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ORIGINAL RESEARCH ARTICLE

Effect of Pergonal® Administration on Semen Characteristics, Hormonal Profile and Biochemical Constituents of the Seminal Plasma of Mature Yankasa Rams

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ABSTRACT

Twenty–four Yankasa rams aged 2 – 2.5 years and weighed between 30.50 and 30.60kg were randomly distributed into 4 groups of 6 animals per group and used to determine the effect of Pergonal® on semen characteristics, hormonal profile and biochemical constituents of seminal plasma. The doses were 0.00, 0.33, 0.66 and 0.99ml Pergonal (R) represented as T_1, T_2, T_3 and T_4 respectively. The group that received 0.00 ml Pergonal(R) served as the control. All the treatments were administered within 3days by intramuscular injection. The results showed that there were significant differences ($P<0.05$) among the treatment groups in semen characteristics. Rams that received 0.66ml Pergonal had the highest values in semen volume (1.60 ml), mass motility (4.0), individual motility (95%), semen pH (7.0), sperm concentration (0.78 $x10^6$/ml), proportion of live (87.25%) and normal (85.10%) sperm cells respectively. Rams on 0.66ml also had the least dead sperm cells (12.75%). There were also significant differences ($P<0.05$) among the treatment groups in their hormonal profiles. Rams on 0.66ml had the highest values in follicle stimulating hormone (12.80nd/ml) and luteinizing hormone (5.16ng/ml) while rams on the control had highest testosterone level (14.21ng/ml) in the serum. The results further showed that there were significant differences ($P<0.05$) among the treatment groups and that rams on 0.66ml had the highest values in urea 42.20mg/dl), glycerlyphosphorylcholine (2.0mg/dl), ascorbic acid (8.0mg/dl), sodium (48mmol/L), potassium (5.85mmol/L), bicarbonate (26.0mmol/L) and fructose (490mmol/L) levels in the seminal plasma. The results of this study showed that Pergonal treatment enhanced sperm quality and was not detrimental to the hormonal profile and biochemical constituents of the seminal plasma of the Yankasa rams.

Keywords: Pergonal, sperm quality, hormones, seminal plasma constituents, 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). The Nigerian Yankasa rams are typically tall, reaching a height of 50-70 cm at the withers and weight of 30-50 kg with an outstanding sexual agility, hence they have been favorite for artificial insemination programmes (Osinowo, 1990).

The primary aim of induction of spermatogenesis is to improve semen quality (Ameh, 2004; Abu *et al.*, 2006). Spermatogenesis requires the stimulation from follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Abu *et al.*,2006). Many of the preparations of FSH and LH (such as recombinant FSH or Gonalfifollistin and other pharmaceutically manufactured FSH and LH) are very expensive perhaps because of the brand names. Some of them require cold chain storage and often deteriorate because of inadequate storage and handling (Herbert *et al.*, 2000).

There is therefore need to examine some generic preparations that could boost spermatogenesis in the animals but at the same time are cheap, readily available and easily managed under developing countries conditions. Pergonal®, a fertility drug (Ferring Labs, USA) also known as Humegon or Mentrophin and with similar constituents as Plusset® is a gonadotrophin lyophilized in vials containing a mixture of gonadotrophins consisting of FSH and 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).

It has not been determined if the administration of the hormone preparation for spermatogenesis would induce any side effects on the hormonal profile and seminal plasma constituents of the Yankasa rams. This study was therefore carried out to determine the effects of Pergonal administration on the semen quality, hormonal profile and seminal plasma constituents of Yankasa rams.

MATERIALS AND METHODS

Experimental Animals and their Management: Twenty-four healthy sexually matured Nigerian Yankasa rams aged 2-2.5 years were used for this study. The animals were purchased from the local markets (Gariki) and housed in clean pens constructed in such a way that the rams could come outside during the day for access to sunlight. The study 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. 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 (containing 2450 kcal/kg) 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 twenty-four rams were divided into 4 treatment groups consisting of 6 rams per group following the completely randomized design (CRD). These groups were assigned to 4 levels of Pergonal as treatments (Table 1). A vial of Pergonal containing FSH 75 I.U and LH 75 I.U. 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 groups received different doses of Pergonal as indicated in Table 1. Pergonal treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01ml graduation. The dosage was based on the manufacturer's prescription.

