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

AB Adewuyi1*, PI Rekwot1, J Rwuaan2, MU Kawu3, M Lawal4, BO Omontese1 & AA Kuje2

1. Artificial Insemination Unit, National Animal Production Research Institute Ahmadu Bello University, Zaria, Nigeria
2. Department of Theriogenology and Production Faculty of Veterinary Medicine Ahmadu Bello University, Zaria, Nigeria
3. Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
4. Department of Surgery and Radiology Faculty of Veterinary Medicine Ahmadu Bello University, Zaria, Nigeria

*Correspondence: Tel.: +2348057373540; E-mail: biyuweda@gmail.com

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), F1 (n=5 received FSH once daily) and F2 (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 F2 than F1 being 16.8 ± 5.0 h and 27.6 ± 4.0 h, respectively. Duration of estrus was shortest for F1 (39.2 ± 11.8 h) and F2 (47.6 ±10.6 h). Estradiol-17β concentrations were elevated in the F1 than F2 1 h after 1st FSH administration, but it was not significant (P > 0.05). Estradiol-17β in F1 (2.7 ± 0.52 pg/ml) was higher than F1 (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 F2 (3.6 ± 3.4) than F1 (0.6 ± 0.9). Medium sized follicles 3 mm - 4.5 mm was higher (P > 0.05) in F2 (2.4 ± 2.6) than F1 (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 F2 and F1 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

Received: 28-12-2016 Accepted: 12-04-2017

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° 8’ N and longitude 7° 45’ E and is about 650 m above sea level. The average annual maximum and minimum temperatures are 31.0 ± 3.2 °C and 18.0 ± 3.7 °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 smutii (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 F2α, 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 F2α, Lutalyse® (Dinoprost tromethamine, Pfizer Animal Health Inc. USA) administered intramuscularly at 10 days' interval starting on day 8. 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 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. Full 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 °C until steroid hormone analysis was conducted.

Hormonal assay

Concentration of Estradiol-17β (E2) was measured in serum using competitive immune-enzymatic colorimetric method for quantitative determination of E2. 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 | F₁ | F₂ |
|-------|----|----|----|
| 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 |
| F₂    | Small    | 18     | 3.6 ± 3.4⁴ |
|       | Medium   | 12     | 2.4 ± 2.6  |
|       | Large    | 12     | 2.4 ± 2.3  |
| F₁    | Small    | 3      | 0.6 ± 0.9⁵ |
|       | Medium   | 3      | 0.6 ± 0.9  |
|       | Large    | 7      | 1.4 ± 1.2  |

Values with different superscripts are significant (P<0.05)

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 F₁ and F₂ 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, F₁, and F₂ 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, F₁ and F₂ groups, respectively (Table 1).

Large sized follicles in group Cn ewes was fewest 1.25 ± 0.5 (Plate I). Group F₁ 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 F₂ 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 F₂ (P<0.05). There was no significant difference (P>0.05) between large sized follicles in groups F₁ and F₂ (Table 2).

Serum concentration of E₂ was consistently elevated in control group Cn during the proestrus sampling period. Nonetheless, E₂ (2.71 ± 0.5 pg/ml) was highest in group F₂ (2.71 ± 0.5 pg/ml) treated animals, 24 hours after treatment commenced. E₂ concentration began to decline in groups Cn and F₁ at 24 hours. At 48 hours post treatment, mean serum E₂ concentration declined in treated ewes (F₂: 1.75 ±0.1 pg/ml vs F₁ 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), E₂ concentrations had increased again in group F₂ (2.3 ± 0.9 pg/ml) while declining in group Cn (2.88 ± 0.2 pg/ml) and group F₁ (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 respectively. (Figure 1).

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$_{20}$. However, in control group, estrus response was 80% similar to Naderipour et al. (2012) in Kalkuhi ewes using PGF$_{20}$. 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 Follitropin$^{a}$-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 F$_2$ 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$_{20}$, 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 1$^{st}$ FSH administration, but it was not significant.

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

The ultrasonic detection showed that fewer follicles were observed for F$_2$, superovulated ewes than F$_1$. 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$_{20}$ (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.

References

Adu IF, Brinckman WL & Kuteyi IS (1979). Reproductive performance of indigenous sheep and their crosses. *Nigerian Journal of Animal Production*, 6(1): 38-47.

Agaviezo BO, Gunn H, Amusan SA & Imumorin IG (2013). Gene flow between Nigerian sheep breeds as revealed by micro satellite DNA markers. *Journal of Animal Production Advances*, 3(2): 35-39.

Ali A, Hayder M & Saifelnaser EOH (2009). Ultrasonographic and endocrine evaluation of three regimes for estrus and ovulation synchronization for sheep in the sub-tropics. *Reproduction in Domestic Animals*, 44(6): 873-878.

