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EFFECT OF GONADOTROPHIN (PERGONAL®) ON HAEMATOLOGY, IMMUNE/LEUCOCYTE STATUS AND SERUM METABOLITES OF MATURE WEST AFRICAN DWARF RAMS TREATED FOR SPERM PRODUCTION

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Summary: *Twenty–four (24)West African Dwarf (WAD) rams aged 2 – 3 years were used to determine the effect of gonadotrophin (Pergonal®) on haematology, immune /leucocyte status and serum metabolites of mature rams treated for sperm production. The Completely Randomized Design (CRD) was used to assign the rams to 4 treatment groups consisting of 6 rams per treatment with one ram per replicate. These groups were assigned to 4 levels of Pergonal® injection as treatments. The doses were 0.00 u/L, 19.26 u/L, 39.00u/L and 57.78 u/L Pergonal® represented as T_1 T_2, T_3 and T_4 respectively. The group which received 0.00 I.U Pergonal® served as the control. All the treatments were administered by intramuscular injections. The results showed significant differences (P < 0.05) among the treatment groups in Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cell (WBC) and Mean Corpuscular Volume (MCV) values. However, there were no significant differences (P > 0.05) among the treatment groups in red blood cells (RBC), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) values. The highest values for PCV (48%), Hb (16.25g/dl),WBC (11.5 $x10^9$/L),RBC (5.35 $x10^{12}$/L) and MCV(90.50fl)were obtained from rams on 39.00 I.U Pergonal(T_3).There were also no significant differences (P>0.05) among the treatment groups in neutrophils, eosinophils, and monocytes values. However, there were significant differences (P<0.05) among the treatment group in lymphocyte values which increased as the administered dosage of Pergona®l increased. The result further showed that there were significant differences (P<0.05) among the treatment groups in serum cholesterol, glucose, calcium, total bilirubin and conjugated bilirubin values. Cholesterol values increased significantly as the dosage of Pergona®l increased. The results of this study showed that apart from RBC, MCH, MCHC, neutrophils, and basophils, the haematological and serum biochemical parameters, and immune status of West African dwarf rams may be affected when 19.26 u/L or more of Pergonal® are used for induction of spermatogenesis, and so they should be constantly monitored*

Key words: *Pergonal, haematology, immune status, serum metabolites, WAD rams.*

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INTRODUCTION

The West African dwarf (WAD) sheep is a hardy animal with a compact body, small and short-legged. It is predominantly found in the humid zone of southern Nigeria. It is the smallest of the Nigerian breeds with a mature body weight of about 15 to 25kg in ewes and 20 – 30kg in rams (Oni, 2002). The breed is believed to be tolerant to trypanosomes because they survive in the heavily tse-tse flies infected forest areas of Southern Nigeria (Charray et al., 1992). They contribute about 11% of the total meat supply in Nigeria (Adu and Ngere, 1979).

The significance of determining hematological and serum biochemical indices of domestic animals have been well documented (Obi and Anoso, 1980; Coles, 1986; Taiwo and Anosa, 1988). Similarly various reports: (Kalamu et al.., 1988; Aba – Adulugba and Joshua, 1990; Esiewo and Moor, 1990; Taiwo and Ogunsami, 2003; Nottidge et al., 1999; Egbe – Nwiiyi et al., 2000; Iheukwumere et al., 2001; Iheukwumere et al., 2004; Iheukwumere et al., 2006; Iheukwumere et al., 2008; Oguike and Ude, 2008) have documented hematological and serum biochemical parameters of domestic species in Nigeria. However, only a few were on sheep particularly the WAD sheep raised in the southern part of Nigeria. Hematological responsiveness of the animal to its external and internal environments may serve as a veritable tool for use in monitoring animal health and nutritional status (Iheukwumere and Okoli, 2002; Esonu et al., 2001; Churias, 2002).

Human Gonadotrophin (Pergonal®), a fertility drug (Ferring Labs., USA) also known as Humagon or Mentrophin with similar constituents as Plusset® is a gonadotrophin lyophilized in vials containing a mixture of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH present in Pergonal play vital roles in the initiation of spermatogenesis (Abu et al., 2006).

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 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 hematology, immune/leucocyte status and serum metabolites of WAD rams.

