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Effectiveness of Administration of Different Dose Rates of FSH + LH (Pergonal®) on Superovulation, Hormonal Profiles and Leucocyte Status of Nigerian Red Sokoto (Maradi) Goats

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Abstract: Sixteen healthy, parous, Red Sokoto (Maradi) goats aged 2-4 years were used to study the effects of varying doses of FSH + LH (Pergonal®) on superovulation, hormonal profiles and leucocyte status. The does were randomly assigned to 4 treatment groups consisting of 19.0 IU, 38.0 IU and 76.0 IU FSH + LH (Pergonal®) and 1.0 mL physiological saline as the control treatment. The treatments were administered intramuscularly for 3 days. The results on the number of corpora lutea on the ovary did not show any significant differences ($p>0.05$) between goats on 19.0 IU (5.36±0.46), 38.0 IU (6.15±1.14) and 76.0 IU (6.60±1.84) FSH + LH. The ovary did not show any significant differences ($p>0.05$) between goats on 19.0 IU (5.36±0.46), 38.0 IU (6.15±1.14) and 76.0 IU FSH + LH (6.60±1.84). However, they differed significantly ($p<0.05$) from goats on the control in the number of corporalutea on the ovary. The number of embryos recovered was not significantly different ($p> 0.05$) between goats on 19.0 IU (4.35±0.82) 38.0 IU (4.50±0.65) and 76.0 IU FSH + LH (4.85±1.38), however, they differed significantly ($p<0.05$) from goats on the control treatment (2.05±0.02). Goats treated on 19.0 IU FSH + LH (63.4±1.35%), 38.0 IU FSH + LH (67.8±1.25%) and 76.0 IU FSH + LH (75.6±2.51%) were similar ($p>0.05$), but, they differed significantly ($p<0.05$) from the control treatment (35.0±0.65%) in embryo recovery rate. There were significant differences ($p<0.05$) between treatment groups in ova/embryo wastage. LH and FSH values were highest at 38.0 IU FSH + LH with (3.80±0.4) and (3.33±0.14) (iu L^{-1}), respectively. Progesterone and oestradiol showed higher values on 38.0 IU FSH + LH (51.91±0.36) and 19.0 IU FSH + LH (11.6±0.7) nmol L^{-1}, respectively. Higher neutrophil counts were observed in goats treated with 76.0 IU FSH + LH (75.64±1.48) nmol L^{-1}. The lymphocyte counts showed significant differences ($p<0.05$) between the treatment groups.

Key words: Goats, superovulation, hormones, leucocyte

INTRODUCTION

The primary goal of superovulation is to obtain consistent high number of viable good quality embryos from each donor (Nowshari *et al*., 1996; Senthilkumer *et al*., 1998; Goel and Agrawal, 2005), Superovulation in goats involves the use of follicle stimulating hormone (FSH), follicle stimulating hormone + Luteinizing hormone (LH + FSH), Pregnant Mare Serum Gonadotrophin (PMGS), synthetic prostaglandin e.g., cloprostenol (Pereira, 1998; Zamfirescu and Sonea, 2000; Iheukwumere *et al*., 2008). Most of these preparations have not been adopted in superovulation of Nigerian Red Sokoto goats, because they are very expensive because of brand name. Herbert *et al*. (2000) indicated that some of them require cold chain storage and often deteriorate because of inadequate storage.

FSH + LH (Pergonal®), a human menopausal gonadotrophin is one of such preparations that induces ovulation in animals (Sugano *et al*., 2001; Herbert *et al*., 2005; Iheukwumere *et al*., 2008; Abu *et al*., 2008). Pergonal® (FSH + LH) is a lyophilized gonadotropin and a preparation of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in a ratio of 1:1 (Dixon and Hopkins, 1996). LH and FSH in pergonal play vital role in the initiation of multiple ovulations in goats (Herbert *et al*., 2005; Iheukwumere *et al*., 2008).

There is paucity of information on the use of these preparations for superovulation in the Nigerian Red Sokoto (Maradi) goats. There is therefore, the need to

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examine some generic preparations that could induce the desired action in the goats, but at the same time are cheap, readily available and easily be managed under our conditions. This study was therefore carried out to evaluate the effect of FSH + LH (Pergonal®) on the superovulatory response, hormonal profiles and leucocyte status of Nigerian Red Sokoto (Maradi goats.

