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Estrus synchronization in sheep using progesterone sponge

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Abstract
In the present experiment 41 ewes were synchronized using progesterone sponge (CSWRI) for 12 days and inj. eCG @200 IU was given on the day of the withdrawal of progesterone sponge. Estrus incidence was observed in 80.49% ewes by the observation of symptoms up to 48 hrs. with the mean estrus onset time of 22.61±1.34hrs. A total of 16 synchronized ewes received fresh embryo transfer of 30 embryos. The conception rate, lambing percentage and gestational embryo loss was 50.00, 50.00 and 50.00 percent respectively. Total 7 lambs were born resulting 23.33% efficacy of the embryo transfer programme.

Keywords: Estrus synchronization, onset of heat, progesterone sponge, embryo transfer, ETT in sheep

Introduction
Assisted reproduction technologies such as Embryo Transfer Technology (ETT) and Artificial Insemination (AI) have been a popular technique for propagation of desired genetics in farm animals (Gordon, 1997) [15]. Precise synchrony in estrus/ovulation is essential for the successful implementation of these techniques. Various estrus synchronization protocols have been developed in ewes, using natural progesterone or synthetic progestagen in combination with gonadotropins and prostaglandins. Synchronized estrus offers homogeneous groups of animals and thus allows the constancy of the results irrespective of the season.

Material and Methods
The present work was conducted on crossbred ewes stationed at Sheep Breeding Farm, Panthal, Reasi, Jammu, India, during the pre-breeding season (August to November). The environmental temperature was fluctuating between a minimum of 8 ^{o}C and a maximum of 30 ^{o}C during this period. All the experimental ewes were reared under loose housing system and were fed daily 6 kg green/dry fodder (Oat/maiz/barseem) and 500 gm concentrate besides the routine grazing of 8-10hrs on lush green pastures. Special attention was made to keep the animal house in a clean and hygienic condition. E Estrus synchronization treatment was given to forty one mature non lactating crossbred ewes using intravaginal progesterone impregnated sponge (procured from CSWRI, Avikanagar) kept in-situ for 12 days and inj. eCG (@200 IU) at the withdrawal of sponge. Estrus detection in donor and recipient ewes was carried out for 30 minutes duration at an interval of every 3 hrs up to 48 hrs after removal of implant using appronized ram. The onset and duration of estrus time and percentage of ewes showing estrus was recorded. Total 16 ewes observed in estrus received 30 freshly collected embryos which were transfer with the assistance of laparoscope. The percent conception rate, gestational loss and lamb survival after birth of lambs were recorded to study the effectiveness of estrus synchronization in the embryo transfer programme.

Results and Discussion
Incidence and Onset of Estrus
Estrus incidence was observed in 80.49% ewes by observation of symptoms up to 48 hrs with the mean estrus onset time of 22.61±1.34hrs (Table 1). The results indicate that the treatment of progesterone implant and eCG has shown high degree of synchrony. The estrus incidence percentage is slightly lower than the other workers (Simonetti *et al*., 1999; Ainsworth and Shrestha, 1983; Crosby *et al*., 1991; O'doherty and Crosby, 1990) [27, 2, 8, 24] who observed estrus up to 72 hrs. The differences in the estrus incidence might be due to the extended observation period in the others work besides the type of protocol used.
The estrus onset time observed in the present work is similar to the work of Smith and Parr (1992) [28] who used CIDR for 14 days with eCG, while Iida *et al.* (2004) [17] reported estrus onset at 25.5±1.28 hrs. by using self-made sponge containing 0.5g progesterone and

Santos *et al*. (2010) [26] who used only MAP sponge to get estrus onset at 27.06±17.46 hrs in their experiments. Other workers has reported longer duration for the estrus onset (Hashemi *et al*., 2006; Das *et al*., 2000; Turk *et al*., 2008) [16, 9, 30] in sheep after treatment with progestin, eCG and PG in different combinations. Differences in the onset of estrus may be due to the difference in the type and amount of progestagen and use of additional hormones, it may also vary due to the breed and seasonal variations.

