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Evaluation of simplified Folltropin-V® (FSH) protocol on follicular turnover in Yankasa ewes

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Abstract

The study was conducted to evaluate the effects of simplifying pFSH (Folltropin-V®) protocol on ovarian follicular turnover in Yankasa ewes. Fifteen Ewes were synchronized for estrus with double injections of 10 mg Dinoprost tromethamine (Lutalyse®) on day 0 and day 10 and randomly allocated to 3 groups. {Cn; control (n= 5, No FSH treatment), F_1 (n=5 received FSH once daily) and F_2 (n=5, received FSH twice daily at 12 hr. interval)}. Folltropin-V® treatments commenced on Day 8 (equivalent to 80 mg, in decreasing doses over 3 days). Blood samples were collected an hour before and after 1st injection of FSH, then every 12 h over the course of treatment, and then every day till end of estrus. Serum was extracted and assayed for estradiol-17β. Ultrasonic scanning of the ovaries was conducted on day 11. Follicles were counted, measured and classified. Onset of estrus was earlier in F_2 than F_1 being 16.8 ± 5.0 h and 27.6 ± 4.0 h, respectively. Duration of estrus was shortest for F_1 (39.2 ± 11.8 h) and F_2 (47.6 ±10.6 h). Estradiol-17β concentrations were elevated in the F_1 than F_2 1 h after 1st FSH administration, but it was not significant ($P > 0.05$). Estradiol-17β in F_2 (2.7 ± 0.52 pg/ml) was higher than F_1 (1.64 ± 0.48 pg/ml) and this was not significant ($P > 0.05$). A significantly higher number ($P < 0.05$) of small follicles < 2 mm were observed in F_2 (3.6 ± 3.4) than F_1 (0.6 ± 0.9). Medium sized follicles 3 mm - 4.5 mm was higher ($P > 0.05$) in F_2 (2.4 ± 2.6) than F_1 (0.6 ± 0.9). Number of large follicles >4.5 mm were similar ($P > 0.05$) being 2.4 ± 2.3 and 1.4 ± 1.2 in F_2 and F_1 respectively. Both single and double daily FSH protocols were equally efficient in inducing multiple follicular developments.

Keywords: Estradiol-17β, Simplified FSH protocol, Superovulation, Ultrasonography, Yankasa ewes

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Introduction

Reproductive indices such as ovulation rate, twinning rate and litter size (1.36 %, 12 % and 1.25 %) of Yankasa sheep are relatively low (Adu *et al*., 1979). Therefore, increase in the ovulation rate and consequent improvement in litter size can be achieved by genetic (long term) or non-genetic (short term) means (Hanrahan & Quirke 1982). Reproductive biotechnologies in small ruminants and other domestic animals have important role in production. The Nigerian sheep is still genetically unimproved (Agaviezor *et al*., 2013). There is need for investigation into novel means of enhancing the capacity of farmers to feed the teeming population. One single factor militating against the capacity of sheep to contribute to the meat supply in the nation's food sector is their low reproductive rates (Adu *et al*., 1979).

Multiple ovulation and embryo transfer (MOET) has been extensively used to multiply genetically superior goats and sheep and many protocols have been developed to optimize its application in research (Bartlewski *et al.,* 2008; Perera *et al.,* 2008). In cross-breeding the benefit has been primarily from male germplasm, ignoring the

potential of the untouched female (Ishwar & Memon, 1996). The successful application of multiple ovulation and embryo-transfer technology largely depends on superovulation for which the essential factor is the treatment with exogenous gonadotropin. FSH is usually administrated in multiple injections twice daily over three to four days, which is more laborious and expensive. Therefore, the superovulation protocols have to be simplified.

Materials and Methods

This study was conducted at the Experimental Unit of the Small Ruminant Research Program, National Animal Production Research Institute, Shika, (NAPRI) Ahmadu Bello University, Zaria, Kaduna state. Shika is located between latitude 11^{o} 8' N and longitude 7^{o} 45' E and is about 650 m above sea level. The average annual maximum and minimum temperatures are 31.0 ± 3.2 ^{o}C and 18.0 ± 3.7 ^{o}C respectively.

