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Effect of exogenous supplementation of GnRH or hCG on fertility in Kenguri ewes

LEENA SAJJAN[1], SURESH S HONNAPPAGOL[2], M K TANDLE[3], DILIPKUMAR[4], M M APPANNAVAR[5] and PRAKASH NADOOR[6]

Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka 585 401 India

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In sheep, 35–40% of fertilized eggs are lost during first 3 weeks of pregnancy; one of the major causes of embryonic loss is likely to be inadequate luteal function (Ashworth and Bazer 1989). The factors responsible for such losses have still to be fully explained. hCG has been administered to ewes at different times during the cycle after AI or breeding in an attempt to reduce embryonic mortality and improve reproductive performance, but the effectiveness of these treatments has not been consistent between studies and the timing of such hormonal treatments also may seem to be important. Another approach in reducing embryonic loss during early pregnancy has been the administration of GnRH which results in a significant increase in systemic progesterone (Sreenan *et al*. 1996). Early pregnancy detection by progesterone estimation would be profitable for sheep breeders; it enables them to adjust nourishment of pregnant ewes according to the individual needs and better sheep management.

Kenguri, a purely mutton purpose Indian breed of sheep and also known as Tenguri (Tenguri after the name of coat colour, "Teng" meaning coconut), is found in hilly tracts of Raichur, Hospet and Bellary districts of Karnataka. There is paucity of literature on the use of hormones in fertility improvement in Kenguri ewes. Hence, this study was conducted to determine the effect of GnRH or hCG treatment on the day of mating and late luteal phase on fertility in ewes, fertility assessment by early pregnancy diagnosis by progesterone estimation and actual lambing, to evaluate the traits like pregnancy rate, lambing rate and multiple birth rates in treated and control groups and to analyze the cost benefit utility of the hormonal treatments in relation to fertility of sheep.

Regular breeder cyclic and non-pregnant ewes (18) and 2–3 rams of Kenguri breed belonging to sheep unit of Veterinary College, Bidar were selected and monitored for 1–2 regular estrous cycles. These ewes were 2–5 years old and had experienced 1–3 lambing. All these animals were kept under the routine management practices with pasture grazing for 6–8 h a day and were accessible to fresh clean water *ad lib*. The animals had around 10–12 h of daylight and 10–12 h of darkness without any artificial light source. The experimental duration was 9 months October 2009 to June (2010). The experimental ewes were allowed to graze with vasectomized rams. The onset of estrus (day 0) was confirmed through close observation of mating with an isolated and vasectomized ram and the ewes that had received paint marking on their brisket region. After confirmation of estrus, the ewes were exposed twice at an interval of 12 h to a fertile ram for breeding purpose. All the 18 Kenguri ewes were randomly allocated equally into 3 experimental groups of which 2 groups were treatment and 1 was untreated control. All the animals were monitored for estrus detection as mentioned earlier. The ewes of group 1 were received with GnRH analogue @ 4µg on day of estrus and repeated on day 12 of post mating, whereas the ewes of group 2 received 200 IU of hCG on the day of estrus and day 12 of post mating. The animals of group 3 did not receive any hormonal treatment but placebo normal saline and served as untreated control group. Blood samples were drawn aseptically from the jugular vein on the days 0, 2, 4, 6, 12, 14, 16 and 30 of estrous cycle. The serum was separated and stored at –20°C until it was assayed for progesterone hormone using standard enzyme linked immunosorbant assay (ELISA) procedure. The serum progesterone concentration was determined by using ELISA technique for knowing progesterone levels on different days of estrous cycle and for pregnancy diagnosis on day 30. The sensitivity of the assay was 0.25 ng/ml to 80 ng/ml with a co-efficient of variation of 3.8 to 5.7 % and the results were obtained within 45 min after submitting the serum samples. The pregnancy rate, lambing rate and multiple birth rate were evaluated for each of the treated and control groups. The cost benefit analysis of various hormonal treatments undertaken in Kenguri ewes in relation to fertility was correlated. The data pertaining to the 2 experimental and control groups was analyzed statistically as per Snedecor and Cochran (1989).

