the highest vas deferens sperm reserve of 12.60 (x10<sup>8</sup>) and this differed significantly ( $P < 0.05$ ) from rams on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub> which were similar ( $P > 0.05$ ) to each other in vas deferens sperm reserve. The lowest value of 9.20 (x10<sup>8</sup>) in vas deferens sperm reserve was observed in rams on T<sub>4</sub> (Table 2).

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 ( $r = 0.76$ ,  $P < 0.01$ ); testicular weight and daily sperm output ( $r = 0.99$ ,  $P < 0.01$ ); testicular weight and caput sperm reserve ( $r = 0.93$ ,  $P < 0.01$ ); testicular weight and gonadal sperm reserve ( $r = 0.99$ ,  $P < 0.01$ ), caput weight and cauda sperm reserve ( $r = 0.86$ ,  $P < 0.01$ ); caput weight and gonadal sperm reserve ( $r = 0.79$ ,  $P < 0.01$ ); cauda weight and daily sperm output per gram testis ( $r = 0.86$ ,  $P < 0.01$ ) and cauda weight and cauda sperm reserve ( $r = 0.97$ ,  $P < 0.01$ ).

## DISCUSSION

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 by Ahemen and Bitto (2007) in West African dwarf rams in Makurdi,

Benue state, Nigeria; Kwari and Waziri (2001) in Balami rams in Maiduguri, Borno state, Nigeria. This could be attributed to the size and nutritional status of the Yankasa rams. The Yankasa rams had been described as one of the large breeds of sheep indigenous to Nigeria (Iheukwumere et al., 2001). The mean values of scrotal circumference, epididymal weight and caput weight values obtained in this study were higher than the values reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams in Umuahia, Abia State, Nigeria. This could be attributed to the positive effect of Pergonal administration on the morphology of the scrotum, testes and epididymis and signifies increased testicular and epididymal mass for sperm production and storage.

However, the mean values of paired testes, corpus, cauda and vas deferens weights obtained in this study were lower than the values reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams in Umuahia, Abia State, Nigeria. This could be attributed to environment, nutritional status of these rams and drug administration (Herbal et al., 2002).

The highest daily sperm output of 4.21 (x10<sup>9</sup>) obtained in this study was lower than the highest value of  $5.16 \pm 0.12$  (x10<sup>9</sup>) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams and lower than 5.29 (x10<sup>9</sup>) reported by Martinez et al. (1994) in temperate breeds. However, the highest daily sperm output value obtained in this study was higher than  $0.60 \pm 0.01$  (x10<sup>9</sup>) reported by Ahemen and Bitto (2007) in WAD rams of similar ages in Makurdi, Benue state, Nigeria. This could be attributed to environment, breed differences and nutritional status of these rams.

The highest daily sperm output/gram/testis obtained in

**Table 3.** Correlation (r) between Testicular Morphometry, Sperm Output Rate, Gonadal and Extragonadal Sperm Reserves in mature Yankasa Rams

| Variables                          | 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                      | 0.76**                             | 0.99**                  | 0.34 <sup>ns</sup>  | 0.30 <sup>ns</sup>   | 0.93**              | 0.99**                | 0.45 <sup>ns</sup> | 0.83**        | 0.33 <sup>ns</sup> |
| Caput weight                       | 0.86**                             | 0.78**                  | 0.86**              | 0.61*                | 0.67 <sup>x</sup>   | 0.79**                | 0.25 <sup>ns</sup> | 0.87**        | -                  |
| Corpus weight                      | 0.39 <sup>ns</sup>                 | 0.30 <sup>ns</sup>      | 0.90 <sup>xx</sup>  | 0.38 <sup>ns</sup>   | 0.46 <sup>ns</sup>  | 0.20 <sup>ns</sup>    | 0.35 <sup>ns</sup> | -             | -                  |
| Cauda weight                       | 0.86**                             | 0.22 <sup>ns</sup>      | 0.97**              | 0.18 <sup>ns</sup>   | 0.21 <sup>ns</sup>  | 0.38 <sup>ns</sup>    | -                  | -             | -                  |
| Gonadal sperm reserve              | 57*                                | 0.11 <sup>ns</sup>      | 0.37 <sup>ns</sup>  | 0.62*                | 0.09 <sup>ns</sup>  | -                     | -                  | -             | -                  |
| Caput sperm reserve                | 0.48 <sup>ns</sup>                 | 0.01 <sup>ns</sup>      | 0.28 <sup>ns</sup>  | 0.53*                | -                   | -                     | -                  | -             | -                  |
| Corpus sperm reserve               | 0.05 <sup>ns</sup>                 | 0.63*                   | 0.25 <sup>ns</sup>  | -                    | -                   | -                     | -                  | -             | -                  |
| Cauda sperm reserve                | 0.20 <sup>ns</sup>                 | 0.38 <sup>ns</sup>      | -                   | -                    | -                   | -                     | -                  | -             | -                  |
| Daily sperm out put                | 0.58*                              | -                       | -                   | -                    | -                   | -                     | -                  | -             | -                  |
| Daily sperm output per gram testis | -                                  | -                       | -                   | -                    | -                   | -                     | -                  | -             | -                  |

\*Significant (P<0.05), \*\*highly significant (P<0.01), <sup>ns</sup>, not significant (P>0.05).

this study ( $0.42 \times 10^9$ ) was lower than the highest value of  $0.98 \pm 0.05$  ( $\times 10^9$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams of similar ages, and lower than the value of  $2.75$  ( $\times 10^9$ ) reported by Martinez et al. (1994) in temperate breeds. This could be attributed to testicular size, nutrition and technique of estimation (Ahemen and Bitto, 2007). Gonadal sperm reserve and caput sperm reserve followed the same pattern as daily sperm output/gram/testis.