Fifteen (n= 15) cycling Yankasa ewes aged 2 to 4 years and weighing 20 – 32 kg were selected for the experiment. Plastic ear tag was used to identify individual ewes. Ewes with body condition score > 2.5 as described by Mendizabal *et al*. (2011) were used. Estrus detection was done using sexually active aproned rams, immobilization of the ewe by the ram was considered to be a sign of the occurrence of estrus (Baril *et al*., 1993). The ewes were fed with *Digitaria smutsii* (wooly finger grass) hay as basal diet and concentrate rations at 0.7 kg /ewe / day was also provided. Fresh clean water was made available at all times.

Experimental design

The ewes were randomly assigned to 3 groups consisting of 5 ewes in each group; The first group was the control group (Cn) and were synchronized with double intramuscular injections of 10 mg Prostaglandin $F_2\alpha$, Lutalyse® (Dinoprost tromethamine, Pfizer Animal Health Inc. USA) at 10 days' interval but did not receive any gonadotropin. The second group (F1, n=5) received double injections of 10 mg Prostaglandin $F_{2\alpha}$, Lutalyse® (Dinoprost tromethamine, Pfizer Animal Health Inc. USA) administered intramuscularly at 10 days' interval starting on day 0. The ewes were injected with 36 mg, 24 mg and 20 mg of Follicle Stimulating Hormone (Folltropin®-V, Bioniche Animal Health, Belleville, ON, Canada) administered intramuscularly once daily, on days 8, 9 and 10 respectively. The third group (F2, n=5) received double injections of 10 mg Prostaglandin $F_{2\alpha}$, Lutalyse® (Dinoprost tromethamine, Pfizer Animal Health Inc. USA) administered intramuscularly at 10 days' interval starting on day 0. The ewes were treated with 18 mg, 12 mg and 10 mg of Follicle stimulating hormone (Folltropin®-V, Bioniche Animal Health, Belleville, ON, Canada) administered intramuscularly twice daily over three days, commencing on day 8, day 9 and day 10.

Ultrasonography of the ovaries

Transabdominal ultrasonography was carried out with the ewes restrained in standing position. Fur in the sub-lumbar fossa was neatly clipped on both the left and right sides. Real-time B-mode ultrasound equipment (Medison SV 600 equipped with a 5.0 MHz convex transducer, Corometrics Medical Systems, Wallingford, Connecticut, USA) was used.

Ovarian structures that are approximately round, anechoic and with sharp outline were designated as follicles. Follicles were counted, measured and categorized. Follicles with equatorial diameter < 3 mm were categorized as small, medium sized (3-4.5 mm), large (>4.6 mm) according to Riesenberg *et al.* (2001) and El-Sherry *et al.* (2011).

Blood collection

Three milliliters (3 ml) of blood was collected using 21 G 5 ml hypodermic syringe by venepuncture of the jugular vein from each ewe starting on day 8. Blood was collected starting 1 h before and 1 h after commencing gonadotropin treatment, twice daily at 12 h interval on days 8, 9 and 10. Blood was collected daily during estrus and 12 h after end of estrus. Serum was extracted from the blood by centrifugation (3000 x g, 10 min) decanted, labeled appropriately and stored at - 20 ^{o}C until steroid hormone analysis was conducted.

Hormonal assay

Concentration of Estradiol-17β (E_2) was measured in serum using competitive immune-enzymatic colorimetric method for quantitative determination of E_2. It was based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The ELISA kits were obtained from Monobind ® Inc, USA. The kits were used according to the manufacturer's specifications.

Statistical analysis

Data about ovarian structures (follicles) were expressed as Mean ± S.D. Intergroup estrus parameters between the groups were compared using Student's T- test. Analysis of variance (ANOVA) and Tukey's post hoc tests were used to determine the main effects of group, day and group by day interaction of estradiol-17β concentration. Graphpad prism version 5.0 for windows (Graph pad Software, San Diego, California, USA) was used for the analyses. P< 0.05 was considered significant.

