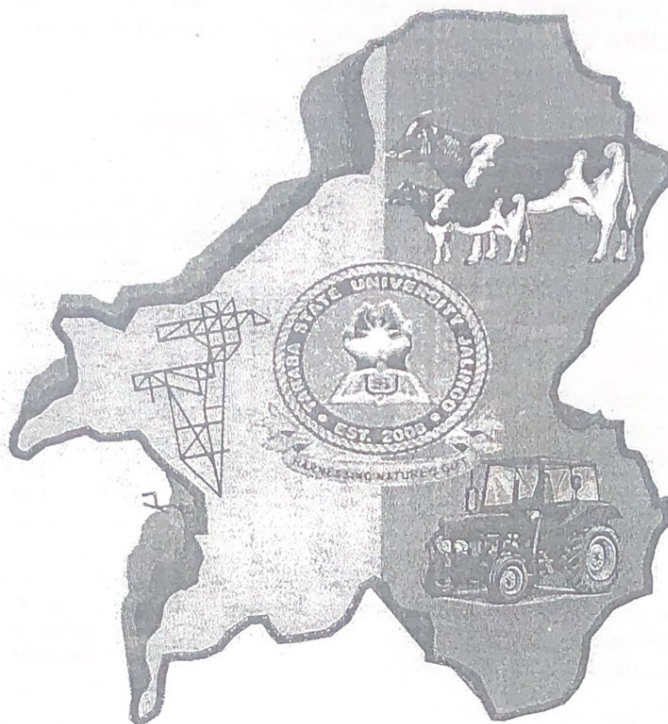


13

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EFFECT OF DEPO PROVERA® ON BLOOD CHARACTERISTICS, ESTRUS SYNCHRONIZATION AND PROGESTERONE LEVEL OF WEST AFRICAN DWARF DOES

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Abstract

Fifteen West African Dwarf Goats (WAD) were used in a completely randomized design to investigate the effect of Depoprovera® on blood characteristics, estrus synchronization and progesterone level of WAD goats. The 15 WAD goats with an initial body weight ranging between 7 and 11kg were injected with 2ml of saline solution (control), 1ml of Depoprovera® (B), 2ml of Depoprovera® (C), 1ml of Depoprovera® + 10 I.U. Oxytocin (D), and 2ml of Depoprovera® + 10 I.U. Oxytocin (E) in a 42day period for observation of estrus. Animals in group D showed signs of synchronized estrus. The results showed that group D had the highest Haemoglobin Concentration, Packed Cell Volume, White Blood Cell count and Mean Corpuscular Volume concentration. While group B had the lowest Haemoglobin Concentration, Packed Cell Volume, Red Blood Cell, White Blood Cell and Mean Corpuscular Volume. The Progesterone level was lowest in group D animals while the highest was in the control group. Also group D had the highest Total Serum Protein and Albumin index. The results suggested that 1ml of Depoprovera® + 10 I.U. Oxytocin can be effectively used to synchronize estrus in WAD goats.

Key Words: West African Dwarf does, estrus, synchronize, Depoprovera®, Oxytocin.

Introduction

In a group of randomly cycling female goats, the time of occurrence of estrus cannot be predicted with any certainty for an individual animal. Running a male of same species with a group of females is the most efficient method for estrus detection and breeding. However, where supervised mating is to be practiced or where artificial insemination is to be used, it is necessary to know when a doe is in estrus. To synchronize estrus in such does will require manipulating the estrous cycles by administering exogenous hormones.

Estrus synchronization (ES) in livestock focuses on the manipulation of either the luteal or follicular phase of the estrus cycle. In does, opportunity for control is greater during the luteal phase, which is of longer duration and more responsive to manipulation. Strategies have been employed to extend the luteal phase by supplying exogenous progesterone with or without accompanying treatments like gonadotropin or prostaglandin, or to shorten it by prematurely regressing the existing corpora lutea (CL) (Wildeus, 2000). Successful techniques must not only establish tight synchrony, but also provide an acceptable

level upon artificial insemination or natural mating (Laboeuf *et al.*, 1998). Most of the recent developments on reproductive technique have used some hormones or imported products that are expensive for producers in the developing countries and among local farmers in terms of availability and cost (Wildeus, 2000).

