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Immunization against androstenedione to improve the reproductive efficiency in Nilagiri and Sandyno ewes

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ABSTRACT

Healthy Nilagiri (90) and Sandyno (90) ewes were selected and allotted to 3 treatment groups of 15 ewes each, viz. AF (Androstenedione immunogen full dose), AH (Androstenedione immunogen half dose) and CC (Control ewes). The experiment was carried out in main and off breeding seasons. The ewes from AF and AH groups were injected with 2 ml (full dose) and 1 ml (half dose) of androstenedione immunogen at 8 and 4 weeks respectively before the start of breeding season. Immunization of ewes against androstenedione had no significant effect on tupping and lambing percentage. The litter size at birth was significantly increased in AF (1.47±0.07) and in AH (1.40±0.07) groups. The litter size was highest in AF Sandyno ewes (1.60±0.09) and in AH Nilagiri ewes (1.43±0.11). The litter size for immunized group ewes was higher during main breeding season (1.40±0.04) than off breeding season (1.23±0.06). Nilagiri (1.31±0.05) and Sandyno (1.33±0.05) ewes had similar litter size at birth. The litter weight at birth and weaning were significantly higher in AF (3.95±0.13 and 13.89±0.61 kg) groups followed by AH (3.80±0.13 and 13.19±0.61 kg) and CC (3.18±0.13 and 10.89±0.66 kg) groups. The weaning percentage/ survivability were similar for all the treatment groups.

Keywords: Androstenedione immunization, Ewes, Litter size, Litter weight, Reproductive efficiency

The number of lambs born per ewe has major effect on the economic efficiency of the sheep production systems and is largely determined by ovulation rate. The litter size is the reflection of ovulation rate. High litter size was attained by selecting highly prolific animals, cross breeding prolific with less prolific breeds (Hanrahan 1985), changing the plane of nutrition (Omar *et al.* 2019) and use of exogenous gonadotrophins (Anilkumar *et al.* 2003, Koyuncu and Altýcekic 2010). All these methods are associated with number of recognized short comings. Hence, an alternative strategy to modulate the activity of the existing endogenous endocrine system is highly essential.

Manipulation of the immune system to confer immunity against infectious organisms is a well-established procedure used in animal production. Similarly, neutralization of the biological actions of an endogenous hormone by antigen-antibody complex formation *in vivo* was used for improvement in litter size in ewes (Crocker *et al.* 2003, Juengel *et al.* 2013, O'Connell *et al.* 2016). The ewes produce antibodies against one of her own hormones and the antibodies thus produced binds to the hormone and inactivate the biological activity of the hormone (androstenedione) leading to changes in the control systems of the ovary (Pell and Aston 1995). Several studies on immunization against androstenedione were recorded and there were no reports of Indian work on androstenedione immunization. Hence a study was carried out to standardize the immunization procedure in Indian breeds of sheep and the influence of breed, breeding season and dose rate of androstenedione immunogen on the reproductive performance of Nilagiri and Sandyno ewes were also carried out.

MATERIALS AND METHODS

Experimental animals: Healthy Nilagiri (90 ewes) and Sandyno (90 ewes) ewes between 2 and 5½ years of age, maintained at Sheep Breeding Research Station (SBRS), Sandynallah, The Nilgiris District of Tamil Nadu were randomly selected. The ewes were allowed for grazing in the natural pasture land from 9.00 AM to 5.00 PM. The ewes were bred in two breeding seasons, viz. main breeding season (September to October) and off breeding season (March to April). The selected ewes (45 ewes in each breed/season) were randomly allotted to three treatment groups of 15 ewes each as follows: Androstenedione immunogen full dose (2 ml) – AF; Androstenedione immunogen half dose (1 ml) – AH and Control ewes – CC. The randomization was done such that there was no significant difference in the age and body weight of ewes

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in different groups.

Location and climate of the research station: Sheep Breeding Research Station, Sandynallah is located at 11°25′ latitude N and 76°46′ longitude E, about 13 km away from Udhagamandalam in the Nilagiri Hills at an altitude ranging from 2,090 to 2,235 meters above mean sea level. The annual rainfall ranges from 848 to 3,000 mm. The farm experiences a temperate climate with a maximum temperature of 24°C during the hottest days. During winter, the night temperature falls to subzero levels.