MATERIALS AND METHODS

This study was carried out at the Livestock

Research Teaching Farm of the Abia State University, Umuahia, Nigeria. This study took place during the dry season between January and April.

Experimental animals: Sixteen clinically, sound, parous Nigerian Red Sokoto goats aged 2-4 years were used in this study. A 2 week pre-experimental period was allowed to enable the animals to adjust to the new environment. The animals used were those that showed good records from their source including evidence of good health and excellent mothering ability. The animals were weighed every week and the weights recorded. The animals were housed in separate pens constructed in such a way that the goats can come outside for sunlight and forage. Routine management practices such as cleaning and deworming were carried out. Fresh browse plants were the main source of feed. Brewer's dried spent grains were used as supplement. The animals were fed twice daily in the monrning and evening. Salt lick was provided as mineral supplement. Water was given liberally to the animals.

Experimental design: Sixteen oestrus does were isolated and randomly divided into four treatment groups identified as T_1, T_2, T_3 and T_4. During the experiment, just prior to use, the contents of the vials of Pergonal® (Ferring Labs, USA) containing 75 IU FSH + 75 IU LH were dissolved in 1 mL of physiological saline solution provided resulting in a solution of 75 IU FSH + 75 IU LH per mL. Each group was then assigned to the following treatments.

T_1 = Was administered with 1.0 mL physiological saline as the control treatment.

T_2 = Each goat received 0.11 mL Pergonal® (equivalent to 19.0 IU FSH + LH) was administered to each goat.

T_3 = Each goat received 0.22 mL Pergonal® (equivalent to 38.0 IU FSH + LH) was administered to each goat.

T_4 = Each goat received 0.44 mL Pergonal® (equivalent to 76.0 IU FSH + LH) was administered to each goat.

The different doses of pergonal® were administered intramuscularly for three consecutive days for three weeks using a mL. syringe with 0.01 mL graduation.

Detection of oestrus goats: The does were watched closely for obvious signs of heat as described by Akusu and Egbunike (1990) and subsequently mated to a virile buck. In order to ensure that mating took place, even at other times, the buck was left with the does throughout the heat period which lasted 2-4 days.

Recovery and evaluation of embryos: The recovery method used in this trial is laparatomy as described by Nowshari and Holtz (1992) and Herbert *et al.* (2005). The parameters evaluated were: number of corpora lutea on the ovary, number of embryos recovered, embryo recovery rate (%) and ova/embryo wastage (%). The embryos were microscopically evaluated at ×70 for identification of uncleaved degenerated or regular embryos and its quality was assessed by the state of development, integrity of the zona pellucida, nature of the shell surrounding the embryo and of the cytoplasm of the embryo. These were carried out at the Histology Unit of the University of Port-Harcourt Teaching Hospital, Port-Harcourt, Nigeria.

Blood collection for leucocyte analysis: The does were bled between 9 am and 10.30 am twice weekly from a punctured jugular vein to aspirate about 7 mL of blood from each doe. Two milliliters of each of blood sample was poured into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for haematological evaluation. Total leucocyte count was carried out using Neubaer haemocytometer after using Natt and Henrick's diluent 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 was counted as described by Jain (1986).

Hormonalysis: Hormones (FSH, LH, estrosen and progesterone) were analyzed using standard ELISA (Enzyme Linked Immunosorbent Assay) kits according to methods described by Alcivar *et al.* (1992) and Nowshari *et al.* (1996). The kits were produced by Immunometrics (London, UK) and obtained from Nzemat (Lagos, Nigeria). However, manufacturer's instructions were strictly followed. The optical density was read using a spectrophotometer at wavelengths between 492 and 550 nm.

Data analysis: All the data collected from this trial were subjected to analysis of variance (Steel and Torrie, 1980). Treatment means where significant were separated by the use of Duncan's New Multiple Range Test as described by Obi (1990).

RESULTS

The results of pergonal® administration for superovulation in Red Sokoto goats are shown in Table 1. The results show no significant differences ($p>0.05$) in the number of corpora lutea observed between the does treated with the varying doses of the drug, however, they differed significantly ($p<0.05$) from the control treatment. Goats treated with 76.0 IU FSH + LH pergonal® showed higher number of corpora lutea, 6.60±1.84 while the untreated goats showed the lowest number of corpora lutea on the ovary, 3.25±0.20.