Improvement in reproductive efficiency will increase the economic viability of livestock enterprise. The reproductive efficiency depends on the duration of pregnancy, survival, viability and duration of reproductive life of the animal (AL-Merestani *et al.*, 2003). To achieve the above factor, the use of modern reproduction tools such as estrus synchronization, super-ovulation amongst others will result in more offsprings. Synchronization techniques offers the opportunity to increase the efficacy of animal production because it reduces time needed for estrus detection, permits mating or artificial insemination at a predetermined time, shortens durations of kidding interval in a flock of goats, and reduces mortality at birth by avoiding breeding during extreme climatic seasons. Consequently, it permits weaning,

fattening and marketing of uniform animal groups. The most common and efficient route of progestogen application in goat is via the intravaginal sponges (Bretzlaff, 1997). The most widely used procedures for synchronization and/or the induction of estrus are 12-21 days of Flugestone acetate (FGA) or Medroxyacetate progesterone (MAP) impregnated intra vaginal sponge treatment (Romano, 1996, Romano and Benech, 1996, Romano, 1998, Romano *et al.*, 2000, Romano, 2002) and an intra muscular injection of pregnant Mare serum gonadotrophin (PMSG) or progestagen withdrawal (Aket *et al.*, 1998, Greyling and Van der Nest, 2000; Motlomeloet *et al.*, 2002) or 11 days treatment with FGA impregnated intra vaginal and an intramuscular injection of PMSG and synthetic prostaglandin (PGF_{2α}) analogue 48 hours before or at sponge withdrawal (Barilet *et al.*, 1993, Laboef *et al.*, 2003).

Even though some studies have found these two progestagen to be equally effective in the induction of estrus, ovulation and fertility (Wildeus, 1993), some researchers have found some differences between the effectiveness of the two types of progestagen sponges (Oyedijiet *et al.*, 1990; Powell *et al.*, 1996).

The use of progestagen in improving fertility, pregnancy and kidding rates will continue to be viable option in reproductive management of goats (Trusellet *et al.*, 1990; Chemineau *et al.*, 1999; Wildeus, 2000).

Estrus synchronization in goats and sheep is limited by the availability of appropriate pharmaceuticals and there is the need for use of product designed and approved for major livestock species or those for birth control in human, one of which is Depo-Provera®.

Depo-Provera® contraceptive injection contains medroxyprogesterone acetate, a derivative of progesterone, as its active ingredient. When administered at the recommended dose to women every 3 months, inhibits the secretion of gonadotropin which, in turn, prevents follicular maturation and ovulation and results in endometrial thinning. Dosage does not need to be adjusted for body weight, though women with lower body weight conceive sooner than women with higher body weights after discontinuing Depo-Provera®. After a single 150mg intramuscular (i.m.) dose of Depo-Provera® contraceptive injection, medroxyprogesterone acetate concentrations

as measured by an extracted radioimmunoassay procedure, increase to reach peak of 1 to 7 mg/ml plasma concentrations in approximately 3 weeks. The levels then decreased exponentially until they become undetectable (<100pg/ml) between 120 to 200 days following injection. Using an unextracted radioimmunoassay procedure for the assay of medroxyprogesterone acetate in serum, the apparent half-life for medroxyprogesterone acetate following i.m. administration of Depo-Provera® contraceptive injection is approximately 50 days (Population Service International, 1994).

The length of estrus cycle in goats is on average of 20 days, the length of estrus is 34-38 hours. Ovulation in goats occurs 34.5±6.6 hours after the onset of estrus (Gonzales-Stagnaro, 1984). Control of estrus in goats can be done in the mating season to synchronize cyclic does or in the non breeding season (Wildeus, 2000).

The aims of the study therefore were:

- To develop the right combination of hormones for WAD goats from readily available human birth control drugs and hormones.
- To develop the best dosage for optimum synchronization and fertilization.

Materials and Methods

The study was carried out at the Small Ruminant Unit of the Animal pavilion of the Faculty of Agriculture, University of Ilorin, Nigeria.