Immunization of ewes, estrus detection, breeding and duration of estrus: The ewes from AF and AH groups were injected with 2 ml (full dose) and 1 ml (half dose) of androstenedione immunogen (Androstenedione-7α-Carboxyethyl thioether: human serum albumin with DEAE dextran immuno-adjuvant, Ovastim, Virbac (Australia) Ltd, New South Wales, Australia) subcutaneously on the neck region. The ewes were immunized with two doses of Ovastim at 8 and 4 weeks before the start of breeding seasons. Similarly, the ewes from control groups were injected with normal saline subcutaneously.

Experimental ewes from AF, AH and CC groups were teased with apronized rams at the ratio of 1:25 daily twice in the morning (6.30 AM to 8.00 AM) and evening (4.30 PM to 6.00 PM). The ewes found in estrum were brought to the breeding pen, weighed and were hand mated with selected rams twice at an interval of 12 h. Teasing of ewes in estrum after breeding was continued once in 4 h using apronized rams. Duration of estrum was calculated as the time interval between the first and last acceptance of the teaser ram by the ewe (Selvaraju 1994). Ewes which were bred 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. The lambs were weaned from the dam at the age of 90 days.

Date of breeding, weight at the time of breeding, date of lambing and weight of ewes at lambing were collected. Particulars pertaining to lambs, viz. number of lambs born, born alive, still born, weight of lambs at birth, number of lambs weaned and weight of lambs at weaning were also collected. Based on these collected data, tupping percentage, lambing percentage, litter size, weaning percentage, litter weight at birth and weaning were calculated.

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. Arcsin transformation was done for tupping and lambing percentage. 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

There was no significant difference in tupping percentage among the treatment groups. Scaramuzzi *et al.* (1993) and Crocker *et al.* (2003) observed no difference in tupping percentage among the treatment groups similar to the present study. Immunization of ewes against androstenedione also had no significant effect on lambing percentage which was in agreement with the findings of Philipon and Driancourt (1987) and Crocker *et al.* (2003). However, lambing percentage was increased in several studies (Wong 1988, Juengel *et al.* 2013, O'Connell *et al.* 2016). The tupping percentage and lambing percentage in ewes bred during main breeding season were significantly (P<0.01) higher (100.00 and 100%) than off breeding season (89.70 and 89.20).

Table 1. Mean (±SE) body weight at breeding, duration of estrum and litter size at birth

Effect	Body weight at breeding (kg)	Duration of estrum (h)	Litter size at birth
Treatment			
AF	28.34±0.45 (50)	27.84±0.68 (50)	1.49±0.06^{b} (50)
AH	28.64±0.45 (50)	27.36±0.68 (50)	1.40±0.06^{b} (50)
CC	28.00±0.45 (50)	27.98±0.68 (50)	1.02±0.06^{a} (50)
Season of breeding			
Main	30.93±0.33^{b} (90)	27.72±0.50 (90)	1.39±0.04^{b} (50)
Off	25.73±0.41^{a} (60)	27.72±0.62 (60)	1.21±0.06^{a} (50)
Breed			
Nilagiri	26.10±0.38^{a} (72)	27.61±0.57 (72)	1.29±0.05 (50)
Sandyno	30.56±0.36^{b} (78)	27.83±0.54 (78)	1.31±0.05 (50)
Overall mean	28.33±0.26 (150)	27.72±0.40 (150)	1.30±0.04 (50)

Means in the same column within categories with different superscript differ significantly.

Duration of estrum: The overall mean duration of estrum in Nilagiri and Sandyno ewes treated with androstenedione immunogen was 27.72±0.40 h (Table 1). There was no significant difference between the breeds, breeding seasons and treatment with androstenedione immunogen. Similar to the present study, Philipon and Driancourt (1987) also recorded no significant difference between the treated and control ewes. This showed that immunization did not affect the expression of estrum. Though the secretion of estrogen per estrogenic follicle is reduced in immunized ewes, the presence of more estrogenic follicle compensates for this and the concentration of estradiol was increased in the circulation there by normal duration of estrum is maintained (Philipon and Driancourt 1987).

