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### **Effect of FSH+LH (Pergonal®) Treatments on Hormonal Profile and Superovulatory Response of West African Dwarf Does**

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**Abstract:** The effect of the administration of varying doses of FSH+LH (Pergonal®, Ferring Labs, USA) was used on 16 clinically, sound, parous, West African dwarf does aged 2-3 years to evaluate the hormonal profiles and superovulatory responses. Four treatment groups were employed consisting of T<sub>1</sub> (administered with physiological saline as the control), T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> given 19.0 IU, 58.0 IU and 82.0 IU FSH+LH, respectively, as intramuscular injections over 3 days. The results on the number of corpora lutea found on the ovary did not show any significant difference ( $p>0.05$ ) between goats on T<sub>2</sub> ( $5.25\pm 2.74$ ) T<sub>3</sub> ( $6.25\pm 2.13$ ) and T<sub>4</sub> ( $6.50\pm 2.74$ ). However, they differed significantly ( $p<0.05$ ) from goats on T<sub>1</sub> ( $3.75\pm 0.30$ ) in the number of corpora lutea on the ovary. The number of embryos recovered was not significantly different ( $p>0.05$ ) between goats on T<sub>2</sub> ( $4.50\pm 0.93$ ), T<sub>3</sub> ( $4.70\pm 0.86$ ) and T<sub>4</sub> ( $4.75\pm 1.27$ ). However, they differed significantly ( $p<0.05$ ) from goats on T<sub>1</sub> ( $2.25\pm 0.01$ ) in number of embryos recovered. Goats treated on T<sub>2</sub> with embryo recovery rate ( $78.30\pm 0.18\%$ ) did not differ significantly ( $p>0.05$ ) from goats on T<sub>3</sub> ( $75.20\pm 0.14\%$ ) and T<sub>4</sub> ( $73.10\pm 0.06\%$ ). However, they differed significantly ( $p<0.05$ ) from goats on T<sub>1</sub> ( $60.00\pm 0.02\%$ ). The ova/embryo wastage was not significantly different ( $p>0.05$ ) between goats on T<sub>2</sub> ( $21.70\pm 0.30\%$ ), T<sub>3</sub> ( $27.80\pm 0.12\%$ ) and T<sub>4</sub> ( $26.90\pm 0.16\%$ ). However, they differed significantly ( $p<0.05$ ) from goats on T<sub>1</sub> ( $40.00\pm 0.01\%$ ) in embryo wastage. LH and FSH were highest at T<sub>3</sub> treatment group with values of ( $3.60\pm 0.02$  and  $3.13\pm 0.14$  (IU L<sup>-1</sup>), respectively. Progesterone and Oestradiol showed higher values on T<sub>4</sub> goats with ( $16.17\pm 1.01$  and  $0.26\pm 0.41$  nmmol L<sup>-1</sup>), respectively. The results of this study indicate that the administration of FSH+LH enhanced embryo production and hormonal levels in West African dwarf goats.

**Key words:** Pergonal, hormone, superovulation, WAD goats

### **INTRODUCTION**

The primary goal of superovulation is to obtain consistent high number of viable good quality embryos from each donor (Nowshari *et al.*, 1996; Senthilkumar *et al.*, 1998; Goel and Agrawal, 2005). Superovulation in goats involves the use of follicle stimulating hormone (FSH), follicle stimulating hormone+Luteinizing hormone (FSH+LH), Pregnant Mare Serum Gonadotrophin (PMSG), synthetic prostaglandin e.g., cloprostenol (Pereira *et al.*, 1998; Zamfirescu and Sonea, 2000). Most of these preparations have not been adopted in superovulation of West African dwarf does, because they are

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very expensive, perhaps because of brand names. Herbert *et al.* (2000) indicated that some of them require cold chain storage and often deteriorate because of inadequate storage.

FSH+LH (Pergonal<sup>®</sup>), a human gonadotrophin is one of such preparations that induces ovulation in domestic animals (Sugano *et al.*, 2001; Herbert *et al.*, 2005). Pergonal<sup>®</sup> (FSH+LH) is lyophilized gonadotrophin 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).