Table 1: Evaluated parameters of Yankasa ewes not treated and treated with simplified and conventional Folltropin-V ® protocol

Group	Cn	F_1	F_2
Estrus response rate (%)	80	100	100
Ovarian follicular response rate (%)	-	40	60
Onset of estrus (h)	24.0 ± 8.6	27.6 ± 4.0	16.8 ± 5.0
Duration of estrus (h)	44.5 ± 6.8	39.2 ± 11.8	47.6 ±10.6

Table 2: Number and Category of Follicles from Ovarian Ultrasonogram on Day 11

Group	Category	Number	Mean ± SD
Cn	Small	-	-
	Medium	-	-
	Large	5	1.25 ± 0.5
F_2	Small	18	3.6 ± 3.4[a]
	Medium	12	2.4 ± 2.6
	Large	12	2.4 ± 2.3
F_1	Small	3	0.6 ± 0.9[b]
	Medium	3	0.6 ± 0.9
	Large	7	1.4 ± 1.2

values with different superscripts are significant (P< 0.05)

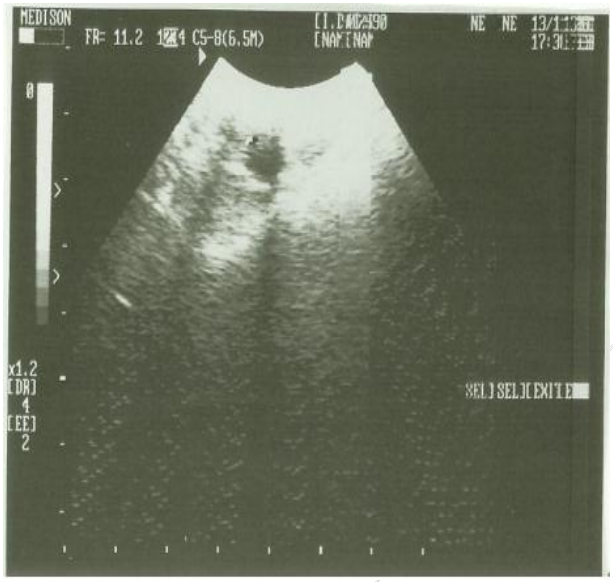

Plate I: Ovarian Ultrasonogram of a ewe in Group Cn showing 1 follicle (Arrow) two days after $PGF_{2\alpha}$ injection

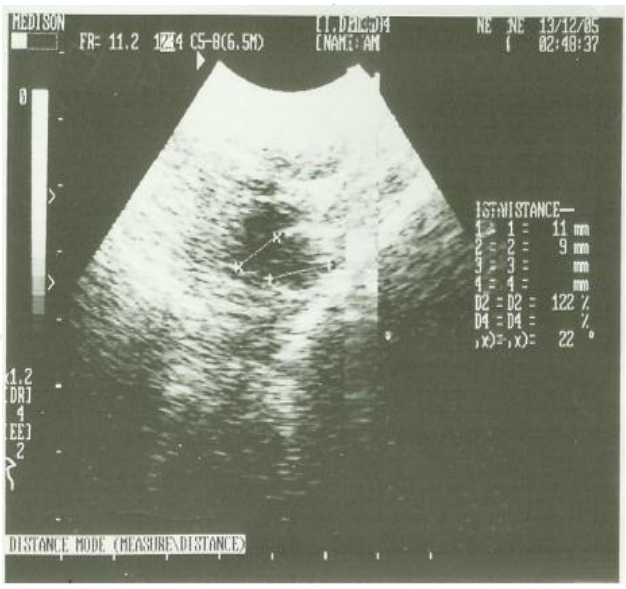

Plate II: Ovarian Ultrasonogram of multiple follicles in a Yankasa ewe treated with pFSH, 2 days after $PGF_{2\alpha}$ injection

Results

Overall estrus response rate was 100 % in treated ewes when compared to the control group with 80%. The response to exogenous gonadotropin treatment was 40 % and 60 % in groups F_1 and F_2 ewes respectively. The Mean ± SD time of onset of estrus was 24.0 ± 8.6 hours, 27.6 ± 4.0 hours, 16.8 ± 5.0 hours for Cn, F_1,and F_2 groups, respectively. Mean ± SD duration of estrus was 44.5 ± 6.8 hours, 39.2 ± 11.8 hours and 47.6 ± 10.6 hours for Cn, F_1 and F_2 groups, respectively (Table 1).