The onset of estrus may vary as per drugs used for synchronization and superovulation. According to Baril *et al*. (1993) [5], early estrus onset is better as it releases oocytes that are appropriate for fertilization and late estrus is associated with lower quality oocytes. Progestagens are metabolized more slowly than progesterone, leading to the late unblocking of the hypothalamic-pituitary axis and consequently delays estrus onset in animals that are treated with progestagens (Flynn *et al*., 2000; Uribe-Velasquez *et al*., 2008) [11, 31].

High levels of progestagens are known to increase follicular turnover (Leyva *et al*., 1998) [20]. The time of onset of estrus and ovulation may be predicted after the withdrawal of progesterone (Bretzlaff, 1997; Leboeuf *et al*., 1998) [6, 19]. Administration of eCG and prostaglandin in various combinations with progestagen treatment favours follicular growth and affect the time of ovulation (Bretzlaff, 1997; Leboeuf *et al*., 1998) [6, 19]. Administration of eCG is useful when it is administered at the time of progestagen removal during the breeding season (Motlomelo *et al*., 2002) [22] whereas, during the non-breeding season, its administration should be 48 hrs before progestagen removal (Baril *et al*., 1993) [5]. The equine chorionic gonadotropin (eCG) administered at the end of P_4 exposure increases the occurrence and speed of follicular development and ovulation (Abecia *et al*., 2011) [1].

Maximum ewes (66.67%) were observed in estrus within 24 hrs of the progesterone implant removal, while 30.30% ewes came in estrus from 24 to 36 hrs duration and a small percentage (3.03%) were detected in estrus from 36-48 hrs duration. The donor and recipient's synchrony and the presence of a functional corpus luteum (CL) are the prerequisite for the embryo transfer (Looney *et al*., 2006) [21]. Success in the assisted reproduction techniques require tight synchrony in estrus induced animals, so as to provide the same uterine environment and proper plasma progesterone support to assure pregnancy after embryo transfer (Rodrigues *et al*., 2017) [25].

Conception rate, and lamb survival after embryo transfer

It is evident from the results (Table 2) that the conception rate was 50% in the embryo transferred ewes, and the lambing rate was in the 50.00% of the conceived ewes. The conception rate observed are quite satisfactory and are better than the previous reports of 29% to 65% pregnancies and 46% to 80% lambing after embryo transfer in sheep (Ishwar and Memon, 1996; Gibbons *et al*., 2019; Folch *et al*., 2000; Dattena *et al*., 2000) [18, 14, 12, 10]. The survival of embryos after transfer in the recipients are influenced by number of factors (Bari, 2003) [4], such as age of the donor, ovulation rate and body condition of the recipients (Alabart *et al*., 2003) [3]. Variations in the conception and lambing rate are atributed to various factors such as the developmental stage of embryo at the time of transfer, synchronization between donor and recipient, age of embryo donor, embryo storage, and embryo production method (*in vivo* or *in vitro*) (Thompson *et al*., 1995; Dattena *et al*., 2000) [29, 10].

There are a great number of environmental factors that influence reproduction (Bronson, 1985) [7] and many of them influence prenatal survival, besides this various maternal and embryonic factors accrues to increased embryonic death after embryo transfer (Wilmut *et al*., 1986) [32]. The embryo transfer efficacy calculated on the basis of lamb born out of total embryos transferred was 23.33% in this experiment (Table 2) is comparable with the results of Naqvi *et al*. (2007) [23], though it is lower than global standard (Gebrehiwot *et al*., 2018) [13] and leaves scope for further investigations in refining this technique.