Fifteen (15) West African Dwarf goats were used for the experiment. The animals (does) were sexually matured and clinically sound, and aged between 2 and 3 years and weighing between 7 and 11 Kg. They were quarantined for 18 days to acclimatize, and treated against external/internal parasites and *Pestes des Petit Ruminatum* (PPR) using Ivermectin injection (i/m) and Tissue Cultured Rinderpest Vaccine (TCRV) respectively at 0.2ml per 10kg body weight. 2 ml of Vitamin B-Complex were administered (i/m) to each goat to aid feed intake while 1ml of long acting oxy-tetracycline injection was given to prevent pneumonia. The experiment was carried out in 90 days to allow for observation of animals that return to estrus.

Housing: The does were housed in pens with slatted floors and little runs were provided for exercise. The pens were cleaned with

detergent and disinfected with Lysol before the arrival of the animals.

Feeding: Water and feed were supplied using plastic bowls and wooden feeding troughs, respectively in their pens and well secure to Table 1: Gross composition of basal diet

Ingredient	% of concentrate feed
Groundnut cake	
Maize	14.00
Wheat Offal	30.00
Premix	33.00
Salt	0.25
Rice bran	1.00
Limestone	20.00
Bone meal	1.00
Total	0.75
Proximate analysis of diet (%)	100.00
Crude protein	
Crude fibre	17.35
Ether extract	10.74
Ash	4.30
	7.30

Experimental design

The does were assigned to five (5) treatment groups with three (3) replicates each in a completely randomized design (CRD). The goats were housed intensively and were checked for palpable deformities of the reproductive organ. Prior to start of the experiment, blood samples were taken from each goat (10ml); 3ml for haematological analysis and 7ml for serum biochemical analysis and subsequently at the second week of hormone administration and at the end of the experiment.

Hormone administration

Animals in Group A (Control) were given 2ml of normal saline. Group B animals were administered 1ml of Depo- provera (equivalent of manufacturer's dose in human). Group C animals had 2ml of Depo- provera. Animals in Group D were given 1ml of Depo-provera + 10 I.U. Oxytocin, while 2ml of Depo- provera + 10 I.U. of Oxytocin were administered to animals in Group E. All hormones were administered intra-muscularly on the hind-leg of each doe using a 1ml syringe with 0.02ml graduation.

Blood sample collection

The does were bled between 9.00am and 10.30am through the jugular vein before administration of test samples. 10ml of blood were collected, 3ml of each blood samples was collected into Ethylene Diamine Tetra-acetic Acid (EDTA) treated Bijou bottles for haematological evaluation. The remaining 7ml

prevent feed wastage and water spillage. The animals were fed at 3% of their body weight with concentrate feed. The composition of the basal diet (concentrate feed) is presented in Table 1.

of each blood sample were deposited in anticoagulant free plastic bottles and allowed to coagulate to produce sera for serum metabolites evaluation. Serum samples were analyzed within 2 hours of their collection.

The different levels of hormones were administered according to different treatment groups, and the animals were closely observed for signs of heat. This observation was done for 7 days, every morning (6 – 8AM) and evenings (5 – 7PM). Animals which showed signs of heat were noted and then the blood samples were collected again during this period.

Haematological, biochemical and serum progesterone analysis

Haematology, serum biochemical and Serum Progesterone concentration tests were carried out at the University of Ilorin Teaching Hospital (UITH) laboratory.

Erythrocyte (RBC) and Leucocytes (WBC) counts were determined as described by Aba-Adalugba and Joshua (1996). Packed Cell Volume (PCV) and haemoglobin (Hb) concentration were measured by the microhaematocrit and cyanmethaemoglobin methods described by Lamb (1991) while the red cell indices- Mean Corpuscular Volume (MCV), and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated as described by Mitruka and Rawnsley (1977) and Jain (1986). The immune system (Neutrophil and Lymphocyte counts) were

determined as described by Jain (1986) and Aba-Adalugba and Joshua (1996).

Serum biochemical evaluation: Total Serum Protein (TSP) was determined as described by Bauer (1982); while albumin was determined by using Bromocresol Green (BCG) method as described by Bell and Petterson (1992). Serum progesterone used levels were computed according to Scott (1955).