Large sized follicles in group Cn ewes was fewest 1.25 ± 0.5 (Plate I). Group F_1 ewes had 0.6 ± 0.9, 0.6 ± 0.9 and 1.4 ± 1.2 follicles as small, medium and large sized follicles respectively. Group F_2 ewes had 3.6± 3.4, 2.4 ± 2.6 and 2.4 ± 2.3 follicles as small, medium and large sized follicles respectively (Plate II). Small sized follicles were more in F_2 ($P<0.05$) There was no significant difference ($P> 0.05$) between large sized follicles in groups F_1 and F_2 (Table 2).

Serum concentration of E_2 was consistently elevated in control group Cn during the proestrus sampling period. Nonetheless, E_2 (2.71 ± 0.5 pg/ml) was highest in group F_2 (2.71 ± 0.5 pg/ml) treated animals, 24 hours after treatment commenced. E_2 concentration began to decline in groups Cn and F_1 at 24 hours. At 48 hours post treatment, mean serum E_2 concentration declined in treated ewes (F_2: 1.75 ±0.1 pg/ml vs F_1 1.65 ± 0.4 pg/ml) while it began to increase in group Cn (3.63 ±0.6 pg/ml). At 84 h mark (24 hours after completing treatment and estrus synchronization), E_2 concentrations had increased again in group F_2 (2.3 ± 0.9 pg/ml) while declining in group Cn (2.88 ± 0.2 pg/ml) and group F_1 (1.6 ±0.4 pg/ml). At 120

h, (60 hours after treatment and estrus synchronization), group Cn had the highest total mean concentrations of E_2 (42.7 pg/ml). Total E_2 for treated ewes was 24.5 pg/ml and 25.7 pg/ml Groups F_2 and F_1 respectively (Figure 1).

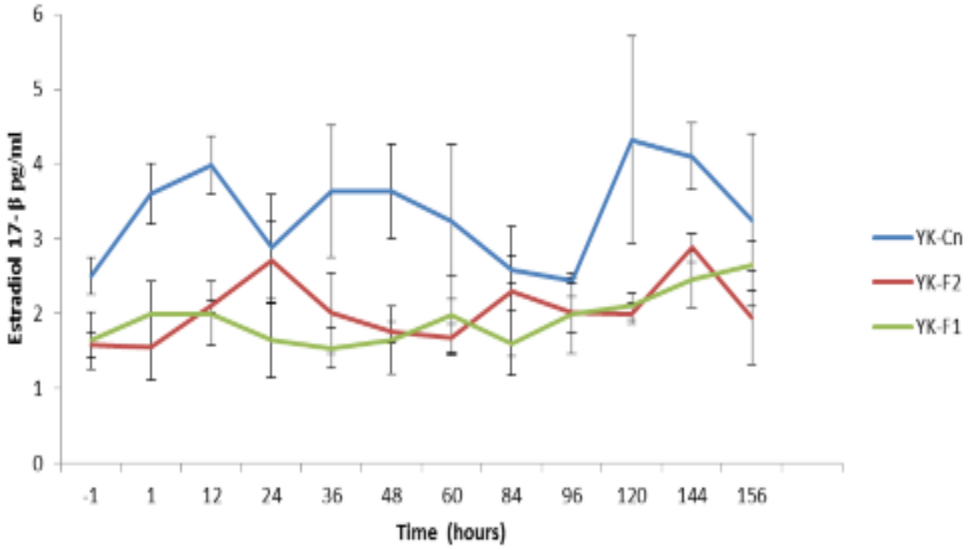

Figure 1: Concentration of serum Estradiol -17β in Yankasa ewes with multiple daily FSH (single versus double) injections