There is paucity of information on the use of these preparations for super ovulation in West African dwarf does. There is therefore, the need to 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<sup>®</sup>) on hormonal profiles and superovulatory response of West African dwarf goats.

## **MATERIALS AND METHODS**

This study was carried out at the livestock Teaching Research Farm of the Federal University of Technology, Owerri, Nigeria. The study took place during the dry season between January and April.

### **Experimental Animals**

Sixteen clinically, sound, parous West African dwarf does aged 2-3 years were used in this study. A two-week pre-experimental period was allowed to enable the animals 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. Brewers' dried spent grains were used as supplement. The animals were fed twice daily in the morning and evening. Salt lick was provided as mineral supplement. Water was given liberally to the animals.

### **Experimental Design**

Sixteen oestrus does were isolated and divided into four treatment groups identified as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. During the experiment, just prior to use, the contents of the vials of pergonal<sup>®</sup> (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<sub>1</sub> was given physiological saline solution for 3 days (control). T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were administered intramuscularly 19.0, 58.0, 82.0 IU FSH+LH for 3 days, respectively.

### **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 laparotomy as described by Nowshari and Holtz (1992) and Herbert *et al.* (2005). The parameters evaluated were (1) number of corpora lutea on the ovary, (2) number of embryos recovered, (3) embryo recovery rate (%) and (4) ova/embryo wastage (%).

The embryos were microscopically evaluated at x70 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 colour 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.

### Hormonal analysis

Hormones 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). Treatments means where significant were separated by the use of Duncan's New Multiple Range Test as described by Obi (1990).

## RESULTS

The results of human menopausal gonadotrophin (Pergonal®) treatments on embryo generation in WAD goats are shown in Table 1. The results showed no significant differences ( $p>0.05$ ) between the does in the number of corpora lutea on the ovary with the varying treatments of FSH+LH. Goats treated with 82.0 IU ( $T_4$ ) recorded the highest number of corpora lutea ( $6.50\pm 2.74$ ) whereas those on  $T_3$  ( $5.75\pm 0.56$ ) showed no significant differences ( $p>0.05$ ) between the treatment groups. However, they differed significantly ( $p<0.05$ ) from  $T_1$  (Control) ( $3.75\pm 0.30$ ) in the number of corpora lutea observed on the ovary.

Goats treated with 82.0, 58.0 and 19.0 IU FSH+LH recorded an average number of embryos recovered as  $4.75\pm 1.27$ ,  $4.70\pm 0.85$  and  $4.50\pm 0.93$ , respectively. These values did not differ significantly ( $p>0.05$ ) between the treatment groups, but were different ( $p<0.05$ ) from the  $T_1$  control ( $2.25\pm 0.01$ ).

The results of the embryo recovery rate observed showed no significant differences ( $p>0.05$ ) between the treatment groups. They however, showed significant difference ( $p<0.05$ ) from the control treatment group. Goats on 19.0 IU FSH+LH treatment showed the highest embryo recovery rate of  $78.30\pm 0.18\%$  while goats on 82.0 IU FSH+LH treatment recorded an embryo recovery rate of  $73.10\pm 0.06\%$ . Goats in the control group showed the lowest embryo recovery rate of  $60.00\pm 0.02\%$ .

The proportions of ova/embryo wastage was highest in the control treatment group ( $40.00\pm 0.01\%$ ) and this differed significantly ( $p<0.05$ ) from goats on 19.0 IU FSH+LH ( $21.70\pm 0.30\%$ ), 58.0 IU FSH+LH ( $27.80\pm 0.12\%$ ) and 82.0 IU FSH+LH ( $26.90\pm 0.06\%$ ) in ova/embryo recovery rate.