Litter size at birth: Litter size at birth, an easily measurable trait, determines the efficacy of any reproductive improvement experiment in small ruminants. The litter size at birth in immunized ewes was significantly increased than control ewes (Table 1). Similar increase in litter size at birth were reported by Zafracas *et al.* (1992), Crocker *et al.* (2003), Juengel *et al.* (2013) and O'Connell *et al.* (2016).

The litter size was non significantly higher in AF group than AH group. Similar observations was made by Smith and McGowan (1986) who have found similar litter size in

Table 2. Mean (±SE) litter size at birth in Nilagiri and Sandyno ewes treated with immunogen

Treatment	Nilagiri			Sandyno		
	Main breeding season	Off-breeding season	Overall	Main breeding season	Off-breeding season	Overall
AF	1.47±0.12 (22/15)	1.25±0.16 (10/8)	1.36±0.16^{b} (32/23)	1.73±0.10 (26/15)	1.44±0.14 (13/9)	1.59±0.09^{c} (39/24)
AH	1.60±0.12 (24/15)	1.25±0.16 (10/8)	1.43±0.10^{b} (33/23)	1.40±0.10 (21/15)	1.33±0.14 (12/9)	1.37±0.09^{b} (33/24)
CC	1.13±0.12 (17/15)	1.00±0.18 (6/6)	1.06±0.11 ^{a} (23/21)	1.00±0.11 (14/14)	1.00±0.13 (10/10)	1.00±0.08^{a} (24/24)
Overall	1.40±0.07^{b} (63/45)	1.17±0.10^{a} (26/22)	1.28±0.06 (88/67)	1.38±0.06 (61/44)	1.26±0.08 (35/28)	1.32±0.05 (96/72)

Means in the same row with different superscripts differ significantly. Numbers in parenthesis indicate number of lambs born / number of ewes lambed.

ewes which were given either 2.0 ml or 1.0 ml immunogen. The highest litter size at birth among the treatment groups was in ewes bred during the main breeding season from AH group (1.60±0.12). Similarly, the season of breeding had a significant ($P<0.05$) effect on litter size at birth in the Nilagiri ewes. The ewes bred during main breeding season (1.40±0.07) had higher litter size than ewes bred during off breeding season (1.17±0.10). The better response to immunization during the main breeding season might be due to better body weight and condition of the animals during this season. Crocker *et al.* (1987) observed that the ewes joined in late December gave better litter size at birth than ewes joined in February.

The Sandyno ewes from group AF had significantly ($P<0.01$) higher (1.59±0.09) litter size than AH (1.37±0.09) and CC (1.00±0.08) groups (Table 2). The highest litter size at birth in Sandyno ewes was in ewes bred during the main breeding season from AF group (1.73±0.11). Litter size at birth in immunized Sandyno ewes was marginally higher than immunized Nilagiri ewes. However, the litter size at birth in Nilagiri ewes (1.12±0.01) was significantly higher than Sandyno (1.05±0.01) ewes (Rajendran 2005). Increase in litter size at birth in Nilagiri and Sandyno ewes was 0.17 and 0.26 respectively. This result showed that, Sandyno ewes responded well to immunization than Nilagiri ewes. The body weight and condition at breeding determined the response for ovulation rate / ovarian stimulation in ewes (Kumar *et al.* 2003). The body weight at breeding was significantly ($P<0.01$) higher in Sandyno ewes (31.54±0.66 kg) than in Nilagiri ewes (26.95±0.64 kg) (Table 1).

In Nilagiri ewes, the litter size at birth in AH group (1.43±0.11) of ewes were higher than AF group (1.38±0.11) (Table 2). Smith and McGowan (1986) observed a higher ovulation rate in full dose than half dose of immunogen treated ewes (2.45 for full, 2.20 for half and 1.86 for control). However, the higher ovulation rate of full dose compared to half dose did not result in more lamb born (lambs born per ewes joined were 1.68 full, 1.65 half and 1.35 control). In addition, the physiological parameter which was found to be genetically correlated with prolificacy is the ovarian sensitivity to stimulation. The Booroola- Merino ewes, which are characterized by high ovulation rate, had a higher ovulatory response than other less prolific Merinos (Bindon and Piper 1986). The Nilagiri ewes being the carrier of *Fec B* gene mutations (Saravanan *et al.* 2020) were prolific than Sandyno ewes (Anilkumar *et al*. 2009) might have had an over stimulation and higher ovulation rate, which the ewes might not be able to convert into lambs and resulted in less litter size at birth as observed in the present study.

