Effect of Gonadotrophin (Pergonal®) on Haematological and Serum Biochemical Parameters of Mature Ouda Rams Treated for Sperm Production

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Abstract— *Twelve Ouda rams aged 2 – 2.6 years and weighed between 40.21 – 40.32kg were randomly distributed into 3 groups of 4 animals with one ram per replicate in a completely randomized design and used to determine the effect of Pergonal® on haematology and serum biochemistry. These groups were assigned to 3 levels of Pergonal® injection as treatments. The injections were 0.00i.u, 49.50i.u, and 99.00i.u Pergonal® represented as T_1 (control), T_2, and T_3, respectively. All the treatments were administered by intramuscular injections. The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. The results of the study showed that apart from Alanine transaminase and eosinophils, the haematological and serum biochemical parameters and immune status of ouda rams may be affected when 49.50i.u or more of Pergonal are used for induction of spermatogenesis. These parameters should be constantly monitored during pergonal administration in ouda rams.*

Keywords— Pergonal, Blood profile, Ouda rams.

I. INTRODUCTION

The Ouda sheep is a large long-legged breed with a convex facial profile found in the Sudan Savannah zone especially in the North Western part of Nigeria. It has a characteristic pie-coat colour pattern of an entirely black or brown head and fore quarters and white hind quarters. The ears are large, long and droopy. Mature males have horns while females are normally polled (hornless). It has thin tail; the rump is short and extremely sloppy. Mature rams measure 30-60kg, while the females can weigh 30-45kg (Oni, 2002).

In order to carry out any sustainable improvement in livestock, there should be methods of ensuring the repeatability and multiplication of desired traits in subsequent generations. Reproduction is a process by which an organism gives rise to a new member of its species. It is a vital factor in determining the efficiency of animal production and its performance is closely related to profitability in livestock enterprise (Iheukwumere *et al.*, 2008). In view of the increasing use of livestock for specialized production, there is need for more practical and better control methods of reproduction.

Sperm formation involves the use of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Iheukwumere *et al.*, 2004). Most of these preparations of FSH and LH are very expensive. Some of them require cold chain storage and often deteriorate because of inadequate storage and handling (Herbert *et al.*, 2000).

Diclair®, also known as Humegon or Mentrophin and with similar constituents as plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of follicle stimulating hormone and luteinizing hormone in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH in Diclair® play vital role in the initiation of spermatogenesis. The hormone preparation is cheap, readily available and does not require cold chain storage (Iheukwumere, 2005).

It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the blood parameters of treated rams. This study was therefore carried out to determine the effect of Pergonal® administration on haematology and serum biochemistry of mature Ouda rams.

II. MATERIALS AND METHODS

2.1 Experimental Animals and their Management

Twelve healthy, sexually matured Balami rams aged 2-3 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 two times within the experimental period. The actual

experimental period lasted for complete 70 days. Routine inspection for cleanliness was carried out. Freshly cut forage consisting of *Panicum maximum, Aspilia africana, Pennisetum purpureun* (Elephant grass) was fed as basal diet and a concentrate ratio 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* to the animals.

2.2 Experimental Design and Drug Administration

The twelve Ouda rams were divided into 3 experimental groups consisting of 4 animals per group which were further divided into two replicates of two rams each in a Completely Randomized Design (CRD). These groups were assigned to 3 levels of Pergonal® as treatments. The levels of Pergonal® were 0.00i.u, 49.50i.u and 99.00i.u represented as T_1, T_2, and T_3 respectively. T_1, which contained no Pergonal® served as the control. The rams were treated by intramuscular injections. The injections were given as follows:

Pergonal was supplied in 5 vials, each vial containing FSH 75i.u and LH 75i.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 75i.u plus PLH 75i.u per ml.

All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one ml syringe with 0.01ml graduation.

TABLE 1
DOSES OF PERGONAL ® ADMINISTERED TO MATURE OUDA RAMS.