MATERIAL AND METHODS

Experimental Animals and their Management. Twenty – four healthy, sexually matured WAD 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 and routine inspection for cleanliness was carried out. Freshly cut forage made up of *Panicum maximum, Aspilia Africana* and *Pennisetum purpureum* was fed as basal diet and a concentrate ration of Grower Mash was used as supplement. The animals were fed twice daily, in the morning and evening. Salt lick was provided as mineral supplement. Water was given *ad libitum* to the animals.

Experimental Design and Drug Administration. Twenty-four WAD rams were divided into 4 treatment groups consisting of 6 rams per group with one ram per replicate in a Completely Randomized Design (CRD). These groups were assigned to 4 levels of Pergonal injection as treatments. The levels of Pergonal were 0.00 u/L,19.26u/L, 39.00u/L and 57.78u/L Pergonal® represented as T_1 T_2, T_3 and T_4 respectively. The group which received 0.00 I.U Pergonal (T_1) served as the control.

Pergonal was supplied in 24 vials, each vial containing FSH 75u/L and LH 75u/L. The content of each vial was dissolved in 1 ml of physiological saline solution immediately prior to use.

Group T_1: Each ram received 1.00ml of physiological saline for 3 days.

Group T_2: Each ram received 5.24 u/L of Pergonal (0.03ml) on the first day. Second day, the group received 7.01 u/L Pergonal (0.04ml),while on the 3rd day, the group also received 7.01 u/L of Pergonal (0.04m) giving a total of 19.26 u/L (0.11ml) Pergonal injections within 3 days.

Group T_3: Each ram received 10.00 u/L of Pergonal (0.06ml) on the first day. Second day, the group received 12.50 u/L of Pergonal (0.07ml). While on the 3rd day, the group received 17.00 u/L of Pergonal (0.09ml) giving a total of 39.00 /u/L (0.22ml) Pergonal injection within 3 days.

Group T_4: Each ram received 19.26 u/Lof Pergonal (0.11ml) on the first day. Second day, the group received 19.26 u/L of Pergonal (0.11ml), while on the third day, the group also received 19.26 u/L of Pergonal (0.11ml) giving a total of 57.78 u/L (0.33ml) of Pergonal injections within 3 days. All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a 1ml syringe with 0.01 ml graduation.

*Blood Collection and Haematological Analysis***.** The rams were bled one week after Pergonal injection between 9am and 10.30am from a punctured jugular vein and aspirated about 12ml 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 10ml 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 3000 rpm 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 haemocytometer 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.

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. Total bilirubin, conjugated bilirubin, cholesterol, glucose and calcium concentrations were determined using the analytical kits of Randox Laboratories Limited Crumin. Co Anthrax, UK at HASTO Medical Laboratory Enugu, Nigeria.

Statistical analysis. Data collected on haematological and serum biochemical parameters of WAD 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).

RESULTS

The results of the haematological parameters of West African dwarf rams treated with gonadrotrophin (Pergonal ®) are presented in Table 1.

Table 1. Effect of Pergonal on the haematology of WAD rams

Parameters	Treatment (Pergonal u/L)				SEM
	T1	T2	T3	T4	
	0.00	19.26	39.00	57.78	
PVC (%)	40.25b	46.00a	48.00a	48.00a	1.85
Hb (x10 g/L)	13.40b	14.58a	16.25a	16.05a	0.84
WBC (x 109/L)	10.33a	7.13b	11.50a	10.40a	0.94
RBC (x 1012g/L)	4.48	4.85	5.35	5.30	0.34
MCV (fL)	84.25b	90.00a	90.50a	90.40a	1.52
MCH (pg)	30.00	30.00	30.00	30.00	0.00
MCHC (x10g/L)	33.30	33.33	33.33	33.33	0.01

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

There were significant differences (P<0.05) among the treatment groups in packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC) and mean corpuscular volume (MCV) values. However, there were no significant differences (P>0.05) among the treatment groups in red blood cell (RBC), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values.Rams treated with Pergonal had higher PCV values which differed significantly ($p < 0.05$) from the rams on the control treatment but were similar ($p>0.05$). Rams that received 39.00 u/L Pergonal (T_3) recorded the highest value of 16.25 (x10 g/L) in haemoglobin and this differed significantly ($P < 0.05$) from rams on T_1 which had 13.40 (x10 g/L). However, there were no significant differences ($P > 0.05$) among rams on T_4, T_3 and T_2 in Hb values.The WBC values did not differ significantly ($P>0.05$) among rams on T_1, T_3 and T_4 but they differed significantly ($p<0.05$) from rams on T_2.Rams on T_3 recorded

the highest RBC value of 5.35 (x 10^{12}g/L), the lowest value was observed in rams on T_1 (4.48 x 10^{12}g/L). There were no significant differences (P>0.05) among the treatments.