The gonadal sperm reserve values obtained in this study were within the range of  $12.15 \pm 1.50$  to  $17.45 \pm 1.64$  ( $\times 10^9$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams of similar ages, but higher than  $2.15(\times 10^9) \pm 0.05/\text{ml}$  for gonadal sperm reserve reported by Ahemen and Bitto (2007) in West African Dwarf rams. However, the gonadal sperm reserve values obtained in this study were lower than the mean value of  $18.80 \pm 1.0$  ( $\times 10^9$ ) for gonadal 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).

The lowest value of ( $2.10 \times 10^8$ ) in extragonadal sperm reserve was recorded by rams on T<sub>1</sub>. The highest extragonadal sperm reserve value of ( $46.44 \times 10^8$ ) obtained in Pergonal treated groups was much higher than the value of  $6.25 \pm 0.54$  ( $\times 10^8$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams and  $14.10 \pm 0.4$  ( $\times 10^8$ ) reported by Kwari and Waziri (2001) in Balami rams. This could be attributed to high capacity for induction of spermatogenesis by Pergonal injection.

The highest value of  $16.40$  ( $\times 10^8$ ) in caput sperm reserve obtained in this study was higher than the highest value of  $4.10 \pm 0.06$  ( $\times 10^9$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams and higher than the highest caput

sperm reserve of  $16.12(\times 10^8)$  reported by Egu et al. (2016) in Ouda rams of similar ages, and higher than  $2.92(\times 10^8)$  for caput sperm reserve reported by Raji and Njidda (2014) in Red Sokoto goats. This suggested high capacity for induction of spermatogenesis by Pergonal injection.

The corpus sperm reserve values obtained in this study were higher than the highest corpus sperm reserve value of  $5.48 \pm 0.63$  ( $\times 10^8$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams. This could be attributed to high capacity for induction of spermatogenesis by Pergonal injection. However, the corpus sperm reserve values obtained in this study were lower than the highest value of  $19.33(\times 10^8)$  reported by Egu et al. (2016) in Ouda rams of similar ages treated with Gonadotrophin (Pergonal®). This could be attributed to breed differences, genotype and testicular size.

Cauda sperm reserve values obtained in this study were higher than the highest cauda sperm

reserve value of  $6.25 \pm 0.54$  ( $\times 10^8$ ) reported by Iheukwumere et al. (2008) in Nigerian Yankasa rams. This could be attributed to high capacity for induction of spermatogenesis by Pergonal injection. However, the cauda sperm reserve values obtained in this study were lower than the value of  $50.25$  ( $\times 10^8$ ) for cauda sperm reserve reported by Egu et al. (2016) in Ouda rams of similar ages treated with Gonadotrophin(Pergonal<sup>®</sup>). This disparity in cauda sperm reserve may not be unconnected to breed differences, genotype, testicular size and nutritional status of the rams.

The vas deferens sperm reserve values obtained in this study were much higher than the range of  $0.45 \pm 0.02$  to  $0.65 \pm 0.04$  ( $\times 10^8$ ) 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. However, the vas deferens sperm reserve values obtained in this study were lower than the highest value of  $13.50(\times 10^8)$  for vas deferens sperm reserve reported by Egu et al. (2016) in Ouda rams of similar ages treated with Gonadotrophin(Pergonal<sup>®</sup>). This disparity in vas deferens sperm reserve could be attributed to breed differences, genotype and testicular size of the rams. Ouda sheep is second to the largest breed of sheep (Balami) in Nigeria and Ouda rams weigh slightly lower than Balami rams (Egu et al., 2016). 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).

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).

In this study, it was observed that Pergonal<sup>®</sup> 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<sup>®</sup> 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 improved 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 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; Egu et al., 2016). Herbert et al. (2000) 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; Egu et al., 2016; Herbert et al., 2005).

The high and positive correlations observed in this study are suggestive of the relationship between the above mentioned parameters and sperm output rate, gonadal and extragonadal sperm reserves. This result is in agreement with the report of Ibrahim (2012) who observed in a study that the paired testes weights and gonadal sperm reserves showed a high significant positive correlation, and that a correlation was observed between testes weights and testicular and epididymal sperm reserves in Ouda, Yankasa and Balami rams; and Perry and Petterson (2001) and Ogbuewu (2008) who documented that testis size is a good indicator of present and future sperm production as well as breeding potential of the male in bulls and rabbits respectively; and Iheukwumere et al. (2008) who reported a high correlation between testicular morphometry and sperm production rate, gonadal and extragonadal sperm reserves in Yankasa rams administered with Clomiphene citrate (Clomid<sup>®</sup>). 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 spermatogenesis and sperm output in Yankasa rams at the level of 99.00i.u without any deleterious effects on the gonadal and extragonadal sperm reserves as the sperm reserves were within the range reported in literature. This level of Pergonal (99.00i.u) is therefore recommended for improvement of spermatogenesis, sperm output and sperm reserves in Yankasa rams.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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