The results on the effect of FSH+LH (Pergonal®) treatments on hormonal profile during super ovulation in West African dwarf does are shown in Table 2. Goats treated with 19.0, 58.0 and

Table 1: Effect of human menopausal gonadotrophin (FSH+LH) on superovulatory response of WAD goats

Parameters	Treatments			
	$T_1$ Control	$T_2$ 19.0 IU FSH+LH	$T_3$ 58.0 IU FSH+LH	$T_4$ 82.0 IU FSH+LH
No. of corpus lutea	$3.75\pm 0.30^b$	$5.75\pm 0.56^a$	$6.25\pm 2.13^a$	$6.50\pm 2.74^a$
No. of embryo recovered	$2.25\pm 0.01^b$	$4.50\pm 0.93^a$	$4.70\pm 0.86^a$	$4.75\pm 1.27^a$
Embryo recovery rate (%)	$60.00\pm 0.02^b$	$78.30\pm 0.18^a$	$75.20\pm 0.14^a$	$73.10\pm 0.06^a$
Ova/embryo wastage (%)	$40.00\pm 0.01^a$	$21.70\pm 0.30^b$	$27.80\pm 0.12^b$	$26.90\pm 0.16^b$

<sup>a, b</sup>: Means within rows having different superscripts are significantly different ( $p<0.05$ )

Table 2: Effect of Human Menopausal Gonadotrophin (FSH+LH) on hormonal profiles of WAD goats

Parameters	Treatments			
	T <sub>1</sub> Control	T <sub>2</sub> 19.0 IU FSH+LH	T <sub>3</sub> 58.0 IU FSH+LH	T <sub>4</sub> 82.0 IU FSH+LH
LH (IU L <sup>-1</sup> )	3.10±0.10 <sup>b</sup>	3.11±0.30 <sup>b</sup>	3.60±0.20 <sup>a</sup>	3.060±0.70 <sup>b</sup>
FSH (IU L <sup>-1</sup> )	2.33±0.16 <sup>b</sup>	2.20±0.01 <sup>b</sup>	3.13±0.14 <sup>a</sup>	2.230±0.07 <sup>b</sup>
Progesterone (nmol L <sup>-1</sup> )	10.53±0.25 <sup>b</sup>	11.20±0.90 <sup>b</sup>	10.93±0.53 <sup>b</sup>	16.170±1.01 <sup>a</sup>
Oestradiol (nmol L <sup>-1</sup> )	0.16±0.62 <sup>b</sup>	0.17±6.25 <sup>b</sup>	0.18±0.31 <sup>b</sup>	0.260±0.41 <sup>a</sup>

<sup>a,b</sup>: Means within rows having different superscripts are significantly different (p<0.05)

82.0 IU FSH+LH treatments showed a luteinizing hormone (LH) level of 3.11±0.3 (IU L<sup>-1</sup>), 3.60±0.20 (IU L<sup>-1</sup>) and 3.06±0.70 (IU L<sup>-1</sup>), respectively. These values did not differ significantly (p>0.05) between treatment groups. However, they differed significantly (p<0.05) from the 58.0 IU FSH+LH, 3.60±0.2 (IU L<sup>-1</sup>) treatment group in LH values. Goats treated with 58.0 IU FSH+LH showed follicle stimulating hormone (FSH) value of 3.13±0.14 (IU L<sup>-1</sup>) which differed significantly (p<0.05) from the (FSH) of goats treated with 19.0 IU FSH+LH, (2.00±0.1 (IU L<sup>-1</sup>), 82.0 IU FSH+LH (2.23±0.07) and the control group with (FSH) value of 2.33±0.16 (IU L<sup>-1</sup>).

Goats treated with 82.0 IU FSH+LH showed progesterone level of 16.17±0.01 (nmol L<sup>-1</sup>) and this differed significantly (p<0.05) from goats treated with 58.0 IU (10.93±0.53 (nmol L<sup>-1</sup>), 19.0 (11.20±0.90 (nmol L<sup>-1</sup>) and the control group (10.53±0.25 (nmol L<sup>-1</sup>). There was significant difference (p<0.05) in oestradiol level of goats treated with 82.0 IU FSH (0.26±0.4 (nmol L<sup>-1</sup>) from goats treated with 58.0 IU (0.18±0.31 (nmol L<sup>-1</sup>), 19.0 IU (0.17±0.25 (nmol L<sup>-1</sup>) and the control group (0.16±0.02 (nmol L<sup>-1</sup>).

