GONADOTROPHIC HORMONE CONCENTRATION AND LITTER SIZE IN EWES IMMUNIZED AGAINST ANDROSTENEDIONE

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ABSTRACT

Immunization against androstenedione is one of the methods of improving the litter size in sheep. The level of gonadotrophin has been said to be controlling factor for ovulation rate. An attempt was made to correlate the level of gonadotrophic hormones with litter size in ewes. Ninety parous healthy Nilagiri (45) and Sandyno (45) ewes were selected and allotted to 3 treatment groups of 15 ewes in each breed (AF-Androstenedione immunogen-full dose, AH-half dose and CC-control group). The ewes from AF and AH groups were injected with androstenedione immunogen (Ovastim, Virbac (Australia) Ltd,) at 8 and 4 weeks before the start of breeding season. Blood samples were collected during Early Follicular Phase (EFP), Late Follicular Phase (LFP) and Mid Luteal Phase (MLP) of the estrous cycle. LH and FSH concentrations were analysed by double antibody radioimmunoassay (RIA). Among the immunized ewes the litter size was higher in Sandyno AF ewes (1.73 ± 0.11) and Nilagiri AH ewes (1.60 ± 0.13). The concentration of LH was significantly higher in immunized groups. The level of LH during EFP (3.91±0.29 çg/ml*)* was significantly higher than LFP *(2.77±0.36 ηg/ml)* and MLP *(2.19±0.28 ηg/ml).* The FSH concentration was higher in EFP *(31.49±2.00 ηg/ml)* and a sharp decline in LFP *(22.70±2.45 ηg/ml)* and again reached the highest value in MLP *(33.01±1.85 ηg/ml).* The Nilagiri ewes *(30.28±1.73 ηg/ml)* had higher FSH concentration than Sandyno ewes *(27.85±1.73 ηg/ml).* The increased plasma LH concentration could be a reason for increased litter size in immunized ewes.

Key words: Ewes, Androstenedione immunization, LH, FSH, RIA and litter size

INTRODUCTION

The fecundity of ewes in sheep farms has a major effect on the economic efficiency and is mainly determined by ovulation rate. Ovulation rate in a sheep is determined by the number of follicles recruited, growth of follicles and number of follicles destined for ovulation, which are under the control of several hormonal factors. Gonadotrophins are the main hormones which initiate the follicular recruitment, its growth and ovulation. The negative feedback effects of gonadal steroids such as estrogen, progesterone etc., have been suggested as a controlling mechanism of gonadotrophins release (Gore-Langton and Armstrong, 1994).

Immunization of ewes against androstenedione a precursor for estrogen is an approach to improve the litter size in ewes (Juengel . *et al.*, 2013 and O'Connell *et al*., 2016). Immunization against gonadal steroids should therefore lead to increased gonadotrophins secretion. However, the mechanism by which the immunization procedure increases ovulation rate remains obscure. It is suggested that the antibodies directed against the androstenedione bind to some of the free androstenedione to alter the steroid feedback relationships between the ovary and the brain (Philipon *et al., 1989). Androstenedione immune ewes have* the steroid feedback relationships between the ovary and the brain (Philipon *et al., 1989).* Androstenedione immune ewes have increased plasma concentrations of LH, oestradiol-*17β,* androstenedione and progesterone. The plasma concentration of FSH was unchanged or significantly lower in the immunized animals (Philipon *et al.,* 1989 and Campbell *et al*., 1991). Contrary to the above findings McNatty *et al.* (1988) found that the concentration of FSH and LH were higher in immunized ewes. The present study was carried out to find out the concentration of gonadotrophins and their role in improvement of litter size in androstenedione immunized Nilagiri and Sandyno sheep.

MATERIAL AND METHODS

Ninety parous healthy Nilagiri (45) and Sandyno (45) ewes were selected and allotted to 3 treatment groups of 15 ewes each (AF-Androstenedione immunogen-full dose, AH-half dose and CC-control group). The ewes from AF and AH groups were injected subcutaneously in the neck, with androstenedione immunogen (Androstenedione-7α-Carboxyethyl thioether: human serum albumin with DEAE dextran immuno-adjuvant, Ovastim, Virbac (Australia) Ltd, New South Wales, Australia) at 8 and 4 weeks before the start of breeding season. Experimental ewes were teased with apronized rams at the ratio of 1:25 twice daily in the morning and evening. The ewes found in estrus were brought to the

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breeding pen, weighed and were hand mated with selected rams twice at an interval of 12 h. Bred ewes were grouped into single flock and maintained under standard managemental condition until lambing. The ewes were monitored closely during lambing and the lambs were marked with their dam immediately after birth and litter size at birth was noted.