Table 1: Incidence and onset of estrus in estrus synchronized ewes

Number of ewes synchronized	**41**
Number of ewes observed in estrus	33
Estrus incidence %	80.49%
Mean onset time after progesterone withdrawal (h)	22.61±1.34
Percent number of ewes in estrus	
0-12 h	21.21%
12-24 h	45.46%
24-36 h	30.30%
36-48 h	3.03%

Table 2: Embryo conception rate in estrus synchronized ewes

Recipient used	**Number of embryos transferred**	**Ewes conceived (%)**	**Lambing/conceived ewes (%)**	**Gestational embryo loss (%)**	**Lamb survival after birth (%)**	**Lambs born**	**Efficacy of embryo transfer**
Total (16)	30	8 (50.00%)	4/8 (50.00%)	4 (50.00%)	6 (85.71%)	7	23.33%

Conclusion

In our study we have achieved satisfactory estrus synchronisation response by use of progesterone impregnated sponge and eCG. There was satisfactory conception rate after embryo transfer. The efficacy of embryo transfer programme calculated on the basis of lamb born out of total embryos transferred was 23.33% in this experiment which is lower than the global standard thus leaves scope for further investigations in refining this technique.

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Declaration of conflicting interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Abecia JA, Forcada F, Gonzalez-Bulnes A. Pharmaceutical control of reproduction in sheep and goats. Veterinary Clinics of North America: Food Animal Practice. 2011;27:67-79.
2. Ainsworth L, Shrestha JNB. Effect of type of intravaginal

