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Oestrus synchronization in sheep using GnRH-$PGF_2\alpha$- GnRH protocol as compared to two injections of $PGF_2\alpha$

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ABSTRACT

In group I (n = 15), the ewes were given 0.004mg of buserelin acetate (Receptal) on day ' O ' followed by 100mg of cloprostenol on 7th day and a second injection of 0.004mg of buserelin acetate on exhibition of oestrus and 3 hours before breeding. In group II (n = 15), two injections of 100mg cloprostenol was given 11 days apart. The percentage of ewes exhibiting oestrus within 48 hours was significantly ($p \leq 0.05$) higher in group I and percentage of ewes exhibiting oestrus after 48 hours was higher in group II.

Lowered plasma progesterone level was significant ($p \leq 0.05$) in group I on the day of oestrus, although there was no significant difference in the oestradoil-17β concentration between the two groups. Conception rate as determined by post-breeding plasma progesterone level and non-return rate in the two groups was 66 and 53 percent respectively.

Key words: Sheep, GnRH, $PGF_2\alpha$, oestrus synchronization

The fact that most sheep, even in agriculturally productive countries, being seasonal breeders often produce smaller lamb crops than the farmer may actually desire. The interval of six months between lambings in such seasonal breeders is a major impediment in realising their full reproductive potential. Controlled breeding in sheep could be expected to cover the full spectrum of lamb production. One of the manipulative techniques is oestrus synchronization. In a new synchronization procedure with timed insemination in cows, GnRH -$PGF_2\alpha$.-GnRH has been used with effective improvement in conception rate. This study presents the effectiveness of this new method of synchronization in sheep .

MATERIALS AND METHODS

In all 42 ewes of UAS breed from Veterinary College, sheep Unit, Bidar, kept under standard feeding and management conditions were randomly selected and divided into the following groups.

In group I (n = 15) , the ewes that were in different stages of oestrous cycle were injected 0.004mg of buserelin acetate (Receptal, Hoechst India Ltd., Mumbai) each on ' O ' day or the day of commencing the experiment followed by an injection of 100mg, cloprosetnol (Sin Cronizador Del,Celo Laboratories Cheminova International , Spain) each. On detection of oestrus or 48 hours after prostaglandin injection, the ewes were again injected with 0.004mg GnRH analogue each and allowed with breeding rams after 3 hours. In group II (n= 15), the ewes were administered two injections of 100mg of $PGF_2\alpha$ analogue each 11 days apart and allowed with breeding rams on observed oestrus or 48 hours after second injection. The control ewes of both groups (n=6) were given a placebo injection at the same time and does schedule as that of treatment groups and were allowed with breeding rams after 48 hours or on observed oestrus.

Blood samples were collected through jugular venipuncture from all the ewes on the following days:

Group I :

1. Fifth day after GnRH injection.
2. On the day of induced oestrus or 48 hours after $PGF_2\alpha$ injection.
3. Twenty first day after allowing for breeding.

Group II :

1. Ninth day after first $PGF_2\alpha$ injection.
2. On the day of induced oestrus or 48hours after second $PGF_2\alpha$ injection.
3. Twenty first day after allowing for breeding.

The oestrus response to synchronization was arrived at by the number and percentage of ewes exhibiting behavioural oestrus and also by peripheral plasma progesterone and oestradoil-17β concentration in the samples collected on the

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day of commencing the experiment and on the day of behavioural oestrus. The conception rates were arrived at by the non-return rates and higher peripheral plasma progesterone concentration in the samples collected on 21st day post breeding. Steroid estimation was done by radioimmunoassay technique and statistical analysis was done as per the procedure explained by Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

Oestrus response in the experimental ewes in the two groups, the mean plasma progesterone levels at different samplings and the mean oestradiol-17β concentration on the day of behavioural oestrus are depicted in Fig.1, Fig.2 and Fig. 3, respectively.