Baril G, Breibon P & Chesne P (1993). Training Manual for Embryo Transfer in Sheep and Goats. Food and Agricultural Organisation, Rome, Italy. Pp 115.

Bartlewski PM, Alexander BD, Rawlings NC, Barret DMW & King WA (2008). Ovarian responses, hormonal profiles and embryo yields in anoestrus ewes superovulated with Follitropin- V after pretreatment with medroxyprogesterone acetate (MAP)- releasing vaginal sponges and a single dose of estradiol-17β (E2-17β). *Reproduction in Domestic Animals*, 43(3): 299-307.

El-Sherry TM, Derar H, Hussein HA, Shahin AY & Fahmy S (2011). Effect of Clomiphene Citrate on follicular recruitment, development and superovulation during the first follicular wave in Rahmani ewes. *International Journal of Endocrinology Metabolism*, 9(3): 403-408.

Forcada F, Ait Amer-Meziane, M, Abecia JA, Maurel MC, Cebrian-Perez JA, Muinor-Blanco T, Asenjo B, Vazquez M & Casao A (2011). Repeated superovulation using simplified FSH/eCG treatment for in vivo embryo production in sheep. *Theriogenology*, 75(4): 769-776.

Hanrahan JP & Quirke JP (1982). Selection for ovulation rate in sheep aided by the use of superovulation and egg transfer. In: Proceedings of World Congress on Sheep and Cattle Breeding. Dunmore press, Palerston North. Pp 329-335.

Ishwar AK & Memon MA (1996). Embryo transfer in sheep and goats. A review: *Small Ruminant Research*, 19(1): 35-43.

Jabbour HN, Ryan JP, Evans G & Maxwell WMC (1991). Effect of season, GnRH administration and Lupin supplementation on the ovarian and endocrine responses of Merino ewes treated with PMSG and FSH-P to induce superovulation. *Reproduction, Fertility and Development*, 3(6): 699-707.

Menchaca A, Villarino M, Crispo M, de Castro T & Rubianes E (2010). New approaches to superovulation and embryo transfer in small ruminants. *Reproduction, Fertility and Development*, 22(1):113-118.

Mendizabal JA, Delfa R, Arana A & Purroy A (2011). Body condition score and fat mobilization as management tools for goats on native pastures. *Small Ruminant Research*, 98(1):121-127.

Naderipour H, Yadi, J, Shad AG & Sirjani MA (2012). The effects of three methods of synchronization on estrus and hormonal profile in Kalkuhi ewes: A comparison study. *African Journal of Biotechnology*, 11(5): 530-533.

Naqvi SMK & Gulyani R (1998). Effect of GnRH and FSH in conjunction with PMSG on superovulatory response in crossbred sheep in India. *Tropical Animal Health and Production*, 30(6): 369-376.

Naqvi SMK & Gulyani R (1999). Ovarian response and embryo recovery to different superovulatory regimens in Rambouillet ewes under semi-arid conditions. *Small Ruminant Research*, 34(2): 127-131.

Perera, GDRK, Pushpakurama, PGA, De Silva, LNA & Alexander, B (2008). Production of genetically superior goats through embryo transfer technology in Sri Lanka. *Tropical Agricultural Research*, 20(1): 177-184.

Riesenberg S, Meinecke-Tillmann S & Meinecke B (2001). Ultrasonic study of follicular dynamics following superovulation with a single application of pFSH, eCG or hMG in goats. *Small Ruminant Research*, 55(4): 83-93.

Ryan DP, Spoon RA & Williams GL (1992). Ovarian follicular characteristics, embryo recovery and embryo viability in heifers fed high-fat diets and treated with follicle stimulating hormone. *Journal of Animal Science*, 70(11): 3505-3513.
Samartzi F, Boscos C, Vainas E & Sakalol P (1995). Superovulatory response of Chios sheep to PMSG during spring and autumn. *Animal Reproduction Science, 39*(3): 215-222.

Sanchez F, Bernal H, del Bosque AS, Gonzalez A, Olivares E, Padilla G & Ledezma RA (2013). Superovulation and embryo quality with porcine follicle stimulation hormone (pFSH) in Kahtadin hair sheep during the breeding season. *African Journal of Agricultural Research, 8*(23): 2977-2982.

Scaramuzzi RJ, Campbell BK, Downing JA, Kendall NR, Khalid M, Munoz Gutierrez M & Somchit A (2006). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. *Nutrition and Reproductive Development, 46*(4): 339-354.

Simonetti L, Forcada F, Rivera OE, Carou N, Alberio RH & Palacin I (2008). Simplified superovulatory treatments in Corriedale ewes. *Animal Reproduction Science, 104*(2): 227-237.

Sosa CA, Gonzalez-Bulnez JA, Abecia F, Forcada F & Meikle A (2009). Short-term undernutrition affects final development of ovulatory follicles in sheep synchronized for ovulation. *Reproduction in Domestic Animals, 43*(6): 1033-1038.