Contrary to Nilagiri ewes, the AF group of Sandyno had higher litter size at birth (1.63±0.09) than AH group of ewes. The body weight of Sandyno ewes was on an average 5 kg higher than Nilagiri ewes. Hence the half dose of the immunogen might not have been effective in Sandyno ewes as noted in Nilagiri ewes. It was suggested that the dose of immunogen may be increased as per the body weight of ewes to achieve greater response in increasing the litter size.

Litter weight at birth and weaning: The ewes from AF group weaned significantly ($P<0.01$) higher litter weight (13.78±0.63 kg) than AH group (13.08±0.64 kg) and was significantly lower in (10.75±0.69 kg) CC group of lambs (Table 3). Zafracas *et al.* (1992) made similar observations that the immunized ewes had higher litter weight than control ewes. The litter weights in immunized groups were higher, because of more twins added up for the litter weight. Sandyno ewes had higher litter weight than Nilagiri ewes. Sandyno lambs were heavier at birth than Nilagiri ewes (Rajendran 2005) and hence the litter weight at birth was more in Sandyno in this study.

The litter weight at weaning was significantly higher in immunized groups than the Control group. The findings in the present study are in consonance with the study made by Zafracas *et al.* (1992). The lambs born during March had higher litter weight at weaning than September. There were more twin lambs during March lambing and hence the total weight of the lambs increased the litter weight in immunized groups.

In the current study, Sandyno lambs were heavier at weaning than Nilagiri lambs. However, the litter size at birth was similar in both breeds. The difference in weaning weight

Table 3. Mean (±SE) litter weight at birth and weaning in ewes treated with immunogen

Effect	Litter weight at birth (kg)		Litter weight at weaning (kg)	
	No.	Mean±SE	No.	Mean±SE
Treatment				
AF	47	3.93±0.13[b]	47	13.78±0.63[b]
AH	46	3.81±0.13[b]	46	13.08±0.64[b]
CC	44	3.16±0.13[a]	40	10.75±0.69[a]
Season of birth				
March	88	3.72±0.09	86	14.08±0.46[b]
September	49	3.55±0.13	47	10.99±0.63[a]
Breed of ewes				
Nilagiri	66	3.46±0.11[a]	65	12.01±0.55[a]
Sandyno	71	3.82±0.11[b]	68	13.06±0.53[b]
Overall mean	137	3.64±0.08	178	12.54±0.39

Means in the same column within categories with different superscripts differ significantly.

was also maintained in litter weight at weaning. Hence, the litter weight was more in Sandyno ewes.

Overall weaning percentage was 95.80 (range: 91.30 to 100%). The weaning percentage was similar in all groups. There was no difference in weaning percentage or survivability among the treatment groups. Present results were in agreement with Crocker *et al.* (1984) who observed weaning percentage of 92 and 93% in control and immunized ewes which had 26 and 60% multiple births. Crocker *et al.* (2003) observed a higher loss of lambs before weaning in immunized ewes. However, Philipon and Driancourt (1987), Juengel *et al*. (2013) and O'Connell *et al.* (2016) observed no difference in the survivability of lambs between control and immunized groups.

Survival rates of twins were often markedly lower than singles (Mutiga and Mukasa-Mugerwa 1992). However, in the present study, the weaning percentage of twins were similar to singles. The better management of the lambs at birth and during the pre-weaning period in semi-intensive system of rearing might be the reason for higher survivability of twins in this study. Hence it is inferred that the survival rates could be high in flock with many twins. The performance of the immunized ewes in this study proved that lamb mortalities need not always increase due to twinning.

In conclusion, it was observed that immunization against androstenedione improves litter size at birth irrespective of breed, breeding season and dose of immunogen without affecting the expression of estrum, tupping percentage, fertility and survivability of lambs. The response for immunization was highest during the main breeding season. The response for immunization was higher in Sandyno ewes than Nilagiri ewes.

REFERENCES

Anil Kumar R, Iyue M and Srinivasan P. 2003. Reproductive efficiency in high and low body weight Nilagiri ewes subjected to short term flushing and PMSG treatments. *Indian Journal of Animal Reproduction* **24**: 24–28.