Oestrus induction

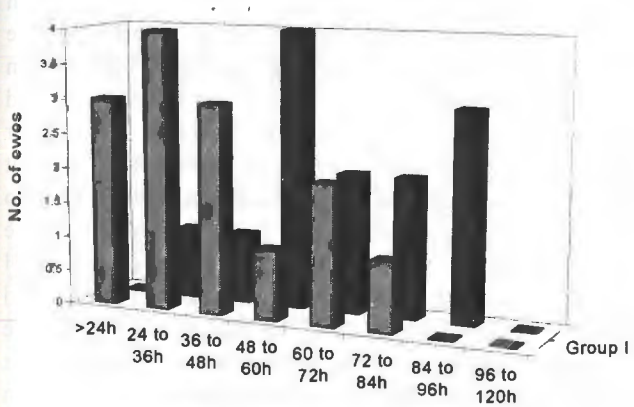

Fig - 1. Oestrus response in the experimental ewes in the two oestrus synchronization regimens adopted

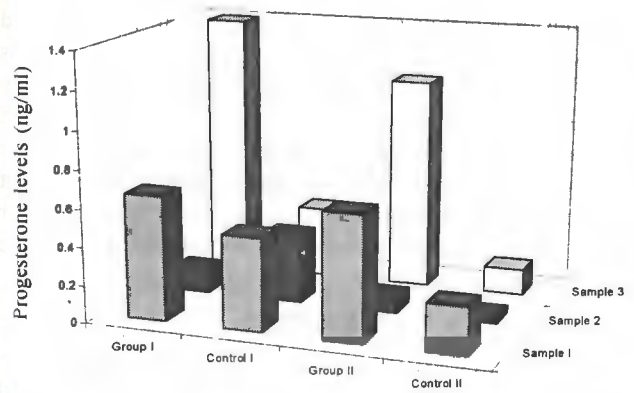

Fig - 2. The mean plasma progesterone levels (ng/ml) in peripheral circulation in oestrus synchronized ewes at different samplings.

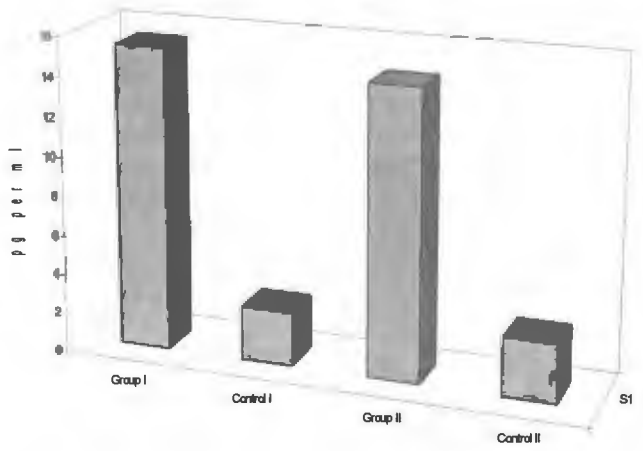

Fig - 3. The mean oestradiol - 17 β concentration (pg/ml) in peripheral circulation on the day of behavioural oestrus.

In the two methods of synchronization adopted in the present study, the number of ewes exhibiting behavioural oestrus within 48h after treatment was much higher in group I (66%) as compared to group II (13.22%) indicating the faster induction of oestrus with GnRH supplementation. This could be attributed to the support of exogenous GnRH for follicular development given before the luteolytic dose of $PGF_2\alpha$ on the day of commencing the experiment. Similar observations were made by Thatcher *et al.* (1989) and Twagiramungu *et al.*(1992) in cows. The increased follicular activity is reflected by higher oestradiol-17β concentration in the ewes of group I on the day of behavioural oestrus. The decrease in the onset of behavioural oestrus in this group may be due to the initiation of a new follicular wave following the first GnRH injection which would have resulted in a new dominant follicle being present at the time of PGF2a injection.

An immediate preovulatory type of LH surge is resulted after a single injection of GnRH which is similar to, but significantly smaller than that of a natural oestrus (McLeod *et al.* 1982). This tonic secretion of LH stimulates the final stages of follicular development and oestrogen synthesis. This LH surge would have occurred immediately after luteolytic action of $PGF_2\alpha$ in group I and the supplementary release of LH initiated by the first injection of exogenous GnRH might have brought about a nearly uniform follicular development and maturation among the ewes of this group, though it was given at random stages of oestrous cycle and thus has brought about a synchrony in exhibition of behavioural oestrus.