Goats on the treatment groups die not show any significant differences ($p>0.05$) between each other in the number of embryos recovered, however, they differed significantly ($p<0.05$) from goats in the control treatment. The results show that goats treated with 76.0 IU FSH + LH recorded an average number of embryos recovered of 4.85±1.38, while the untreated goats had the lowest number of embryos recovered of 2.05±0.02.

The results of the embryo recovery rate observed did not show any significant ($p>0.05$) difference between the treatment groups. Goats on 76.0 IU FSH + LH treatment showed higher embryo recovery rate of 75.6±2.15% while goats on 19.0 IU FSH + LH treatment showed the lowest embryo recovery rate of 63.4±1.35%.

The proportion of ova/embryo wastage did not show any significant differences ($p>0.05$) between the treatment groups. The values obtained for goats on 76.0 IU FSH + LH treatment was 28.5±0.04%, goats on 19.0 IU FSH + LH treatment was 38.4±0.3% while goats on 38.0 IU FSH + LH 34.2±0.06%,

The results on the effects of FSH + LH Pergonal® treatment on hormonal profiles during superovulation in Red Sokoto Goats (RSG) are shown in Table 2. The results show that goats treated with 38.0 IU FSH + LH differed significantly ($p<0.05$) from goats treated with 19.0 IU, 76.0 IU FSH + LH and the control treatment in Luteinizing Hormone (LH) values of the serum.

The Follicle Stimulating Hormone (FSH) values of the serum was higher in goats treated with 38.0 IU FSH + LH with a value of 3.33±0.14 IU L^{-1} and this differed significantly ($p<0.05$) from goats treated with 19.0 IU and 76.0 IU FSH + LH and the control treatment with 2.13±0.11, 2.43±0.05 and 2.43±0.14 iu L^{-1}, respectively.

A higher serum progesterone level of 51.91 nmol L^{-1} was observed in goats treated with 38.0 IU FSH + LH, while the lowest values were observed in goats treated with 19.0 IU FSH + LH and the control group. However, progesterone levels did not differ significantly ($p>0.05$) between goats treated with 38.0 IU and 76.0 IU FSH + LH treatments.

Serum estradiol level was higher in goats treated with 19.0 IU FSH + LH, 11.80±0.64 nmol L^{-1} and this was significantly different ($p<0.05$) from goats treated with 38.0 IU, 76.0 IU FSH + LH and the control treatment group in serum extradoil values.

The results of the effects of FSH + LH treatment on the leucocyte count of Red Sokoto goats are shown in Table 3. The Red Sokoto goats treated with 76.0 IU FSH + LH recorded the highest Neutrophil count 75.64±1.49% and this differed significantly ($p<0.05$) from goats treated with 19.0 IU, 38.0 IU FSH + LH and the control treatment with 62.35±1.03, 63.64±1.68 and 61.24±0.56%, respectively.

Table 1: Effect of pergonal (R) dosage on superovulation of Red Sokoto (Maradi) goats

	Treatments Pergonal(R) (FSH + LH)			
Parameters	Physiol saline (Control)	19.0 IU	38.0 IU	76.0 IU
No. of goats flushed	4	4	4	4
No. of corpora lutea	3.25±0.20^{b}	5.36±0.46^{a}	6.15±1.14^{a}	6.60±1.84^{a}
No. of embryo recovered	2.05±0.02^{b}	4.35±0.82^{a}	4.50±0.65^{a}	4.25±1.38^{a}
Embryo recovery rate (%)	35.0±0.65^{b}	63.4±1.35^{a}	65.8^{a}±1.25^{a}	75.6±2.51^{a}
Ova/embryo wastage (%)	56.0^{a}±1.01^{a}	38.4^{b}±0.13^{b}	34.2±0.06^{b}	28.5b±0.04^{b}

a b: Means within row with different superscripts are significantly different ($p<0.05$)

Table 2: Effect of FSH + LH treatment on hormonal profiles of (RSG) goats during superovulation

	Treatments (Pergonal(R))			
Parameters	Physiol saline (Control)	19.0 IU FSH + LH	38.0 IUFSH + LH	76.0 IUFSH+LH
LH (iu L^{-1})	3.40±0.3^{b}	3.35±0.2^{b}	3.80±0.4^{a}	3.08±0.85^{b}
FSH (iu L^{-1})	2.43±0.14^{b}	2.13±0.11^{b}	3.33±0.14^{a}	2.43±0.05^{b}
Progesterone (nmol L^{-1})	9.15±0.35^{b}	11.6±0.7^{b}	51.91±0.56^{a}	39.18±0.7^{a}
Estradiol (nmol L^{-1})	0.13±0.01^{b}	11.80±0.64^{a}	0.26±0.02^{b}	0.17±0.04^{b}

a b: Means within row with different superscripts are significantly different ($p<0.05$)