	Treatment Dosage (ml)		
Day	**T_1**	**T_2**	**T_3**
1	0.00	0.11	0.22
2	0.00	0.11	0.22
3	0.00	0.11	0.22
Total	0.00	0.33	0.66

TABLE 2
CONCENTRATION OF PERGONAL®ON MATURE OUDA RAMS.

	Concentration of Personal® (i.u)		
Day	**T_1**	**T_2**	**T_3**
1	0.00	16.50	33.00
2	0.00	16.50	33.00
3	0.00	16.50	33.00
Total	0.00	49.50	99.00

2.3 Blood Collection and Haematological Analysis

The rams were bled one week after Diclair® injection between 9am and 10.30am from punctured jugular vein and aspirated about 7ml of blood from each ram. Two milliliters of each blood sample were poured into Bijou bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematological evaluation. The remaining 5mls of each blood sample were allowed to coagulate to produce sera for blood chemistry analysis.Blood samples were analyzed within 2 hours of their collection for packed cell volume (PCV) and haemoglobin (Hb). Erythrocyte or red blood cells (RBC) and leucocyte counts were determined as described by Jain (1986). Erythrocyte count was done in a haemocytometer chamber placed under a light microscope. Packed cell volume was determined by the microhaematocrit method (Jain, 1986) with 75 x 16mm capillary tubes filled with blood and centrifuged at 3000rpm for 5 minutes. Haemoglobin concentration was also determined by the cyanmethemyoglobin method (Jain, 1986). The various red cell indices like mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from RBC, Hb and PCV (Lazzaro, 2003). Total leucocyte count was carried out using a Neubaer haemocytometre placed under a light microscope under x 10 magnification, after using Natt and Henricks dilution to obtain a 1:200 blood dilution. Differential leucocyte count was achieved using blood smears stained with Wright's dye and each type of cell (neutrophil, lymphocyte, eosinophil, monocyte and basophil) was determined with a counter.

2.4 Evaluation of Blood Chemistry

The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for biochemical evaluation, urea, calcium, cholesterol, total bilirubin, conjugated bilirubin, Alkaline phosphatase, Alanine transaminase and Aspartate transaminase concentrations were determined using the analytical kits of Randox Laboratories Limited Crumin. Co. Anthrax, UK at MOUAU Medical Laboratory Umuahia, Nigeria.

2.5 Data Analysis

Data collected on haematology and serum biochemistry of Ouda rams were subjected to 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).

III. RESULTS AND DISCUSSION

The results of Pergonal® administration on haematological parameters of mature Ouda rams are shown in Table 3.

There were significant differences ($P<0.05$) among the treatment groups in haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC), red blood cells (RBC) means corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) values.

Rams on T_3 recorded the highest value of 13.20 (g/dl) in Hb and this differed significantly ($P < 0.05$) from rams on T_1 which were similar ($P > 0.05$) to rams on T_2 in Hb values. There was no significant difference ($P > 0.05$) between rams on T_3 and T_2 in Hb values. The lowest value in Hb was observed in rams on T_1 (11.17 g/dl). The Hb values obtained in this study were within the normal range of (9.0 – 15.0 g/dl) reported by Radostits *et al.* (1997) in sheep. Haemoglobin concentration in the blood has been associated with availability of nutrients to the animal (Esonu *et al.,* 2001; Iheukwumere and Herbert, 2002).

Rams on T_3 recorded the highest value of 44.67% in PCV and this differed significantly ($P<0.05$) from rams on T_1 which were similar ($P>0.05$) to rams on T_2 in PCV values. There was no significant difference ($P>0.05$) between rams on T_3 and T_2 in PCV values. The PCV values obtained in this study were within the normal range of 27.0–45.0% reported in sheep by Radostits *et al*. (1997). This suggests that the test drug did not have adverse effect on the physiological state of the animals.