The highest MCV value (90.50fL) was recorded in rams on T_3which did not differ significantly (P>0.05) from T_2 (90.00 fL) and T_4 (90.40) but they differed significantly (P<0.05) from rams on T_1 (84.25fL). There were no significant differences (P>0.05) among the treatment groups in mean corpuscular haemoglobin (MCH). All the treatments had the same value (30.00pg). There were also no significant differences (P > 0.05) among the treatment groups in MCHC values.They allhad a common value of 33.00 (x10 g/L).

The effects of Pergonal on differential leucocyte count of WAD rams are presented in Table 2.

Table 2. Effect of Pergonal on differential leucocyte count of WAD rams

Parameters	Treatment (Pergonal u/L)				SEM
	T_1	T_2	T_3	T_4	
	(0.00)	(19.26)	(39.00)	(57.78)	
Neutrophils (%)	43.25	41.00	40.00	41.66	1.73
Lymphocytes (%)	54.50b	55.00b	58.00a	58.24a	0.95
Eosinophils (%)	1.75	2.50	1.25	1.20	0.30
Monocytes (%)	0.50c	1.50a	0.75bc	1.46ab	0.25
Basophils (%)	0.00	0.00	0.00	0.00	--

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

Rams on the control treatment (T_1) recorded the highest neutrophil (43.25%) followed by T_4(41.66%), T_2(41.00%) and T_3 (40.00%) respectively. The values did not differ significantly (p > 0.05). Lymphocyte count increased as the amount of Pergonal administered on the rams increased. Rams on T_4 recorded the highest lymphocyte count (58.24%) which did not differ significantly (p>0.05) from rams on T_3 (58.00%) but they differed significantly (p<0.05) from T_1 (54.50%) and T_2 (55.00%).Eosinophil counts in the rams increased from 1.75% in T_1 to 2.50% in T_2, and then declined to 1.20% in T_4. Significant differences (p>0.05) were not observed among the treatment groups. Monocyte count was highest in rams on T_2(1.50%) and this differed significantly (P<0.05) from rams on T_1 and T_3 which were similar (P> 0.05) to each other. However, there was no significant difference (P>0.05) between rams on T_2 and T_4 in monocyte values. The lowest value in monocyte count was observed in rams on T_1 (0.50%).

The results of serum metabolites of WAD rams treated with gonadotrophin (Pergonal®) are shown in Table 3.

Table 3. Effects of Pergonal on Serum Metabolites of West African Dwarf Rams

Parameters	Treatment (Pergonal u/L)				SEM
	T_1	T_2	T_3	T_4	
	0.00	19.26	39.00	57.78	
Urea (mmol/L)	3.08[a]	2.70[b]	2.73[b]	3.05[a]	0.10
Glucose (mmol/L)	3.68[a]	2.48[b]	3.18[b]	2.30[b]	0.32
Cholesterol (mmol/L)	1.48[b]	1.33[b]	2.10[a]	2.30[a]	0.24
Calcium (mmol/L)	2.11[b]	2.21[b]	2.30[a]	2.30[a]	0.05
Total bilirubin (mmol/L)	6.50[a]	8.45[a]	8.40[a]	5.35[b]	0.76
Conjugated bilirubin (mmol/L)	3.58[a]	3.58[a]	3.53[b]	3.53[b]	0.01

[ab]Means in the same row with different superscript are significantly (P < 0.05) different.SEM =Standard error of means