Table 3: Effect of FSH + LH treatment on leucocyte count of Red Sokoto goats

	Treatments Pergonal(R)			
Parameters	Physiol saline (Control)	19.0 IU FSH + LH	38.0 IU FSH + LH	76.0 IU FSH + LH
Neutrophil (%)	61.24±0.56^{b}	62.35±1.03^{b}	63.60±1.68^{b}	75.64±1.48^{a}
Eosonophil (%)	-	2.50±0.40	-	-
Monocyte (%)	-	4.56±0.68	-	4.56±0.68
Lymphocytes (%)	43.12±0.56^{a}	36.0±0.12 ^{ab}	32.66±0.54^{b}	25.50±0.56^{b}

a b: Means within row with different superscripts are significantly different ($p<0.05$)

This eosinophi, count was observed only in goats treated with 19.0 IU FSH + LH. The monocyte count was observed in goats treated with 19.0 IU and 76.IU FSH + LH. However, there was no significant difference ($p>0.05$) between the treatment groups in monocyte count.

Goats treated with 38,0 IU FSH + LH showed no significant difference ($p>0.05$) from goats treated on 76.0 IU FSH + LH, however, they differed significantly ($p<0.05$) from the control treatment group. The basophil count did not show any significant difference ($p>0.05$) between the treatment groups.

DISCUSSION

The highest number of corpora lutea was observed in goats treated with 76.0 IU FSH + LH, however, the values were similar between the treatment groups, but differed significantly from the control treatment. The CL value obtained in this study are comparably lower than the CL number reported by Pereira *et al.* (1998) in goats, but higher than the CL number reported by Goel and Agrawal (1996) in goats. The CL number obtained in this study are comparable to that reported by Senthilkumar *et al.* (1998) in Malabari goats and Iheukwumere *et al.* (2008) in West African dwarf goats. The observed similarity in CL numbers of goats treated with different doses of FSH + LH (Pergonal®) indicates enhancement of ovarian activity. This observation is in agreement with the findings of Lazano *et al.* (2000), Briggadike *et al.* (2000) and Iheukwumere *et al.* (2008).

The number of embryos recovered were similar in goats treated with the varying doses of FSH + LH (Pergonal®) and was within the range reported by Goel and Agrawal (1996). This value was also comparable to the findings of Iheukwumere *et al.* (2008) in West African dwarf goats. However, the values were lower than embryo numbers obtained in FSH treated goats reported by Rathore *et al.* (1998). Goats on the control treatment administered with physiological saline showed lower number of embryos recovered when compared with goats treated with varying doses of FSH + LH (Pergonal®). The low number of embryos recovered in the control group of goats might be due to excessive oestradiol level in the circulation during early luteal phase (Goel and Agrawal, 1996) and for premature release of $PGF_{2\alpha}$ (Pereira *et al.*, 1998). However, in this study it was observed that goats on the controls group showed the lowest serum oestradiol when compared with the goats on Pergonal® (FSH + LH) treatment. These results confirmed the efficacy of this gonadotrophin in inducing superovulation and enhancing ovarian activity. These results also agreed with the reports of Sugano *et al.* (2001), Iheukwumere (2004) and Iheukwumere *et al.* (2008).

The higher embryo recovery rate 75.6% observed in goats treated with 76.0 IU FSH + LH (Pergonal ®) was comparably within the value 75% reported by Rathore *et al.* (1998). However, the value was lower than embryo rate of 85% reported by Pereira *et al.* (1998) in goats treated with prostaglandin $F_{2\alpha}$ before flushing.

Goats on the control group showed higher ova/ embryo wastage 73.0%. This value is comparably higher than 56% reported by Herbert *et al.* (2005) in goats. The low ova/embryo wastage and similarities observed in goats treated with varying doses of FSH + LH (Pergonal®) indicates efficacy of the gonadotrophin in superovulation of Red Sokoto goats. This observation is in agreement with the reports of Iheukwumere *et al.* (2008) in goats treated with FSH + LH) (Pergonal®) and also Herbert *et al.* (2005) in goats treated with FSH + LH (Pergonal®) supplemented with concentrate diets and Lazano *et al.* (2000) in ewes.