Rams on T_2 recorded the highest value of 4.83 ($x10^9$/L) in WBC and this differed significantly ($P<0.05$) from rams on T_3 which were similar ($P>0.05$) to rams on T_1 in WBC values. There was no significant difference ($P > 0.05$) between rams on T_2 and T_1 in WBC values. The WBC values obtained in this study were within the normal range of 4-12 ($x10^9$/L) reported in sheep by Radostits *et al.* (1997). This was an indication that Pergonal injections were tolerated by the rams. However, the WBC values obtained in this study were lower than the value of 7.38 ($x10^9$L) reported in West African Dwarf sheep by Oguike and Ude (2008) and lower than the value of 11.70 ($x10^9$/L) reported by Taiwo and Ogunsami (2003) in WAD sheep in South Western Nigeria.

Abnormal production of white blood cells in the blood of animals is usually associated with immune response by animals due to the presence of an antigen (foreign body) in the body. The normal values of WBC obtained in this study depicts absence of infections since elevation of white blood cells suggests infection by microorganisms especially bacteria (Aka *et al.,* 2008; Sowande *et al.,* 2008).

TABLE 3
EFFECT OF PERGONAL® ON THE HAEMATOLOGY OF MATURE OUDA RAMS

Parameters	Treatment Pergonal® i.u			
	T_1 0.00	T_2 49.50	T_3 99.00	SEM
Hb (g/dL)	11.17^b	12.33^{ab}	13.20^a	0.59
PCV (%)	42.00^b	43.00^{ab}	44.67^a	0.78
WBC ($x\ 10^9$/L)	4.70^{ab}	4.83^a	4.67^b	0.05
RBC ($x10^{12}$/L)	11.80^b	11.93^b	12.30^a	0.12
MCH (Pg)	9.47^b	10.34^{ab}	10.73^a	0.37
MCV (fl)	27.93^b	28.67^{ab}	29.55^a	0.47
MCHC (g/dl)	33.90^b	36.04^{ab}	36.32^a	0.77

^{ab}: Means within row having different superscript are significantly ($P < 0.05$) different. SEM = Standard error of means.

Rams on T_3 recorded the highest value of 12.30 ($x10^{12}$/L) in RBC 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 RBC values. The lowest value of 11.80 (x 10^{12}/L) in RBC was values observed in rams on T_1. The RBC values obtained in this study were within the normal range of 9.0- 15.0 ($X10^{12}$/L) Reported by Radostits *et al*. (1997) in sheep, but were hiher than the range of 2.32± 0.18-3.17±0.09 (x10 ^{12}/L) reported by sowande *et al.* (2008) in WAD sheep. This may be attributed to differences in breed and nutritional status of the rams. It could also be that the test drug increased efficient utilization of nutrient since the lowest value in RBC was observed in rams on the control treatment (T_1) ($11.80X10^{12}$/L).

Rams on T_3 recorded the highest value of 10.73 (pg) in MCH and this differed significantly (P<0.5) from rams on T_1which were similar (P>0.05) to rams on T_2 in MCH values. There was no significant difference (p >0.05) between rams on T_3 and T_2 in MCH values. The lowest value in MCH was observed in rams on T_1 (9.47 pg). The MHC values obtained in this study were within the normal ranges of 8-12 (pg) reported in sheep by Radostits *et al.* (1997).

Rams on T_3 recorded the highest value of 29.55 (fl) in MCV and this differed significantly (P<0.5) from rams on T_1 which were similar (P>0.05) to rams on T_2 in MCV values. There was no significant difference (P>0.05) between rams on T_3 and T_2 in MCV values. The lowest value in MCV was observed in rams on T_1 (27.93fl). The MCV obtained in this study were within the normal range of (28-40fl) reported for sheep (Radostits *et al.,* 1997).

Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro, 2013).

Rams on T_3 recorded the highest value of 36.32 (g/dl) in MCHC and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_2 in MCHC values. There was no significant difference (P>0.05) between rams on T_3 and T_2 in MCHC values. The lowest value in MCHC was observed in rams on T_1 (33.90 g/dl). The MCHC values obtained in this study were within the normal range of 31.0-34.0(g/dl) reported in sheep by Radostits *et al.,* (1997).

The results of differential leucocyte count of Ouda rams treated with gonadotrophin (Pergonal®) are shown on Table 4.

There were significant differences (P<0.05) among the treatment groups in neutrophil, lymphocyte and monocyte counts. Eosonophil counts were similar (P>0.05) among the treatment groups.