progestagen treatment of estrous response and reproductive performance of ewes. Theriogenology. 1983;19(6):869-875.
3. Alabart, JL, Folch, J, Fernández-Arias A, Ramón JP, Garbayo JM, Cocero MJ. Screening of some variables influencing the results of embryo transfer in the ewe part II: Two-day-old embryos. Theriogenology. 2003;59:1345-1356.
4. Bari F. Factors affecting the survival of sheep embryos after transfer within a MOET program. Theriogenology. 2003;59(5-6):1265-1275.
5. Baril G, Brebion P, Chesnc P. Training Manual for Embryo Transfer in Sheep and Goats. FAO, Rome; c1993. ISSN 1014-1019.
6. Bretzlaff K. Control of the estrous cycle. In: Current therapy in large animal theriogenology. Ed. Youngquist RS, W.B. Saunders Company, Philadelphia; c1997. p. 510-514.
7. Bronson FH. Mammalian reproduction: an ecological perspective. Biology of Reproduction. 1985;32:1-26.
8. Crosby TF, Boland MP, Gordon I. Effect of progestagen treatments on the incidence of oestrus and pregnancy rates in ewes. Animal Reproduction Science. 1991;24(2):109-18.
9. Das GK, Naqvi SMK, Gulyani R, Pareek SR, Mittal JP. Effect of two doses of progesterone on estrus response and fertility in a cycling crossbred Bharat Merino ewes in a semi-arid tropical environment. Small Ruminant Research. 2000;37(1-2):159-163.
10. Dattena M, Ptak G, Loi P, Cappai P. Survival and viability of vitrified *in vitro* and *in vivo* produced ovine blastocysts. Theriogenology. 2000;53:1511-1519.
11. Flynn ID, Duffy P, Boland MP, Evans AC. Progestogen synchronisation in the absence of a corpus luteum results in the ovulation of a persistent follicle in cyclic ewe lambs. Animal Reproduction Science. 2000;62(4):285-296.
12. Folch J, Olivera J, Aguilar B, Alabart JL, Sanchez P, Echegoyen E, *et al*. Resultados obtenidos em la transferência de embriones dentro del programa genético de la U.P.R.A. carnes Oviaragon. In: Resumenes de las XXV Jornadas SEOC; c2000. p. 559-561.
13. Gebrehiwot HW, Tsegaye Y, Gebrekidan B. A Review on Current Status of Embryo Transfer Technology in Sheep and Goats. E C Veterinary Science. 2018;3.1:250-259.
14. Gibbons A, Bruno-Galarraga M, Fernandez J, Gonzalez-Bulnes A, Cueto M. Vitrified embryo transfer in Merino sheep under extensive conditions. Animal Reproduction. 2019;16(2):297-301.
15. Gordon I. Controlled reproduction in Sheep and Goats, CAB International, Wallingford, 1997, 450.
16. Hashemi M, Safdarian M, Kafib M. Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. Small Ruminant Research. 2006;65(3):279-283.
17. Iida K, Kobayashi N, Kohno H, Miyamoto A, Fukui Y. A comparative study of induction of estrus and ovulation by tree different intravaginal devices in ewes during the nonbreeding season. Journal of Reproduction and Development. 2004;50:63-69.
18. Ishwar AK, Memon MA. Embryo transfer in sheep and goats: A review. Small Ruminant Research. 1996;19:35-43.
19. Leboeuf B, Manfreidi E, Boué P, Piacère A, Brice G, Baril G. Artificial insemination of dairy goats in France. Livestock Production Science. 1998;55(3):193-203.
20. Leyva V, Bnckrell BC, Walton JS. Regulation of follicular activity and ovulation in ewes by exogenous progestagen. Theriogenology. 1998;50:395-416.
21. Looney CR, Nelson JS, Schneider HJ, Forrest DW. Improving fertility in beef cow recipients. Theriogenology. 2006;65:201-209.
22. Motlomelo KC, Greyling JPC, Schwalbach LMJ. Synchronization of oestrus in goats: the use of different pro-gestagen treatments. Small Ruminant Research. 2002;45:45-49.
23. Naqvi SMK, Joshi A, Kumar D, Gulyani R, Maurya VP, Saha S, *et al*. Developmental competence, birth and survival of lambs following transfer of twin or triple embryos of dwarf size prolific donor into large size non-prolific recipient sheep. Journal of Cell and Animal Biology. 2007;1(5):082-086.
24. O'Doherty JV, Crosby TF. The effect of progestagen type, PMSG dosage and time of ram introduction on reproductive performance in ewe lambs. Theriogenology. 1990;33:1279-1286.
25. Rodrigues M, Bonotto A, Acosta D, Boligon A, Corrêa M, Brauner C. Effect of oestrous synchrony between embryo donors and recipients, embryo quality and state on the pregnancy rate in beef cattle. Reproduction in Domestic Animals. 2017;53(1):152-156.
26. Santos IW, Binsfeld LC, Weiss RR, Kozicki LE. Fertility Rates of Ewes Treated with Medroxyprogesterone and Injected with Equine Chorionic Gonadotropin plus Human Chorionic Gonadotropin in Anoestrous Season. Veterinary Medicine International; c2010. p. 1-4.
27. Simonetti L, Ramos G, Gardon JC. Estrus presentation and distribution in ewes treated with intravaginal sponges impregnated with medroxyprogesterone acetate (MAP) in combination with pregnant mare serum gonadotropin (PMSG) Brazilian Journal of Veterinary Research and Animal Science. 1999;36(5):00. https://doi.org/10.1590/S1413-95961999000500002.
28. Smith JF, Parr J. Effect of oestrogen pre-treatment and duration of CIDR@ treatment on pattern of onset of oestrus in ewes. Proceedings of the New Zealand Society of Animal Production. 1992;52:257-260.
29. Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during *in vitro* pre-elongation development of ovine embryos. Biology of Reproduction. 1995;53:1385-1391.
30. Turk G, Gur S, Sonmez M, Bozkurt T, Aksu EH, Aksoy H. Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with pro-gestagen-PMSG-PGF2a combination. Reproduction in Domestic Animals. 2008;43:308-313.
31. Uribe-Velasquez LF, Oba E, Souza MIL. Efeitos da progesterona exógena sobre o desenvolvimento folicular em ovelhas. Arquivos Brasileiro de Medicina Veterinaria. 2008;60:58-65.
32. Wilmut I, Sales DI, Ashworth CJ. Maternal and embryonic factors associated with prenatal loss in mammals. Reproduction. 1986;76(2):851-864.