Goats treated with different doses of FSH + LH (Pergonal®) were not similar between treatment groups in LH concentration levels. Goats administered with 38.0 IU FSH + LH showed higher LH value of 3.80±0.4 (UL^{-1}). The high LH value observed in this study is in agreement with the reports of Price *et al.* (1999) and Iheukwumere *et al.* (2008) who indicated the effect of superovulation on LH secretion is an inhibition or complete absence of preovulatory LH surge. The reason for this is unknown, but as noted by these authors it may be related to an observed down regulation of pituitary GnRH receptors.

The Follicle Stimulating Hormone (FSH) in the serum was higher in goats treated with 38.0 IU FSH + LH (Pergonal) showing a level of 3.33±0.14 (IU L^{-1}). This observation is in agreement with the findings of Herbert *et al.* (2005) who reported increased (FSH) levels in superovulated goats.

A higher progesterone concentration of the serum was observed in goats treated with 38.0 IU FSH + LH, 51.91±0.56 (nmol L^{-1}). The most well known effect of superovulation is the increase in plasma progesterone (Alcivar *et al*., 1992). Progesterone concentration increases during the luteal phase of the cycle and remains higher in equine Chorionic Gonadotrophin (eCG) stimulated animals, even after prostaglandin induced luteolysis (Alcivar *et al*., 1992). The similarity in progesterone levels of goats treated with varying doses of FSH + H (Pergonal®) indicate that these levels of the gonadotrophin do not affect progesterone levels in Red Sokoto goats. This observation is in agreement with the findings of Herbert *et al.* (2005) and Iheukwumere *et al.* (2008) in West African dwarf goats.

Goats treated with 76.0 IU FSH + LH showed higher oestradiol level in this study. Syuperovulation increases plasma oestradiol concentration and the number of growing follicles. Thus, the increase in plasma oestradiol concentration could be the result of an increase in healthy estrogen secreting follicles (Camillo *et al*., 2000) or it could be due to a direct stimulation of steriodogenesis in the follicles present (Driancourt and Fry, 1992). The similarity in oestradiol concentration of goats administered with 19.0 IU FSH + LH and 38.0 IU FSH + LH indicate that these doses of FSH + LH (Pergonal®) did not affect oestradiol levels in goats. This observation is in agreement with the findings of Briggadike *et al.* (2000) and Herbert *et al.* (2005) in goats.

Neutrophil percentage was more in goats treated with 76.0 IU FSH + LH. The value obtained in this study was higher than the value 57.95±0.23% reported by Mahmood *et al.* (1994) in Pashmina goats treated with FSH-P during superovulation and 57.68% reported by Abu *et al.* (2008) in West African dwarf goat.

Eosinophil percentage was only observed in goats treated with 19.0 IU FSH + LH. The percentage value of eosinophils was higher than the value 2.15±0.34% reported by Mahmood *et al.* (1994) in Pashmina goats and lower than 3.92±1.49% reported by Tambuwal *et al.* (2002) in Red Sokoto goats.

Monocyte percentage was recorded only in goats treated with 19.0 IU and 76.0 IU FSH + LH. The values obtained were similar between the treatment groups. These values are lower than 7.35±1.70% reported by Tambuwal *et al.* (2002) in Red Sokoto goats, but, higher than 2.47±0.41% reported by Mahmood *et al.* (1994) in Pashmina goats.

The lymphocyte percentage count were not similar for all treatments. Goats treated with 38.0 IU FSH + LH recorded the lowest lymphocyte percentage of the blood. The values obtained in this study are within the average values 37.50±3.0 and 36.40±2.53% reported by Mahmood *et al.* (1994) and Tambuwal *et al.* (2002), respectively in goats. The basophil percentage did not show any values between the treatment groups. This is in contrast with the findings of Mahmood *et al.* (1994) and Abu *et al.* (2008) who reported values for basophil percentage in Pashmina goats and West African dwarf goats, respectively. The variability in leucocyte counts observed in this study may be due to differences in physiological status of the goats as was reported by Egbe-Nwiinyi *et al* (2000) and Abu *et al.* (2008).

CONCLUSION

The results of this study indicate that the administration of FSH + LH (Pergonal®) enhanced embryo generation while LH was higher in 38.0 IU FSH + LH treated Red Sokoto goats. The effect of increasing embryo production in Red Sokoto goats would receive a boost with the introduction of hormonal administration in these indigenous low reproductive animals.

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