Table 1: Doses of Pergonal Administered on Mature Yankasa Rams

	Treatment Dosage (ml)			
Day	**T_1**	**T_2**	**T_3**	**T_4**
1	0.00	0.09	0.18	0.33
2	0.00	0.12	0.21	0.33
3	0.00	0.12	0.27	0.33
Total	**0.00**	**0.33**	**0.66**	**0.99**

Semen Collection and Evaluation

Semen collection was done by Electro-ejaculation method (Noakes *et al.,* 2001) after one week of Pergonal administration and continued at 2 weeks interval for 9 weeks. The semen was collected between 8.00am and 9.00am with a standard precision electronic model (Electro-ejaculator, USA). A transparent graduated tube immersed in a protective jacket containing water at 37^{o} C, with a funnel was used to collect the semen. The animal was restrained in an upright position by an assistant. The Vaseline lubricated probe was inserted gently into the rectum. The rhythmic stimulation of the ampullae and sacral nerve plexus caused erection and subsequently ejaculation within few minutes.

Semen evaluation was done as promptly as possible post collection as described by Rodriguez-Martinez and Barth (2007) for qualitative and quantitative parameters such as semen volume, sperm concentration, live sperm percentage, sperm motility and semen pH.

Hormonal Assay

Blood samples (5ml each) were obtained with needle and syringe by jugular vein puncture of the 24 rams on day 7 after the Pergonal injection, for testosterone, FSH and LH evaluation. It was cooled immediately in iced water and transferred to the laboratory, refrigerated at 4^{0}C for 1 hour and the serum separated by centrifugation at 5,000rpm for 10 minutes. The serum was stored immediately at -20^{0}C until enzyme immuno assayed (EIA) with Immunometrics Limited Kit (UK) for

testosterone, FSH and LH as described by Micallef *et al.* (1995).

Biochemical Constituents of Seminal Plasma
Semen samples used for estimation of biochemical constituents of seminal plasma were centrifuged at 15,000 rpm for 15 minutes (Iheukwumere *et al.*, 2001). The seminal plasma samples were immediately subjected to laboratory analysis for the following biochemical parameters; urea, non-protein nitrogen, glycerylphosphorylcholine, fructose, ascorbic acid, sodium, potassium and bicarbonate. Sodium and potassium concentrations were estimated with a flame photometer on samples suitably diluted with deionized water, while bicarbonate and urea concentrations were determined according to the method of Baker and Silverton (1986). Fructose concentration in the plasma was determined according to the procedure of Singgh (2004). Ammonia concentration was determined according to the procedure of Cheesbrough (2004). Magnetic resonance spectroscopy was used to determine the concentrations of glycerlyphosphorylcholine, lactic acid and ascorbic acid (Robert *et al.*, 2000).

Data Analysis:
Data obtained on semen characteristics, hormonal assay and seminal plasma constituents of Yankasa rams 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 Pergonal administration on semen characteristics of Yankasa rams are shown in Table 2. There were significant differences ($P<0.05$) among the treatment groups in all the parameters measured. Rams on 0.66 ml Pergonal (T_3) recorded the best results in most of the parameters evaluated. The lowest value for semen volume (0.60 ml) was obtained in rams on the control treatment. The highest semen volume of 1.60ml (T_3) obtained in this study was higher than the mean ejaculate volume of 1.25±0.7ml reported by Iheukwumere and Okere (1990) in Yankasa rams of similar ages. The lowest value of 2.30 for mass motility was observed in rams on the control treatment. Mass motility values obtained in this study fall within the normal range of 1 to 5 reported by Bracket (2005).