COLLECTION OF BLOOD SAMPLES:

Five ewes of each breed from AF, AH and CC groups were selected randomly and estrus was synchronized by two doses of Prostaglandin $F_2\alpha$ (PG-I and PG-II of 10 mg of Dinoprost Tromethamine - Lutalyse, Pharmacia, Pfizer Animal Health, Pfizer Centre, Mumbai- 400 102, India) administered at 10 days interval. The ewes were teased with apronized rams from 48 hours after PG-II. Three ewes from each group and breed, which showed standing estrus, were selected for blood sampling. A polyvinyl intra jugular cannula (Mediflon (18X1.3X45mm) – Eastern Medikit Ltd, Gurgaon, India) was fixed to all ewes on day 10 of the estrous cycle to collect the blood samples. All ewes were injected with PG $F_2\alpha$ (PG-III) at 15.00 h on the day of cannulation.

Blood samples were collected at 30 minutes interval during early follicular phase (EFP- from 21 to 27 hours after PG-III), and late follicular phase (LFP - from 38 to 42 hours after PG-III). Twelve days after PG-III the ewes were re-cannulated and blood samples were collected for 6 hours at 30 minutes interval (Mid Luteal Phase - MLP). Three ml of blood was collected in centrifuge tubes containing 100 IU Heparin (Beparine, Biological E. Limited, Hyderabad, India). Plasma was separated by centrifugation at 2000 rpm for 20 minutes at room temperature. The plasma was stored in plasma vials at –20°C until assayed.

ESTIMATION OF GONADOTROPHINS

Plasma LH and FSH concentrations were determined by Radioimmunoassay (RIA), which was performed at the National Institute of Animal Nutrition and Physiology, Adugudi, Bangalore t as per the procedure of Niswender *et al.* (1969).

LUTEINIZING HORMONE

RIA kit for estimation of LH was supplied by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Hormone and Peptide Program (NAPP), Torrance, U.S.A. The ovine LH used for iodination was NIDDK-oLH-1-4(AFP-8614B). The antiserum used was NIDDK - anti - oLH-1 (AFP-192279). The antiserum exhibited low cross reactivity reactions with FSH–(NIDDK-RP-1) = 0.054 %; oGH - (NIDDK-1-4) = 0.006%; oPRL - (NIDDK-1-2) = 0.00005 % and bTSH - (NIDDK-1-1) = 0.0015 %. Each plasma sample (0.2ml) was assayed in duplicate (100 µl per sample). The minimum detectable level of LH was 0.1 ng/ml plasma. The intra and inter-assay co-efficient of variation were d”10 and d” 13 per cent respectively.

FOLLICLE STIMULATING HORMONE

Plasma samples collected at one-hour interval were analyzed for FSH concentrations by radioimmunoassay (RIA). RIA kit was supplied by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Hormone and Peptide Program (NAPP), Torrance, U.S.A. The ovine FSH (oFSH) used for iodination was NIDDK-oFSH-19-SIAFP (AFP4117A). The oFSH reference preparation was NIDDK-oFSH-RP-2. The antiserum used was NIDDK – anti – oFSH -1 (AFP - C5288113). The sensitivity of the assay was 0.1 ng/ml plasma. At a final dilution of 1:80,000 the antiserum exhibited cross reactivity reactions with LH (NIDDK-23) = 0.00167%; oGH – (NIDDK-AFP-5285C) = 0.00027% and oPRL - (NIDDK- AFP-4328C) = 0.00001 %. Each plasma sample (0.2ml) was assayed in duplicate (100 µl per sample). The intra and inter-assay co-efficient of variation were d”10 and d” 13 per cent respectively.

STATISTICAL ANALYSIS

Least square procedure (Harvey, 1990) was used to study the effects of treatment and other factors on various traits. All possible interactions with set of fixed effects were fitted initially and insignificant interaction effects were omitted. The linear statistical model was used for analysis of various traits. The differences between the least square means for subclasses under a particular effect were tested by Duncan’s multiple range test modified by Kramer (1957).

RESULTS AND DISCUSSION

Litter size at birth

The litter size at birth was significantly higher ($P<0.01$) in ewes treated with immunogen than in control ewes (Table 1). Similar increases in litter size at birth were reported by Crocker *et al.* (2003), Juengel *et al.* (2013) and O’Connell *et al.* (2016). In Nilagiri ewes, AH group had slightly higher litter size than AF group. Sensitivity of the ovaries for stimulation (Androstenedione immunogen) is higher in genetically prolific sheep. The Booroola- Merino ewes, which are characterized by high ovulation rate, had a higher ovulatory response to androstenedione immunogen (to what?) than other less prolific Merinos (Bindon and Piper, 1986). The Nilagiri ewes being the carrier of Fec B Gene mutations (Saravanan *et al*., 2020) are more prolific than Sandyno ewes (Anilkumar, *et al.,* 2009) and might have had an over stimulation with full dose of immunogen and higher ovulation rate and resulted in small litter size at birth as observed in the present study. In contrary, the AF group of Sandyno ewes had significantly higher litter size at

birth than AH group of ewes. The body weight of Sandyno ewes was on an average 5 kg higher than Nilagiri ewes and hence, the half dose of the immunogen might not have been as effective as full dose in Sandyno ewes.