Discussion

The study revealed that the efficacy of using Lutalyse (Dinoprost tromethamine) for estrus synchronization in superovulated Yankasa ewes was 100 %. This agrees with Naqvi & Gulyani (1998) and Naqvi & Gulyani (1999) who also reported 100 % response in estrus synchronization of fine wool sheep and Bharat merino ewes respectively. Sanchez *et al.* (2013) also reported 100 % success in estrus response in Katahdin sheep using the natural $PGF_{2\alpha}$. However, in control group, estrus response was 80 % similar to Naderipour *et al.* (2012) in Kalkuhi ewes using $PGF_{2\alpha}$. This may be due to good body condition of the ewes at the time the experiment was conducted and the fact that there is no definitive breeding season for Nigerian sheep breeds; they can be bred throughout the year. After superovulatory induction with Folltropin®-V, it was observed that the time to onset of estrus was shortest for the F_2 group (16.8 ± 5.0 h). This is earlier compared to the report of 20.1 ± 2.1 h onset of estrus in Kahtadin sheep following superovulation with 80 mg FSH reported by Sanchez *et al.* (2013). Onset of estrus differed significantly between treated groups ($P < 0.05$). This was attributed to the initial elevation in estradiol concentration in the F2 group. This observation is in contrast to reports of Jabbour *et al.* (1991) (in Merino ewes), Simonetti *et al.* (2008) (in Corriedale ewes) and Forcada *et al.* (2011) (in Ojalada Soriana ewes. Treated animals synchronized with $PGF_2\alpha$ for superovulation showed estrus earlier than control animals, similar to report of Samartzi *et al*. (1995). The study showed that there was no significant difference ($P>0.05$) in total estradiol concentrations in both F_2 and F_1 (24.51 pg/ml and 25.73 pg/ml respectively). E_2 concentrations were elevated in the F_1 than F_2 1 h after 1st FSH administration, but it was not significant.

The ultrasonic detection showed that fewer follicles were observed for F_1 superovulated ewes than F_2. Observations of follicular development 12 h after completing the protocol, showed that F_2 ewes produced more small sized follicles (6 ± 0.57) than F_1 (1.5 ± 0.5). Medium sized follicles were also higher in the F_2 (4 ± 1.52) than F_1 (1.5 ± 0.5), however the number of large follicles was similar in both protocols.

Ryan *et al.* (1992), Scaramuzzi *et al.* (2006) and Sosa *et al.* (2009) reported a positive correlation between BCS of ewes, estrogen levels and estrus rates. This suggests that there is an active conversion of cholesterol to E_2 in the current study. At first sample, serum E_2 concentrations were observed to have risen within 1 h of induction of treatment in the F_2 group. This led to the initial E_2 peak before the end of the treatment regimen in the F_2 group. This increase is correlated with increasing size and functional maturation of the dominant follicles. A similar observation of this early E_2 peak was made by Riesenberg *et al*. (2001). Maximal E_2 concentration was lowest for F_1 group of ewes ($P>0.05$) in agreement with Jabbour *et al*. (1991) and Menchaca *et al*. (2010). An alteration in the ability of the treated ewes to synthesize E_2 in this study was observed. The peak E_2 concentrations among the treated ewes are therefore indicative of relative differences in follicular activity. No significant ($P>0.05$) differences were recorded in the E_2 concentrations between superovulated ewes. This suggests that frequency of administering FSH has no significant effect on development of follicles. This is confirmed by results of ovarian ultrasonograms in which number of large sized follicles didn't differ among treated ewes, that responded to ovarian super stimulation. The injection of $PGF_{2\alpha}$ (48 h) caused E_2 concentrations to increase within 12- 36

h in agreement with Ali *et al*. (2009). This is probably due to the GnRH surge at the hypothalamo-pituitary axis due to removal of negative feedback of progesterone.

In conclusion, the number of follicles at the end of superovulatory induction in Yankasa ewes with 80 mg FSH administered as double injections versus single injection did not differ significantly. Therefore, single daily injections of FSH may be as effective as double daily administration of FSH Yankasa ewes.

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