Table 2: Effect of Pergonal on semen characteristics of Yankasa rams

	Treatment (Pergonal) I.U.				
	T_1	T_2	T_3	T_4	
Parameters	**0.00**	**57.78**	**118.50**	**173.34**	**SEM**
Semen volume (ml)	0.60^{c}	1.30^{ab}	1.60^{a}	0.80^{bc}	0.23
Mass motility	2.30^{b}	3.00^{ab}	4.00^{a}	3.00^{ab}	0.34
Individual motility (%)	85.00^{ab}	70.00^{b}	95.00^{a}	95.00^{a}	5.20
pH	8.00^{a}	6.00^{b}	7.00^{ab}	7.00^{ab}	0.65
Sperm Concentration ($x10^6$/ml)	0.31^{b}	0.64^{a}	0.78^{a}	0.78^{a}	0.10
Proportion of live sperm cells (%)	83.00^{a}	83.20^{a}	87.25^{a}	68.53^{b}	4.18
Proportion of normal sperm cells (%)	82.50^{a}	78.23^{a}	85.10^{a}	62.53^{b}	5.06
Proportion of dead sperm cells (%)	17.00^{b}	16.80^{b}	12.75^{b}	31.76^{a}	4.18

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

The lowest score of 70.00% in individual motility was observed in rams on 0.33 ml Pergonal (T_2). The highest score in individual motility obtained in this study (95.00 %) was higher than the score of 85.0±7.5% reported by Iheukwumere *et al.* (2001) in Yankasa rams of similar ages. Sperm motility is affected by frequency of semen collection (Iheukwumere *et al.*, 2008).

Semen pH values obtained in this study were within the normal range of 7 – 8 reported by Meacham (2002) except in rams on 0.33ml Pergonal whose semen pH (6.0) was lower than the normal range. The measured pH depends on the length of time since ejaculation and it tends to increase shortly after ejaculation as a result of loss of CO_2 (Meacham, 2002).

The highest value for sperm concentration obtained in this study (0.78×10^6) is similar to the highest value for sperm concentration [0.79±0.7 ($x10^6$)] reported by Iheukwumere *et al.* (2001) for Yankasa rams of similar ages. However, this was lower than the normal range of 200 – 5000 ($x\ 10^6$) for rams. Normally an increase in the semen collection frequency is associated with a decrease in spermatozoa concentration (Aroita *et al.*, 2000; Iheukwumere and Okere, 1990).

There were significant differences ($P<0.05$) among the treatment groups in the proportion of live sperm cells, normal sperm cells and percentage dead sperm cells. The lowest proportion (68.53%) of live sperm cells was observed in rams on 0.99ml Pergonal (T_4). The highest percentage of live sperm cells obtained in this study (87.25%) falls within the range of 82.4±3.8 – 87.0±8.2% reported by Iheukwumere *et al.* (2001) in Nigerian Yankasa rams.

Percentage normal sperm cells followed the same pattern as live sperm cells. However, there were no significant differences ($P>0.05$) among rams on 0.66ml Pergonal, the control (T_1) and 0.33ml Pergonal (T_2) treatments. The lowest proportion of normal sperm cells (62.53%) was observed in rams on 0.99ml Pergonal (T_4). The highest percentage of normal sperm cells obtained in this study was slightly lower than the value of 87.0±8.2% reported by Iheukwumere *et al.* (2001) in Nigerian Yankasa rams.

Rams on 0.99ml Pergonal (T_4) recorded the highest percentage of dead sperm cells (31.76%) and this differed significantly ($P<0.05$) from rams on the control treatment (T_1), 0.33ml Pergonal (T_2) and 0.66mlPergonal (T_3) which were similar ($P>0.05$) to each other in percentage dead sperm cells. The lowest value of dead sperm cells (12.75%) was observed in rams on 0.66ml Pergonal (T_3).

The observation in this study that the group that received higher dose of the test drug recorded the lowest percentage of live sperm cells, normal sperm cells, and highest percentage of dead sperm cells suggests that a higher dose of the drug such as 0.99ml / ram within 3 days could have deleterious effect on sperm cells.