CONCENTRATION OF LH

Treatment Groups: Plasma LH concentrations in AF *(3.83 ± 0.31 ηg/ml)* and AH groups *(3.12 ± 0.31 ηg/ml)* were significantly higher than the control ewes *(1.92 ± 0.31 ηg/ml).* Numerous studies have demonstrated an increased secretion of LH in androstenedione immune ewes (Philipon *et al.,* 1989; Campbell *et al.,* 1990 and Campbell *et al.,* 1991). Estrogenic follicles in immunized ewes produce less estrogen per follicle (Scaramuzzi and Hoskinson, 1984) because of inhibition of the androstenedione by immunogen. This decrease in estrogen production per follicle appeared to be offset by more number of estrogenic follicles in immunized ewes. This results in increased level of LH secretion in immunized ewes (Campbell *et al.,* 1991), which might be due to the increased concentration of estrogen .

Phase of Estrous Cycle: LH concentration was significantly (P<0.01) higher in early follicular phase (3.91 ± 0.29 *ηg/ml)* and there was a marked reduction during late follicular phase (2.77 ± 0.36 *ηg/ml) and t* its level was the least during the mid-luteal phase (2.19 ± 0.28 *ηg/ml).* Similar trend of LH concentration was observed by Campbell *et al.* (1991) in immunized ewes. The concentration of LH is more during the follicular phase and is controlled by estrogen and progesterone. The presence of estrogen during the follicular phase, particularly at the preovulatory stage causes an LH surge and ovulation in ewes (Pathiraja *et al.,* 1984).

LH concentration in all three phases of the estrous cycle in AF group was significantly higher than the other two groups (AF and CC groups). Similarly, the concentration of LH was the least in all three phases of the estrous cycle in animals of control group. The results from the study indicated that administration of immunogen (full and half dose) definitely increased the LH concentration in all 3 phases of the cycle

Breed of Ewes: LH concentration in Sandyno ewes (3.63 ± 0.26 *ηg*/ml) was significantly higher than the Nilagiri ewes *(2.29 ± 0.26 ηg*/ml). However, the difference in the LH concentration was minimal among the Nilagiri (1.89 ± 0.07 *ηg*/ml) and Sandyno ewes (1.95 ± 0.62 *ηg/* ml) of control group, which clearly indicated that the immunization resulted in increased secretion of LH in both breeds.

Plasma LH concentration increased significantly in immunized ewes (AF and AH groups) than in that of control groups. Further this increase in LH concentration was more pronounced in Sandyno ewes than in Nilagiri ewes. This might be the possible reason for the increased response in terms of litter size in Sandyno ewes than in Nilagiri ewes. More so, the study indicated that the LH concentration was increased with increasing dose of immunogen. But in Nilagiri ewes although the LH concentration in AF group was more than AH group, the litter size was higher in AH group. This might be due to the low body weight of the Nilagiri ewes and higher prolificacy than Sandyno ewes. The Nilagiri ewes being more prolific might have had an ovarian over stimulation in response to full dose of immunogen with higher ovulation rate, by virtue of which ewes failed to convert into lambs and resulted in small litter size at birth (Bindon and Piper, 1986) as observed in the present study.

CONCENTRATION OF FSH

Treatment Groups: The level of FSH was non-significantly higher in control ewes (29.80 ± 2.12 *ηg*/ml) than in AF (28.93 ± 2.12 *ηg*/ml) and AH (28.46 ± 2.12 *ηg/* ml) groups. Similar observations were reported by Philipon *et al.* (1989); Campbell *et al.* (1990) and Campbell *et al.* (1991). However, McNatty *et al.* (1988) observed an increased level of FSH in androstenedione immune ewes.

The FSH concentration is under dual control of estradiol and ovarian peptide inhibin (Martin *et al.,* 1988). Immunization against androstenedione causes a transient decrease in estrogen and thereby increased the concentration of FSH. However, the peripheral concentration of inhibin was higher in androstenedione immune ewes (Campbell *et al.,* 1990). The transient increase in FSH would be counteracted by the increased inhibin secretion thus leading to unchanged FSH concentration. This might be the possible reason for the lower level of FSH in immunized ewes compared to control ewes.