Statistical Analysis

All data collected were subjected to analysis using General Linear Model (GLM) Type III of Statistical Analysis System (SAS, 2002). The levels of significance were determined using the Dunnett's Multiple Range Test (Duncan, 1955).

Results and Discussion

The results of the effect of Depo-provera® on haematological parameters are shown in Table 2 while Table 3 shows the effect of Depo-provera® on the serum biochemical parameters. It was worth noting that more than 70% of the parameters evaluated showed differences but they were not significant ($p > 0.05$).

Medroxyprogesterone acetate had no significant effect on the packed cell volume which was highest in group treated with 1ml of Depo-provera+ 10 I.U. of oxytocin (group D) and lowest in group injected with 1ml of Depo-provera (group B). The high values of PCV could be linked to suggestions by Daramola *et al.* (2005) that WAD goats have tendency for compensatory accelerated production of PCV to help return the body to normal during infection. It is also of note that PCV varies proportionately with total serum protein corroborating the fact that it could be used to assess the nutritional and physiological status of the animal (Esonuet *et al.*, 2001).

Haemoglobin concentration was highest in group A (control), followed by group D and lowest in group B. The values were similar to the high value (13.2 ± 2.3 g dL⁻¹) reported by Taiwo and Ogunsemi (2003) in WAD goats. However all the values were within the range reported for average value 10.07 ± 9.26 g dL⁻¹ by Iheukwumere *et al.* (2005) in West African Dwarf goats. Haemoglobin concentration in the blood has been associated with availability of nutrients in the animal body (Esonuet *et al.*, 2001). It is possible that progesterone or its analogue increase metabolism and efficiency of nutrient utilization.

Erythrocyte counts (RBC) indicate no significant variation among the levels of administration of medroxyprogesterone, highest was in group C administered with 2ml of Depo-provera while the least was in group B treated with 1ml of Depo-provera.

White blood cell (WBC) count was highest in groups A and E, though only group D showed signs of heat with a value of $13.9 \pm 4.44 \mu\text{L}^{-1}$ and the lowest in group B with a value of $6.8 \pm 4.44 \mu\text{L}^{-1}$. However the values obtained in this study were higher than the value $7.29 \pm 0.56 \times 10^3 \mu\text{L}^{-1}$ reported by Iheukwumere (2006) in West African Dwarf goats, but falls within the range $4.0 - 13.7 \times 10^3 \mu\text{L}^{-1}$ reported by Oduyeet *et al.* (1976) for goats.

Mean Corpuscular Volume (MCV) was the same in A and E (105.50 ± 9.41 fL), though not significant. MCV was highest in D (114.20 ± 9.41 fL) and lowest in B (83.00 ± 9.41 fL) followed by C (96.00 ± 9.41 fL). MCV values obtained in this study ranged from B (83.00 ± 9.41 fL) to D (114.20 ± 9.41 fL). This value was lower than 37.50 ± 1.50 fL reported by Aba-Adulugba and Joshua (1990). When compared to the erythrocyte counts (RBC) in this study, it could be observed that MCV is an indication of the average volume of a red blood cell (Oduyeet *et al.*, 1976).

Mean Corpuscular Haemoglobin Concentration (MCHC) was not significantly different in all the treatment groups. MCHC value ranged from 33.50 ± 0.32 g dL⁻¹ in A, C and E to 34.00 ± 0.32 g dL⁻¹ in B and D. The highest value of MCHC was in groups A, C and E which were the same. This value was higher than 32.30 ± 0.5 g dL⁻¹ reported by Tambuwa *et al.* (2002) in Red Sokoto goats but closer to values of 36.60 ± 2.30 g dL⁻¹ reported by Iheukwumere *et al.* (2006) in West African Dwarf goats.

There were no significant differences ($p > 0.05$) between treatment groups in Neutrophil values. Does in treatment groups A and E ($25.80 \pm 9.30\%$) had the same value. The highest Neutrophil values was in Group B ($39.00 \pm 9.30\%$), though below the value $42.9 \pm 1.30\%$ reported by Daramola *et al.* (2006) for adult West African Dwarf goats (doe) but within the range $15.6 - 43.9\%$ reported by Mitruka and Rawnsley (1977). This was followed by D ($28.30 \pm 9.30\%$), while the lowest was in Group C ($24.5 \pm 9.30\%$).