In group II where only $PGF_2\alpha$ was used for

synchronization of oestrus there was a wide range in the onset of behavioural oestrus (32 to 120 h). This wide variation may be due to the time to administration of $PGF_2\alpha$ as the onset of oestrus is reported to be related to the day of the cycle on which luteal regression was induced. Thus, the time from administration of $PGF_2\alpha$ to the onset of oestrus is clearly related to the day of the cycle on which luteal regression was induced (Houghton *et al.* 1995).Wales and Fairnie (1984) have also reported similar observation of wide range of oestrus in response to $PGF_2\alpha$. Where as Fairnie and Wales (1978) had reported higher oestrus in response to two injections of $PGF_2\alpha$ regimen of synchronization if the two injections in the protocol are spaced eight days apart than in those treated at longer intervals indicating a wide variation in the treatment regimen also.

The occurrence of oestrus within a particular time interval was higher in GnRH treated group and thus it is advantageous over the two injections of $PGF_2\alpha$ protocol of synchronization for monitoring the behavioural oestrus and controlled breeding (Pursley *et al.* 1995) .

Hormonal profile : There was no difference in progesterone concentration between the two groups indicating that though GnRH increased follicular activity it would not have induced multiple ovulation and hence the oestrus response with GnRH can be considered to be similar to naturally occurring oestrus cycle and also to double does $PGF_2\alpha$ induced cycle. Beck *et al.* (1996) also reported similar findings with regard to oestrus synchronization and fertility in ewes synchronized by GnRH with $PGF_2\alpha$ and two injections of $PGF_2\alpha$.

The oestradiol-17β concentration in the plasma samples collected on the day of behavioural oestrus though was non significant in difference between the two groups the level in group I was slightly higher when compared to the level in group II. This may be attributed to increased follicular activity resulting from LH release consequent to GnRH injection in group I (Keisler, 1994). In group II lack of exogenous GnRH support would have resulted in the development of follicles at varied times leading to difference in oestradiol-17β concentration.

Return rate: In the two groups the return rate after synchronization was 20 and 30 percent respectively. This is in contrast with the findings of Beck *et al.* (1996) where in the number of ewes returning to service after synchronization was more in single GnRH and $PGF_2\alpha$ combined protocol than in two injections of $PGF_2\alpha$ regimen. However, McMillan *et al.* (1986) reported that an injection of a GnRH analogue after mating improved pregnancy rate to first mating at a synchronized oestrus in ewes. Thus, second GnRH injection added in the present protocol might have brought about increased synchrony of ovulations.

In the presence of a functional corpus luteum the first GnRH supplementation though would have enhanced follicular activity the follicles would not have reached the point of ovulation due to the inhibitory effect of progesterone on hypothalamus. Hence the rate of return to service after synchronization might have been more in the synchronization trials of Beck *et al.* (1996) using a single GnRH and $PGF_2\alpha$ regimen. The second GnRH included in the present study might have been advantageous for better rate of induction of oestrus and ovulation of pre ovulatory follicles at a precise time.

Conception Rate : The peripheral plasma concentration of more than 1ng per on day 20 post breeding can be taken as diagnostic tool for confirming pregnancy as was indicated in the present study and also as reported by Gvozdic and Ivkov (1994). Ten of the 11 ewes in the first group and 8 of the 10 ewes in the second group which had plasma progesterone concentration of more than 1ng per ml on the 20th day post breeding, lambed successfully after completion of gestation. The higher conception rate in group I, than group II clearly indicates the higher ovulatory response in group I which is attributable to the GnRH supplementation . This is in agreement with the findings of McMillan *et al.* (1986) where in treatment with GnRH given around breeding time after synchronizing oestrus had improved pregnancy rates and the lower conception rate reported by Beck *et al.* (1996) with single GnRH and $PGF_2\alpha$ synchronization could be due to lack of the second GnRH in their protocol.

Based on the present study it can be concluded that synchronization of oestrus in sheep using GnRH- $PGF_2\alpha$ -GnRH regime is effective and higher conception rates could be achieved probably due to increased follicular activity and synchrony of ovulation at a precise time interval. The undesired variability in onset of behavioural oestrus ion the two injections of $PGF_2\alpha$ synchronisation regimen could also be over come by the above protocol. More work by monitoring the steroid and gonadotrophin profile throughout the experiment and monitoring follicular dynamics during the course of experiment using ultrasonography or laparoscopy could lead to better understanding of the effectiveness of the protocol and precise ovulation time.

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