Phase of Estrous Cycle: The concentration was significantly (P<0.01) higher in early follicular phase (31.49 ± 2.00 *ηg*/ml) followed by a sharp decline during the late follicular phase (22.70 ± 2.00 *ηg*/ml) and raised in mid luteal phase (33.01 ± 2.00 *ηg*/ml). Similar fluctuations during the different phases of estrous cycle were observed by many authors (Martin *et al.,* 1988; Philipon *et al.,* 1989; Campbell *et al.,* 1990 and Campbell *et al.* 1991) who found that the level of FSH was the lowest during LFP and the highest during MLP.

Breed of Ewes: The Nilagiri ewes (30.28 ± 1.73 *ηg*/ml) had a higher concentration of FSH than Sandyno ewes (27.85 ± 1.73 *ηg/*ml). Generally, the sheep with inherited high ovulation rate may be maintaining a higher level of FSH as observed in D'man sheep (Pathiraja *et al.,* 1984) or produce less feedback hormone per egg shed as in Booroola gene (Cummins *et al.,* 1983). Similarly, The Nilagiri ewes being the carrier of Fec B Gene mutations (Saravanan *et al*., 2020) are more

prolific than most of the Indian breeds, might have a higher level of FSH than Sandyno ewes.

In conclusion, the immunization against androstenedione has increased the litter size in both Nilagiri and Sandyno ewes. Plasma LH concentration significantly increased in immunized ewes and it was more pronounced in Sandyno ewes. The increase in LH concentration could be the reason for the improvement in litter size at birth in immunized ewes.

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Table 1 : Mean (± SE) liter size at birth in ewes treated with immunogen

Treatment	Nilagiri	Sandyno
AF	1.47 ± 0.13 (22/15)[a]	1.73 ± 0.11 (26/15)[a]
AH	1.60 ± 0.13 (24/15)[a]	1.40 ± 0.11 (21/15)[b]
CC	1.13 ± 0.13 (17/15)[b]	1.00 ± 0.11 (14/14)[c]

Means in the same column between the rows with different superscripts differ significantly

Table 2 : Mean (± SE) concentration of Luteinizing Hormone and Follicle Stimulation Hormone in ewes treated with immunogen

Hormone	LH		FSH	
Effects	**Number**	**Mean ± SE (ηg/ml)**	**Number**	**Mean ± SE (ηg/ml)**
Treatments	**		NS	
AF	198	3.83 ± 0.31 [b]	102	28.93 ± 2.12
AH	198	3.12 ± 0.31 [b]	102	28.46 ± 2.12
CC	198	1.92 ± 0.31 [a]	102	29.80 ± 2.12
Phase of the estrous cycle	**		**	
Early follicular phase **(EFP)**	216	3.91 ± 0.29 [b]	108	31.49 ± 2.00[b]
Late follicular phase **(LFP)**	144	2.77 ± 0.36 [a]	72	22.70 ± 2.45 [a]
Mid luteal phase **(MLP)**	234	2.19 ± 0.28 [a]	126	33.01 ± 1.85 [b]
Breed	**		NS	
Nilagiri	297	2.29 ± 0.26 [a]	153	30.28 ± 1.73
Sandyno	297	3.63 ± 0.26 [b]	153	27.85 ± 1.73
Treatment X Phase	NS		NS	
AF x EFP	72	4.98 ± 0.51	36	32.57 ± 3.47
AF x LFP	48	3.99 ± 0.62	24	21.76 ± 4.27
AF x MLP	78	2.52 ± 0.49	42	32.46 ± 3.21
AH x EFP	72	4.72 ± 0.51	36	29.89 ± 3.47
AH x LFP	48	2.42 ± 0.62	24	23.62 ± 4.27
AH x MLP	78	2.22 ± 0.49	42	31.89 ± 3.21
CC x EFP	72	2.03 ± 0.51	36	32.03 ± 3.47
CC x LFP	48	1.90 ± 0.62	24	22.71 ± 4.27
CC x MLP	78	1.83 ± 0.49	42	34.67 ± 3.21
Breed X Phase	*		NS	
NILAGIRI x EFP	108	2.03 ± 0.51 [a]	54	33.54 ± 2.83
NILAGIRI x LFP	72	1.90 ± 0.62 [a]	36	23.08 ± 3.47
NILAGIRI x MLP	117	1.83 ± 0.49 [a]	63	34.23 ± 2.62
SANDYNO x EFP	108	4.72 ± 0.51 [b]	54	29.44 ± 2.83
SANDYNO x LFP	72	2.42 ± 0.62 [a]	36	22.31 ± 3.47
SANDYNO x MLP	117	2.22 ± 0.49 [a]	63	31.79 ± 2.62
Overall mean	**594**	**2.96 ± 0.18**	**306**	**29.07 ± 1.22**

Means in the same column between the rows with different superscripts differ significantly

*(P<0.05) ** (P<0.01) NS – Not significant