The mean serum progesterone levels from the day of

Present address: [3]Professor and Head (tandlemk @yahoo.co.in), Department of Veterinary Gyneacology and Obstetrics.

estrous cycle till the 30th day of post mating in the ewes supplemented with exogenous GnRH ranged between 0.89±0.19 and 5.29±1.03 with gradual increase from the 0 day of estrus till 30th day of post mating. The mean serum progesterone levels during the estrous cycle though higher as compared to the control group but were nonsignificant ($P < 0.05$). This was also reflected in terms of improved lambing percentage of 83.33 in the treated Kenguri ewes with 5 ewes getting pregnant as against 2 out of 6 in the control group. Improved pregnancy rates were reported following the exogenous supplementation of GnRH in sheep and this improvement was attributed to the better ovulation in treated ewes (Zain and Mousa 1999). The improved fertility in the ewes following GnRH supplementation on day 12 post mating may be on account of luteotrophic, luteoprotective and anti-luteolytic responses evoked by exogenous GnRH through endogenous LH release (McCracken *et al*. 1984) thereby increasing the maternal recognition of pregnancy that occurs around day 13 in sheep (Short 1969). The administration of GnRH to sheep results in an increase in plasma LH concentration and hence systemic progesterone and much of this rise in serum progesterone is due to the induction of accessory corpora lutea (Cam *et al.* 2002).

The mean serum progesterone levels from the day of estrous cycle until the 30th day of post mating in the ewes supplemented with exogenous hCG ranged between 0.49±0.07 and 4.55±0.50 with gradual increase from the 0 day of estrus till 14th day of post mating and then decreased. The mean serum progesterone levels during the estrous cycle in treated and control groups did not exhibit any significant difference ($P < 0.05$) although the levels were marginally higher in treated group compared to the control. This is further supplemented by the fact that all the 6 ewes were confirmed pregnant in the treated group as against 2 in the control. Similarly, Nephew *et al*. (1994) reported increased pregnancy rates in ewes supplemented with hCG. However, Ishida *et al*. (1999) did not encounter any improvement in the fertility following hCG treatment in ewes.

The mean serum progesterone level on the day of estrus though differed significantly ($P < 0.05$) between the treated group but showed no significant difference during the post mating period.

The mean serum progesterone profiles of pregnant and non-pregnant animals among the control group where the serum progesterone levels in the pregnant group gradually increased from day 0 to 16th day of post mating and sudden decrease on 30th day of post mating and similar trend was noticed in non pregnant animals. The serum progesterone levels between pregnant and non pregnant animals among hCG treated group was not analyzed as all animals were pregnant. Nephew *et al*. (1994) reported that hCG transiently increased the concentrations of progesterone and oestradiol in plasma. Tandle *et al*. (2004) also recorded increased progesterone level to 1.67±0.49 ng/ml on day 23 in ewes treated with hCG at estrus. Similar findings were also recorded by Tandle *et al.* (2008) and Moeini *et al*. (2009).

The ewes of group 2 that had received hCG registered considerably highest lambing (100%) followed by groups 1 (GnRH) and 3 (control) with the respective values being 83.33 and 33.3% respectively. In the present study, multiple lambing was not recorded in any of the treatment groups. On the contrary, Mohammed *et al*. (2000) reported twinning and higher litter size in GnRH treated Barki ewes (36.84 vs 17.6%; $P<0.09$ and 1.23 vs 1.44; $P<0.05$). Gaffari Turk (2008) also noticed that GnRH treatment immediately after artificial insemination increased the multiple birth rates of Awassi ewes synchronized with progestogen-PMSG-PGF2α combination.

The cost of GnRH treatment per ewe was ₹ 109.70 and the lambing percentage was 83.33, whereas the cost of hCG therapy per ewe was ₹ 69.70 with 100 lambing percentage. The present findings of lambing percentage with GnRH treatment and cost of hormone (83.33% vs ₹ 46.90) and for hCG (66.67% vs ₹ 29.00) are somewhat higher/lower with the findings of Tandle (2001). However, there are no more reports available on cost benefit analysis of exogenous hormone supplementation to improve fertility and fecundity in ewes.

SUMMARY

The estimation of steroid hormone profile at different days of estrous cycle is indicative of the ovarian status of the animal. The administration of hCG on day 0 of estrous cycle and day 12 of post mating improved lambing percentage and also fertility. Among the 2 exogenous hormonal supplementations hCG therapy on day 0 and day 12 of post mating appeared to be the best in improving fertility in Kenguri ewes. The hormonal therapies are cost effective and are of great economic benefit to the farmers involving in sheep rearing.

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