Rams on T_1 recorded the highest urea value, 3.08 (mmol/l) and this differed significantly (P<0.05) from rams on T_2 (2.70 mmol/L) and T_3 (2,73mmol/L) which were similar (P > 0.05) to each other. However, there was no significant difference (P>0.05) between rams on T_1 (3.08 mmol/L) and T_4 (3.05 mmol/L) in urea values.The highest glucose value (3.68 mmol/L) was recorded in rams placed on T_1 and this differed significantly (P<0.05) from rams on T_2 (2.48 mmol/L), T_3 (3.18 mmol/L) and T_4 (2.30 mmol/L) which were similar (P>0.05) to each other.There was no significant difference (P> 0.05) between rams on T_4 (2.30mg/dl) and T_3 (2.10 mg/dl) in serum cholesterol level.Rams on T_4 recorded the highest serum cholesterol value (2.30 mg/dl) and this differed significantly (P<0.05) from rams on T_1 (1.48 mg/dl) and T_2 (1.33 mg/dl).Rams on T_3 and T_4 recorded the highest serum calcium value of 2.30 (mmol/L) each which is similar to 2.30 – 2.90 (mmol/L) reported for sheep (Merck, 2010) but this differed significantly (P < 0.05) from the values in rams on T_1 (2.11 mmol/L) and T_2, (2.21 mmol/L) which were similar (P>0.05).The rams placed on T_1 and T_2 recorded the highest conjugated bilirubin value of 3.58 (mmol/L) each and this differed significantly (P<0.05) from rams on T_3 and T_4 which were similar (P>0.05) to each other in conjugated bilirubin values.There were no significant differences (P > 0.05) among rams on T_3, T_2 and T_1 in total bilirubin values. Rams on T_2 recorded the highest total bilirubin value of 8.45 (mmol/L) and this differed significantly (P<0.05) from rams on T_4 (5.35 mmol/L).

DISCUSSION

The packed cell volume values obtained in the Pergonal treated groups were higher than the normal range of 27.0 – 45.0 (%) reported in sheep (Radostits et al., 1997), and higher than 44.33% reported in West Africa dwarf sheep (Oguike and Ude, 2008), 35.5% reported by Taiwo and Ogunsami (2003) in WAD Sheep. This disparity in the results may not be unconnected to the differences in the sex and physiological status of these WAD sheep since the study of Oguike and Ude (2008) was on lactation in WAD sheep.

The Hb values obtained in this study were higher than the normal range of 9.0 – 15.0 (g/dl) for sheep (Radostits et al., 1997). It is possible that higher level of gonadotrophin injection increased metabolism and efficient utilization of nutrients. Haemoglobin concentration in the blood had been associated with availability of nutrient to the animal (Esonu et al., 2001; Iheukwumere and Herbert, 2002).

The white blood cell values obtained in this study were within the normal range of 4 – 12 (x 10^9/L) reported for sheep (Radostits et al., 1997). This was an indication that Pergonal injections were tolerated by the rams. The highest white blood cell value 11.50 (x10^9/L) obtained in this study (T_3) was higher than 7.38 (x 10^9/L) reported in WAD sheep (Oguike and Ude, 2008) in South Eastern Nigeria but close to 11.70 (x10^9/L) reported in WAD sheep in South Western Nigeria(Taiwo and Ogunsami, 2003). Abnormal production of white blood cells in animals is usually associated with immune response by animals due to the presence of antigen (foreign bodies) in the animal. The normal values of white blood cell obtained in this study also depicted the absence of infections since elevation of white blood cells suggests infection by micro-organisms especially bacteria (Akaet al., 2008; Sowande et al., 2008).It could be that high dose of the drug (Pergonal ®) increased efficient utilization of nutrients. The RBC values obtained in this study were higher than 2.32$\pm$0.18 – 3.17$\pm$0.09 (x10^{12}/L) reported in WAD sheep (Sowande et al., 2008).

The MCV values obtained in this study were higher than the normal range (27–40 fl) for sheep(Radostits et al., 1997; Reece and Swenson, 2004). Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro, 2003).The MCH value obtained in this study (30.00 pg) was higher than the normal range (8–12 pg) in sheep(Radostits et al., 1997; Reece and Swenson, 2004). The common MCHC value of 33.00 (g/dl) obtained in all the treatments was within the normal range of 31.0–34.0 (g/dl) reported insheep (Reece and Swenson, 2004). The observed variations in MCV and MCH values may not be unconnected to the differences in season, nutritional and physiological status of the animals as suggested by Esonu et al. (2001).