The Lymphocyte values observed though different but not significant ($p < 0.05$) in all the treatment groups. It ranged from $61.00 \pm 8.70\%$ in Group B to $75.00 \pm 8.70\%$ in Group C. The highest value was observed in group C ($75.00 \pm 8.70\%$) followed by D ($71.20 \pm 8.70\%$) while the lowest was in group B ($61.00 \pm 8.70\%$). Groups A and E had similar values ($69.20 \pm 8.70\%$). These values were higher than the value 56.6 ± 2.1 reported by Daramola *et al.* (2005) for WAD goats (doe) but falls within the range $43.9 - 81.2\%$ reported by Iheukwumere *et al.* (2005). Immune status is a function of the leucocytes, neutrophils and lymphocytes. Lymphocytes are known to play key roles in immune defense system in both man and animals. The similarity observed in neutrophils and lymphocyte values of all the treatment groups was an indication that the hormone was not detrimental to the normal function of the immune system. This agrees with the reports of Butchar and Miles (2004) that most immunological abnormalities observed in malnutrition could be corrected after nutritional rehabilitation.

The results of the effects of Depo-provera^R on biochemical parameters of West African Dwarf does as shown in Table 4.2 reveals that there were no significant differences ($p > 0.05$) in total serum protein between goats in all the treatment groups. The total serum protein ranged between $42.00 \pm 9.34 \text{ g dL}^{-1}$ in Group A to $65.00 \pm 9.34 \text{ g dL}^{-1}$ in Group D. The highest value was observed in group D ($65.00 \pm 9.34 \text{ g dL}^{-1}$), followed by groups B ($59.00 \pm 9.34 \text{ g dL}^{-1}$), E ($50.50 \pm 9.34 \text{ g dL}^{-1}$) and C ($49.00 \pm 9.34 \text{ g dL}^{-1}$) respectively, with the lowest value recorded in A ($42.00 \pm 9.34 \text{ g dL}^{-1}$), though different but not significant ($p > 0.05$). It has been reported that serum protein content depends on both the quantity and quality of protein supplied in the diet (Iyayi and Tewe, 1998).

The highest serum albumin was observed in WAD does in group D ($39.00 \pm 3.05 \text{ g dL}^{-1}$), this was higher than the value $33.1 \pm 1.1\%$ reported by Daramola *et al.* (2005) for WAD goats

(doe) suggesting that the animals had good clotting ability of the blood and adequate ability to prevent hemorrhage (Robert *et al.*, 2000). The lowest value of $32.5 \pm 3.05 \text{ g dL}^{-1}$ was observed in group A does. The serum albumin levels of groups B ($34.50 \pm 3.05 \text{ g dL}^{-1}$), C ($35.00 \pm 3.05 \text{ g dL}^{-1}$) and E ($36.00 \pm 3.05 \text{ g dL}^{-1}$) were slightly different, though not significant ($p > 0.05$).

Chlorine ion was the same in groups A and C ($107.00 \pm 11.63 \text{ g dL}^{-1}$) which was observed to be the highest, while the lowest value was in D ($66.50 \pm 11.63 \text{ g dL}^{-1}$). The CL value of groups B and E were $88.50 \pm 11.63 \text{ g dL}^{-1}$ and $94.50 \pm 11.63 \text{ g dL}^{-1}$ respectively, there were no significant difference ($p > 0.05$) in all the treatment groups.

Similarly, HCO_3^- were the same in treatment group A and D ($20.50 \pm 1.39 \text{ g dL}^{-1}$) which happens to be the lowest while the highest value was observed in group C ($23.00 \pm 1.39 \text{ g dL}^{-1}$) followed by group B ($22.00 \pm 1.39 \text{ g dL}^{-1}$) and E ($21.00 \pm 1.39 \text{ g dL}^{-1}$) respectively. There were no significant difference ($p > 0.05$) in HCO_3^- in all the treatment groups.