## DISCUSSION

The results of the effect of FSH+LH (Pergonal<sup>®</sup>) treatments on the superovulatory response of West African dwarf does indicate that the number of corpora lutea on the ovary were similar between treatment groups. However, higher number of corpus luteum was observed in goats treated with 82.0 IU FSH+LH (6.50±2.74). The corpus luteum number observed in this study was in agreement with the findings of Senthilkumar *et al.* (1998) in Malaban goats. The observed corpus luteum number was higher than 5.50±0.96 reported by Goel and Agrawal (1996). The CL number in this study was also comparably lower than the CL number of 9.2±3.7 reported by Pereira *et al.* (1998) in goats. The observed similarity in CL numbers of goats treated with different doses of FSH+LH (Pergonal<sup>®</sup>) indicates enhancement of ovarian activity. This observation is in agreement with the findings of Briggadike *et al.* (2002) and Lazano *et al.* (2000).

The number of embryos recovered were similar in goats treated with the varying doses of FSH+LH (Pergonal<sup>®</sup>) and was within the range (4.00±0.94) reported by Goel and Agrawal (1996). This value was also lower than embryo numbers (6.0) obtained in FSH treated goats reported by Rathore *et al.* (1998). Goats on the control group administered with physiological saline showed lower number of embryos recovered when compared with goats treated with varying doses of FSH+LH (Pergonal<sup>®</sup>). 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<sub>2</sub>α (Pereira *et al.*, 1998). However, in this study it was observed that goats on the control group showed the lowest serum oestradiol when compared with the goats on FSH+LH (Pergonal<sup>®</sup>) treatment. These results confirmed the efficacy of this gonadotrophin in inducing super ovulation and enhancing ovarian activity. These results also agree with the reports of Sugano *et al.* (2001) and Iheukwumere (2004).

The embryo recovery rate ( $78.30 \pm 0.18\%$ ) of goat treated with 19.0 IU of FSH+LH (Pergonal<sup>®</sup>) was comparably higher than 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 of  $40.00 \pm 0.01\%$ . This value is comparably lower than  $56.0 \pm 0.10\%$  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<sup>®</sup>) indicate efficacy of the gonadotrophin in super ovulation of West African dwarf does. This observation is in agreement with the reports of 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<sup>®</sup>) were not similar between treatment groups in LH concentration levels. Goats administered with 58.0 IU FSH+LH showed higher LH value of  $3.60 \pm 0.2$  (IU L<sup>-1</sup>). The high LH value observed in this study is in agreement with the reports of Price *et al.* (1999) who indicated the effect of super ovulation 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 follicular LH receptors or potentially to a down regulation of pituitary GnRH receptors.

The Follicular Stimulating Hormone (FSH) in the serum was higher in goats treated with 58.0 IU FSH+LH, showing a level of  $3.13 \pm 0.14$  (IU L<sup>-1</sup>). This observation is in agreement with the findings of Herbert *et al.* (2005) who reported increased (FSH) levels in super ovulated goats.

A higher progesterone concentration of the serum was observed in goats treated with 82.0 IU FSH+LH,  $16.17 \pm 1.01$  (nmol L<sup>-1</sup>). The most well known effect of super ovulation 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+LH (Pergonal<sup>®</sup>) indicate that these levels of the gonadotrophin do not affect progesterone levels in West African dwarf goat. This observation is in agreement with the findings of Oliveira-Tiho *et al.* (2000) and Herbert *et al.* (2005).

Goats treated with 82.0 IU FSH+LH showed higher oestradiol level in this study. Super ovulation 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 steroidogenesis in the follicles present (Driancourt and Fry, 1992). The similarity in oestradiol concentration, of goats administered with 19.0 IU FSH+LH and 58.0 IU FSH+LH indicate that these doses of FSH+LH (Pergonal<sup>®</sup>) 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.

## CONCLUSION

The results of this study indicate that the administration of FSH+LH (Pergonal<sup>®</sup>) enhanced embryo generation while LH was higher in 58.0 IU FSH+LH treated West African dwarf goats. The effect of increasing embryo production in West African dwarf goats would receive a boost with the introduction of hormonal administration in these indigenous low reproductive animals.

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