Rams on T_1 had the highest neutrophil value of 44.80% and this differed significantly (P<0.05) from rams on T_2 which were similar (P>0.05) to rams on T_2 in neutrophil values. There was no significant difference (P>0.05) between rams on T_1 and T_3 in neutrophil values. The neutrophil values obtained in this study were within the normal range of 10-50% (Radostits *et al.,* 1997) or 0.70-6.00 ($x10^3$ µL) reported for sheep (Merck, 2010). Neutrophils have phagocytic and bactericidal capabilities which mean that they play an important role in inflammatory conditions. They are very important for defense whenever acute infection is present (Banerjee, 2005).

Results showed that rams on T_3 had the highest lymphocyte value (53.50%) and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_2. There was no significant difference (P>0.05) between rams on T_3 and T_2 in lymphocyte values. The lymphocyte values obtained in this study were within the normal range (40–75%) for sheep (Merck, 2010). This suggests that the administration of the test drug was not detrimental to the functioning of the immune system (Iheukwumere *et al.,* 2008). White blood cell and lymphocyte counts are known to increase during infection. The highest eosinophil count was recorded by rams on T_2 and it did not differ significantly (P> 0.05) from rams on T_1 and T_3 which were also similar (P>0.05) to each other. These values were within the normal range of 1–10% for sheep (Merck, 2010). This is an indication that administration of Pergonal® did not trigger allergic reactions in the rams.

Monocyte counts showed that rams on T_2 had the highest value (3.25%) and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to those on T_3. There was no significant difference (P>0.05) between rams on T_2 and T_3 in monocyte values. The monocyte values obtained in this study were within the normal range of 0 – 6% or 0–0.75 ($x10^3$/L) reported for sheep (Radostits *et al.,* 1997; Merck 2010). The observed variations in monocytes counts may be attributed to other physiological factors (Mahmood *et al.,* 1994; Egbe-Nwiiyi *et al.,* 2000) rather than Pergonal®.

Basophils were not detected among the treatment groups.

The results of serum biochemical parameters of Ouda rams are shown in Table 5.

Rams on T_2 had the highest urea value (5.67mmol/L) and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_3. There was no significant difference (P>0.05) between rams on T_2 and T_3 in urea values. Serum urea values obtained in this study were within the normal range of 2.85–7.14 (mmol/L) reported in sheep (Kaneko *et*

al., 1997). High level of urea in the blood have been reported to indicate a lowered utilization of protein, poor protein quality or excess protein catabolism associated with protein deficiency (Oduye and Adadevoh, 1976; Oduguwa *et al.,* 1999; Ahamefule *et al.,* 2005).

Serum glucose concentration was highest in rams on T_3 (3.86mmol/L) and this differed significantly ($P<0.05$) from rams on T_2 which were similar ($P>0.05$) to rams on T_1 in glucose values. There was no significant difference ($P>0.05$) between rams on T_3 and T_1 in glucose values. The values did not follow a definite trend but were within the normal range of 2.78–4.44 (mmol/L) reported for sheep (Kaneko *et al.,* 1997). Glucose is one of the metabolites measured as an indicator of the energy status of animals. Normal glucose levels in the rams indicate adequate synthesis in the liver from proprionate metabolism as the major glucose precursor (Sowande *et al.,* 2008).

TABLE 4
EFFECT OF PERGONAL® ON DIFFERENTIAL LEUCOCYTE COUNT OF MATURE OUDA RAMS

Parameters	Treatment (Pergonal® i.u)			
	T_1 0.00	T_2 49.50	T_3 99.00	SEM
Neutrophils (%)	44.80^{a}	40.50^{b}	43.34^{ab}	1.26
Lymphocytes (%)	52.50^{b}	53.30^{ab}	53.50^{a}	0.31
Eosinophils (%)	2.60	2.95	2.61	0.12
Monocytes (%)	0.10^{b}	3.25^{a}	0.55^{ab}	0.98
Basophils (%)	0.00	0.00	0.00	0.00

[ab]:Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

TABLE 5
EFFECT OF PERGONAL®ON SERUM BIOCHEMICAL PARAMETERS OF MATURE OUDA RAMS.