Serum Potassium (K) level was the same in animals in group B and E ($5.30 \pm 0.41 \text{ mmol L}^{-1}$) which were the highest value, followed by D ($4.65 \pm 0.41 \text{ mmol L}^{-1}$) and C ($4.45 \pm 0.41 \text{ mmol L}^{-1}$) respectively. These were higher than the value $4.3 \pm 0.1 \text{ mmol L}^{-1}$ reported by Daramola *et al.* (2005) in WAD goats (doe), indicating probable electrolyte balance in the animal body caused by progesterone administration. This observation is in agreement with the reports of Iheukwumere *et al.* (2004) in goats. The lowest serum Potassium value was observed in group A ($3.90 \pm 0.41 \text{ mmol L}^{-1}$).

Serum Progesterone level was highest in the A ($3.40 \pm 1.66 \text{ mmol L}^{-1}$) while the lowest value was observed in group D ($0.90 \pm 1.66 \text{ mmol L}^{-1}$), followed by group B ($1.00 \pm 1.66 \text{ mmol L}^{-1}$). Animals in treatment groups C and E had ($3.25 \pm 1.66 \text{ mmol L}^{-1}$) and ($3.00 \pm 1.66 \text{ mmol L}^{-1}$) respectively.

Table 2: Effect of Mpa (Depo-Provera®) On Haematological Parameters Of West African Dwarf Does.

Haematological Indices	A Control	B 1ml of Depo	C 2ml of Depo	D 1ml of depo+10 I U Oxytocin	E 2ml of depo+10 I U Oxytocin	± SEM
Hb(g/dl)	13.67	10.48	11.85	13.10	11.68	1.69
PCV(%)	34.25	31.00	32.25	38.75	34.75	2.13
RBC(μL^{-1})	3.05	2.93	3.65	3.45	3.16	0.34
WBC(μL^{-1})	14.80	6.80	12.40	13.90	10.60	4.44
MCV (fL)	105.50	83.00	96.00	114.20	110.50	9.41
MCH(pg)	33.50	36.75	32.50	34.00	33.50	1.93
MCHC(g/dL)	33.50	34.00	33.50	34.00	33.50	0.32
PLAT(%)	821.00	930.00	923.00	875.00	630.00	175.20
NEUT(%)	25.80	39.00	24.50	28.30	22.80	9.30
LYMPH(%)	69.20	61.00	75.00	71.20	72.20	8.70

Table 3: effect of MPA (Depo-Provera®) on Biochemical Parameters of West African Dwarf Does.

Serum biochemical indices	A Control	B 1ml of Depo	C 2 ml of Depo	D 1ml of Depo+10 I U of Oxytocin	E 2ml of Depo+10 I U of Oxytocin	± SEM
TP (g dL ⁻¹)	42.00	59.00	49.00	65.00	50.50	9.34
ALB (g dL ⁻¹)	32.500	34.50	35.00	39.00	36.00	3.05
CL ⁻ (g dL ⁻¹)	107.00	88.50	107.00	66.50	94.50	11.63
HCO ₃ ⁻ (g dL ⁻¹)	20.50	22.00	23.00	20.50	21.00	1.39
K (mmolL ⁻¹)	3.90	5.30	4.45	4.65	5.30	0.41
PROG (mmolL ⁻¹)	3.40	1.00	3.25	0.90	3.00	1.66

Conclusion and Recommendation

From the experiment, the efficacy of the use of medroxyprogesterone acetate to synchronize estrus in WAD does can be confirmed and it can be said to be technically simple and economically cheap for easy adoption for smallholder farmers. The study indicates that 1ml of progesterone in combination with 10 I.U of Oxytocin can be used in synchronizing WAD does. This confirms the report of Powell *et al.* (1996) that estrus synchronization can be achieved in WAD does under its native environment with just a single injection of 100mg of progesterone or lower dosage in combination with 10 i u of Oxytocin or 0.04mg of ethinylestradiol. Variation between treatments regarding onset of estrus can be due to amount of estrogen in blood arising from induced luteolysis as estrogen level in blood is presumed to bring the animal in estrus and a depressed effect on progesterone.

Results of the present study shows that administration of Depo-provera^R at different levels of up to 2ml + 10 I.U of Oxytocin has

no delirious effect on serum biochemical and haematological parameters of WAD doe, and their health status were not compromised. I therefore recommend that further research should be done using Depo-provera^R in fertility improvement and other reproductive performance of WAD does.

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