The neutrophil counts obtained in this study were within the normal range of 10 – 50% or 0.7 – 6.0($x10^3/\mu L$) reported for sheep (Radostits et al., 1997; 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 (Reece and Swenson, 2004; Banerjee, 2005).

The values of lymphocytes obtained in this study were within the normal range of 40 – 50% or 2.0–9.0 ($x10^3/\mu L$) reported for sheep(Radostits et al., 1997; Merck, 2010) but lower than 60 – 65% reported by Reece and Swenson (2004). However, the results of this study suggest that the immunity of the rams was not compromised by the administration of Pergonal. The values of eosinophil obtained in this study were within the normal range of 0 – 10% or 0 – 1.0($x10^3/\ \mu L$) reported in sheep(Radostits et al., 1997; Merck, 2010). This is an indication that the treatment did not trigger allergic reactions in the rams.

Monocyte values obtained in this study were within the normal range of 0 – 6% or 0– 0.7($x10^3/\mu L$) reported for sheep (Radostits et al., 1997; Merck, 2010). Basophils were not detected among the treatment groups.The higher and lower leucocyte counts observed in this study may be due to differences in physiological status of the rams as was reported by Mahmoodet al. (1994) and Egbe – Nwiiyi et al. (2000).

Serum urea values obtained in T_2 and T_3were lower than the normal range of 2.85–7.14 (mmol/L) reported in sheep (Kaneko et al., 1997) or 4.20 ± 1.60 (mmol/L) reported for WAD sheep (Oduye and Adadevoh, 1976; Daramola et al., 2005). It could be that Pergonal administration at these levels reduced metabolism and efficient utilization of nutrients. It has been observed that serum urea content depends on both the quantity and quality of protein supplied in the diet (Iheukwumere and Herbert, 2002).

Glucose values obtained in T_1, T_2 andT_3 were within the normal range of 2.40 – 4.50 (mmol/L) reported in sheep (Kaneko et al., 1997; Merck, 2010). The value for T_4 (2.30 mmol/L) was below the normal range for apparently healthy sheep. 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 propionate metabolism as the major glucose precursor (Sowande et al., 2008). It was also observed that the administration of Pergonal on rams lowered the glucose levels in the rams

Serum cholesterol values obtained in this study were within the normal range(1.10 – 2.30mmol/L) reported in sheep (Kaneko et al., 1997; Merck, 2010). Higher levels of Pergonal treatment increased cholesterol values in the animals. Cholesterol in the serum has been associated with the quantity and quality of fat supplied in the diet (Esonu et al., 2001).

Serum calcium values obtained in this study were lower than the normal range of 2.88 – 3.20 (mmol/L) reported in sheep (Kaneko et al., 1997) and 9.60 ± 1.60 (mmol/L) reported for WAD sheep (Oduye and Adadevoh, 1976; Daramola et al., 2005). The similarity observed in rams on T_3 and T_4 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. It also suggests that the administration of Pergonal did not interfere with calcium metabolism in the rams.

The total bilirubin values obtained in this study were within the normal range of 0.70 – 8.60 (mmol/L) reported in sheep(Kaneko et al., 1997; Merck, 2010). Total and conjugated bilirubin are indicators of protein adequacy. The normal values of total bilirubin in this study suggest adequate or sufficient protein and good quality of protein in the diet which supported basic metabolic and physiological activities.Conjugated bilirubin values obtained in this study were within the normal range of 0 – 4.6 (mmol/L) reported in sheep (Kaneko et al.,1997). High levels of conjugated bilirubin indicate that bile is not being properly excretedwhich might result in obstructions in the bile duct or gall bladder. Administration of Pergonal on rams did not produce any negative effect.

CONCLUSION

The results of this study showed that apart from RBC, MCH, MCHC, neutrophils and basophils, the haematological parameters and serum metabolites as well as immune status of West African Dwarf rams may be affected when 19.26 u/Lor more of Pergonal are used for induction of spermatogenesis. Though most of the haematological and serum biochemical values fall within the normal ranges for adult sheep, there is the need to constantly monitor the blood profile of WAD rams under Pergonal treatment for spermatogenesis.

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