Parameters	Treatment (Pergonal® i.u)			
	T_1 0.00	T_2 49.50	T_3 99.00	SEM
Urea (mmol/L)	4.33^{b}	5.67^{a}	5.16^{ab}	0.39
Glucose (mmol/L)	3.56^{ab}	3.20^{b}	3.86^{a}	0.19
Cholesterol (mg/dl)	57.00^{a}	45.00^{b}	48.67^{ab}	3.55
Calcium(mmol/L)	2.89^{b}	3.18^{a}	3.19^{a}	0.09
Alkaline	68.50^{b}	70.30^{ab}	73.42^{a}	1.44
Alanine transaminase (iu/L)	30.33	30.33	30.33	0.00
Aspartate transaminase (iu/L)	112.00^{b}	124.00^{a}	118.00^{ab}	3.47

[ab]: Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Rams on T_1 had the highest level of cholesterol (57.00mg/dl) and this differed significantly ($P < 0.05$) from rams on T_2 which were similar ($P > 0.05$) to rams on T_3. There was no significant difference ($P > 0.05$) between rams on T_1 and T_3 in cholesterol levels. Serum cholesterol values obtained in this study were within the normal range of 40 – 58 (mg/dl) reported in sheep by Kaneko *et al.* (1997). Cholesterol level in the serum has been associated with the quality and quantity of fat in the diet (Esonu *et al.,* 2001). High cholesterol level in the serum has been implicated in the etiology of arteriosclerosis and other heart diseases in man (Mc Donald *et al.,* 1995; Ramos *et al.,* 2003).

Serum calcium values obtained in this study were within the normal range of 2.88 – 3.20 (mmol/L) reported in sheep (Kaneko *et al.,* 1997) but lower than the value 9.60 $\pm$ 1.60 reported for WAD sheep (Oduye and Adadevoh, 1976). The similarity observed in rams on T_2 and T_3 indicates probable electrolyte balance in the animals' body caused by gonadotrophin administration. This observation is in agreement with the report of Iheukwumere *et al.* (2004) in goats.

Rams on T_3 had the highest value of 73.42 (iu/L) in Alkaline phosphatase and this differed significantly ($P<0.05$) from rams on T_1 which were similar ($P>0.05$) to rams on T_2. There was no significant difference ($P>0.05$) between rams on T_3 and T_2 in Alkaline phosphatase values. The values obtained in this study were within the normal range of 68 – 387(iu/L) reported in sheep by Radostits *et al.,* (1997) but higher than the value (49.67 iu/L) reported by Oguike and Ude (2008) in WAD ewes. Alkaline phosphatase assay is useful in the diagnosis of obstructive liver diseases (Murray *et al.,* 2003).

Alanine transaminase values did not differ significantly (P>0.05) among the treatment groups. The values obtained in this study were within the normal range of 30 $\pm$ 4.0 (iu/L) reported in sheep by Kaneko *et al.*, (1997). Increase in Alanine transaminase value would signify necrosis or myocardial infarction (Sokumbo and Egbunike, 2000). Alanine transaminase assay is important in the diagnosis of liver damage caused by drug toxicity or harmful chemicals (Nelson and COX, 2005).

Rams on T_2 recorded the highest value of 124.00 (iu/L) in Aspartate transaminase and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_3. There was no significant difference (P > 0.05) between rams on T_2 and T_3 in Aspartate transaminase values. The values obtained in this study were within the normal range of 60 – 280 (iu/L) reported in sheep (Kaneko *et al.*, 1997). In this regard, Pergonal® can be said to be considered safe for the rams.

IV. CONCLUSION

From the results of this study it can be concluded that Gonadotrophin (Pergonal®) had no deleterious effects on haematological and serum biochemical parameters of Ouda rams. Though most of the values obtained fall within the normal ranges for adult sheep, the variations observed suggest the need to constantly monitor blood profile of Ouda rams under Pergonal treatment for sperm production.

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