Anilkumar R, Chandrahasan C, Selvaraju M and Dinakaran A M. 2009. Reproductive performance of Nilagiri and Sandyno ewes. *Indian Journal of Animal Reproduction* **30**: 26–28.

Bindon B M and Piper L R. 1986. The reproductive biology of prolific sheep breeds. *Oxford Reviews of Reproductive Biology* **8**: 414–51.

Crocker K P, Cox R I, Johns M A, Johnson T J and Salertian M. 1984. Influence of time of injection of Fecundin on the reproductive performance of Merino ewes in Western Australia. *Reproduction in Sheep*. Cambridge University press, Cambridge, UK. pp. 329–331.

Crocker K P, Cox R I, Johnson T J and Salertian M. 1987. The reproductive performance of naturally mated and artificially inseminated ewes treated with Fecundin at various intervals before the start of breeding. *Theriogenology* **27**: 869–77.

Crocker K P, Davidson R, Highman D and Hart K. 2003. Increasing lambing percentages with Ovastim. *Ovine Observer* **23**: 10–11.

Hanrahan J P. 1985. Immunization of ewes against androstenedione. Animal Production, report from Dunsinea Moorepark and Western Research Centers, Research report (1985). Cited from *Animal Breeding Abstract* (1986) **54**: 3002.

Harvey W R. 1990. Mixed Model Least-squares and Maximum Likelihood Computer Programme. PC-2 version. Ohio State University, Columbus.

Juengel J L, Proctor L E, Wearne K, Olliver D, Hudson N L, Jensen D, Davis G H, Johnstone P D and McNatty K P. 2013. Effects of immunization against androstenedione or bone morphogenetic protein 15 (BMP15) on reproductive performance in sheep. *Journal of Animal Sciences* **91**: 5946–53.

Koyuncu M and OzisAltýcekic S. 2010. Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. *Asian Australasian Journal of Animal Sciences* **23**: 308–11.

Kramer C Y. 1957. Extension of multiple range tests to group correlated adjusted means. *Biometrics* **13**: 13–18.

O'Connell A R, Demmers K J, Smaill B, Reader K L and Juengel J L. 2016. Early embryo loss, morphology, and effect of previous immunization against androstenedione in the ewe. *Theriogenology* **86**: 1285–93.

Omar A E, Gharib H S and Said E N. 2019. Effect of feeding different concentrate roughage ratio on growth, reproductive performance and behavior of sheep. *Slovakian Veterinary Research* **56**(Suppl 22): 433–43.

Mutiga E R and Mukasa-Mugerwa F. 1992. Effect of the method of estrus synchronization and PMSG dosage on estrus and twinning in Ethiopian Menze sheep. *Theriogenology* **38**: 727–34.

Pell J M and Aston R. 1995. Principles of immuno modulation. *Livestock Production Science* **42**: 123–33.

Philipon P and Driancourt M A. 1987. Potential of active immunization against androstenedione to improve fecundity in sheep. *Animal Reproduction Science* **15**: 101–112.

Rajendran R. 2005. 'Genetic analysis of reproduction traits and survivability of Nilagiri sheep and its Synthetic crosses'. PhD Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

Saravanan R, Kumarasamy P, Anil Kumar R, Thiruvenkadan A K and Murali N. 2020. Molecular screening of *Fec* B gene mutation through PCR RFLP method in different sheep breeds of Tamil Nadu. *International Journal of Genetics* **12(4)**: 725–27.

Scaramuzzi R J, Nancarrow C D, Murray J D and Walton J R. 1993. Reproductive wastage in the androstenedione-immune ewe. *Journal of Reproduction and Fertility* **98**: 617–24.

Selvaraju M. 1994. 'Studies on oestrus synchronization in Tellicherry goats'. M.V.Sc. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India.

Smith J F and McGowan L T. 1986. The effect of dose level of steroid immunogen on the reproduction performance of ewes. *Proceedings of the New Zealand Society for Animal Production* **46**: 165–170.

Wilkins J F. 1997. Method of stimulating ovulation rate in Merino ewes may affect conception but not embryo survival. *Animal Reproduction Science* **47**: 31–42.

Zafracas A, Avidi M and Alifakiotis T. 1992. Increasing lambing percentage by androstenedione immunoneutralization in synchronized ewes with or without PMSG. *Therigenology* **37**: 1341–44.