The results of Pergonal administration on hormonal profile of Yankasa rams are presented in Table 3. There were significant differences ($P<0.05$) among the treatment groups in follicle stimulating hormone (FSH) levels in the serum. Rams on T_3 recorded the highest FSH value of 12.80 ng/ml which did not differ significantly ($P>0.05$) from those on T_4 (12.45 ng/ml) but they differed significantly ($P<0.05$) from rams on treatments T_1 and T_2 which had the same value of 12.25 ng/ml The observation in this study that the FSH value in the rams treated with 0.66ml Pergonal (T_3) was numerically higher than in the group which received a higher dose of the drug (T_4) suggests that a higher dose of the drug, such as 0.99ml/ram within 3 days given in this study could excite suppressive effect on the hypothalamus. This observation is in agreement with the report of Iheukwumere (2005) in goats.

There were significant differences ($p<0.05$) among the treatment groups in Luteinizing hormone (LH) levels in the serum. Rams on the control treatment (T_1) recorded the highest LH value of 5.24ng/ml and this differed significantly ($P<0.05$) from rams on 0.33ml Pergonal (T_2) and 0.99ml Pergonal (T_4) which were similar ($P>0.05$) to each other in LH values. The observation in this study that the LH value in rams on the control treatment was higher ($P<0.05$) than in the group which received higher dose of Pergonal (0.99ml/ram) suggest that higher dose of the drug within 3 days given in this study could excite suppressive effect on the hypothalamus. 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.*, 2000).

Table 3: Effect of Pergonal on hormonal profile of Yankasa rams

Parameters	Treatment (Pergonal I.U)				SEM
	T_1 0.00	T_2 57.78	T_3 118.5	T_4 173.34	
FSH(ng/ml)	12.25[b]	12.25[b]	12.80[a]	12.45[ab]	0.13
LH (ng/ml)	5.24[a]	5.08[b]	5.16[ab]	5.04[b]	0.04
Testosterone (ng/ml)	14.21[a]	14.20[a]	14.10[a]	13.21[b]	0.23

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

The testosterone values decreased non-significantly (P>0.05) from 14.21ng/ml (T_1) to 14.10ng/ml (T_3)) before declining significantly (P<0.05) in T_4 (13.21ng/ml). Rams on the control treatment (T_1) recorded the highest testosterone value of 24.21 (ng/ml) and this differed significantly (P<0.05) from rams on T_4. Testosterone values obtained in this study were within the normal range (0-20ng/ml) reported in sheep by king *et al.* (1993). The observation in this study that the testosterone value in the control group was higher (P<0.05) than in the group that received the highest dose (0.99ml/ram) of Pergonal injection suggests that higher doses of Pergonal such as 0.99ml/ram administered within 3 days in this study could excite suppressive effects on the hypothalamus that caused progressive decrease in the testosterone level.

The results of Pergonal administration on biochemical constituents of seminal plasma of Yankasa rams are presented in Table 4. There were significant differences (P<0.05) among the treatment groups in urea, ammonia glycerylphosphorylcholine, fructose, ascorbic acid, sodium, potassium and bicarbonate levels in the seminal plasma.

The lowest value of 34.10 (mg/100ml) in urea was observed in rams on the control treatment. The highest value (42.20mg/100ml) for urea in seminal plasma obtained in this study was within the normal range of 43-75mg/100ml reported by Roller *et al.* (1982) to be normal for rams. Cortada *et al.* (2000) reported that sharp increase in plasma urea level could result in gonadal degeneration and infertility, with reduced sperm production and loss of libido.

There were no significant differences (P>0.05) among rams on 0.66ml, 0.99ml and 0.33ml Pergonal treatments in ammonia levels. The lowest value (1.80mg/100ml) was observed in rams on the control treatment.

Table 4: Effect of Pergonal Administration on biochemical constituents of seminal plasma of Yankasa rams.

Parameter	Treatment (Pergonal I.U)				SEM
	T_1 0.00	T_2 57.78	T_3 118.5	T_4 173.34	
Urea (mg/dl)	34.10[b]	34.30[b]	40.20[ab]	42.20[a]	2.06
Ammonia (mg/dl)	1.80[b]	1.85[ab]	2.00[a]	2.00[a]	0.05
Glycerylphosphoryl Choline (mg/ dl)	925.00[b]	935.00[b]	1020.00[a]	1030.00[a]	2758
Fructose (mmol/L)	450.00[c]	460.00[bc]	480.00[ab]	490.00[a]	9.13
Ascorbic acid (mg/dl)	5.60[c]	6.20[bc]	7.40[ab]	8.00[a]	0.55
Sodium (mmol/L)	43.00[b]	43.00[b]	45.00[ab]	48.00[a]	1.18
Potassium (mmol/L)	4.12[b]	5.65[a]	5.75[a]	5.85[a]	0.41
Bicarbonate (mmol/L)	22.20[b]	23.15[b]	25.00[a]	26.00[a]	0.86

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

Glycerylphosphorylcholine values for rams on T_3 (1020.00mg/100ml) and T_4 (1030mg/100ml) were similar (P>0.05) but differed significantly (P<0.05) from the values for rams on T_1 (925.00mg/100ml) and T_2 (935.00mg/100ml) which were equally similar (P>0.05). The level of glycerophosphocholine in all the groups indicated that sperm maturation and motility were enhanced by the administration of Pergonal injection (Kidd, 2005).Rams on T_4 recorded the highest value (490.00mg/100ml) in seminal plasma fructose while the lowest value of 450.00mg/100ml in seminal plasma fructose was observed in rams on the control treatment (T_1). Significant differences (P<0.05) existed among treatment groups. Owen and Katz (2005) reported that fructose is a measure of seminal vesicle function, being a source of energy to the sperm. Significant differences (P<0.05) were observed among the treatment groups in the levels of ascorbic acid in the seminal plasma. Rams on T_4 recorded the highest value (8.00mg/100ml) while the lowest value of 5.60mg/100ml in ascorbic acid was observed in rams on the control treatment (T_1). Studies have shown that vitamin C plays a vital role in increasing semen volume, sperm concentration and motility in rams and goats (Sonmez and Demirci, 2003; Fazeli *et al*., 2010) and keeping them strong by protecting them from free radicals (Dawson *et al*., 1992; Fazeli *et al*., 2010). Results of this study indicate that the administration of Pergonal in rams enhanced the concentration of ascorbic acid in the seminal plasma which is very vital in assessing semen quality and fertility in male animals.

There were significant differences (P>0.05) among the treatment groups in sodium, potassium and bicarbonate levels in the seminal plasma. The highest values for sodium (48.00mmol/l), potassium (5.85mmol/l) and bicarbonate (26.00mmol/l) were recorded in rams on T_4. The lowest values for sodium (43.00mmol/l), potassium (4.12mmol/l) and bicarbonate (22.20mmol/l) were recorded in rams on T_1. A positive and significant correlation has been established between sodium concentration and sperm concentration in rams (Akpa *et al*., 2013). This trend was equally observed in this study. For potassium, the values also increased as the level of Pergonal administered. Increasing potassium concentration in the seminal plasma is negatively correlated with progressive motility of sperm in rams and bucks while sodium has the opposite effect (Abdel-Rahman *et al*., 2000; Akpa *et al*., 2013). Bicarbonate values in the seminal plasma increased significantly (P<0.05) as the dosage of Pergonal increased and were higher than 20.00mmol/l recorded by Okamura *et al.* (2006), who also inferred that sodium bicarbonate in seminal plasma stimulates sperm motility. This is in agreement with the findings of this study. It was observed that apart from non-protein nitrogen, other biochemical constituents of seminal plasma increased significantly (P<0.05) in value as the level of Pergonal administered increased.

CONCLUSION

The results of this study indicate that the administration of gonadotrophin (Pergonal®) enhanced semen quality of the Yankasa ram at the level of 0.66mls. The main intention of the administration of Pergonal® was to enhance spermatogenesis and improve semen quality. The administration was not detrimental to the hormonal profile and seminal plasma constituents of the rams. Though most of the values obtained fall within the normal ranges for adult sheep, there is need to continuously monitor hormonal profiles of Yankasa rams under Pergonal treatment for spermatogenesis.

CONFLICT OF INTEREST

There is no conflict of interest with regards to the publication of this study

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