# SUPEROVULATION RESPONSE, NON-SURGICAL RECOVERY AND MORPHOLOGY OF SAHIWAL COW EMBRYOS

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## ABSTRACT

The study was designed to observe superovulation and non surgical recovery of embryos in Sahiwal cows. With FSH-P the mean corpus luteum formation and embryo recovery was 8.75 ± 0.35 and 6.25 ± 1.06 whereas with PMSG these values were 4.93 ± 1.51 and 3.00 ± 1.41, respectively. The differences between the two hormonal treatments were significant ($P < 0.05$). Embryos were recovered on day 7 of the oestrous cycle with two-way Foley's catheter flushing with Dulbeco's phosphate buffered saline. With FSH-P the recovered embryos were in compact morula (37.5%), blastocyst (34.37%), morula (12.50%), degenerated (9.37%), growth retarded (3.12%) and unfertilized (3.12%) forms. With PMSG the embryos were in the form of compact morula (31.25%) blastocyst (25.00), morula (18.75), degenerated (12.50%), growth retarted (6.25%) and unfertilized (6.25%).

## INTRODUCTION

In embryo transfer, outstanding cows are used for the production of genetically superior embryos. The technique involves superovulation, insemination and recovery of the embryos from the donor female and their transfer to the synchronized recipient females. Although embryo transfer is not as powerful genetic tool as artificial insemination but when the two tools are combined and used optimally, genetic progress for milk production can be upto 15 per cent faster than artificial insemination alone (Seidel, 1981). Information on superovulation and non-surgical recovery of embryos in Sahiwal cows under local environment and managemental conditions is scarce. Such information is needed for better understanding of multiple ovulations and embryo transfer technology in this breed of cattle.

The present project was, therefore, designed to compare the superovulation response with porcine pituitary follicle stimulating hormone (FSH-P) and pregnant mare serum gonadotropin (PMSG) in Sahiwal cows and non-surgical recovery of embryos on day 7 of the estrus. Morphological forms of the collected embryos were also studied.

## MATERIALS AND METHODS

Six clinically normal Sahiwal cows in 2nd to 4th lactations with normal reproductive tract, were divided into two equal groups for two superovulatory regimens. In first regimen, FSH-P (Shering, U.K.) was injected intramuscularly in dioestrus (day 10-11) for 4 days at 12 hours interval at a dose rate of 5mg per injection. For luteolysis, 500 and 250 μg cloprostenol (Estrumate, ICI, U.K.) were injected with 6th and 7th injection of FSH- P. respectively. In second regimen, 2000 IU of pregnant mare serum gonadotrophin (Antex-Leo Pharmaceutical products, Denmark) was injected intramuscularly on day 10-12 of the oestrous cycle, followed by 500 μg of cloprostenol on day 3rd of treatment and a second dose of 250 μg 12 hours later. A second attempt for superovulation was made at 50-60 days interval in both the regimens.

The cows were observed closely for signs of oestrus after second cloprostenol injection and those found in oestrus were inseminated twice at 12 hours interval with liquid semen containing at least 20 x $10^6$ spermatozoa.

Collection of the embryos was attempted on day 7 of the oestrous cycle. Before collection, both ovaries were palpated per rectum to estimate the number of corpore lutea (CLs). Flushing of each uterine born was performed with 500 ml of Dulbeco's phosphate buffered saline (PBS) enriched with one per cent fetal calf serum and gentamicin (0.08 mg/ml) using a two-way Foley's catheter (16-20).

Recovery of PBS from each uterine horn was recorded and flushing was collected in a graduated cylinder. After collection the cylinder was placed at the room temperature for 30 minutes so that the embryos settle down. All the fluid, except the bottom 75-100 ml, was siphoned off into another cylinder. The remaining fluid was poured into 2-3 scored flat bottom Pertridishes and evaluation of embryos was done under a stereomicroscope (20x) and embryos were classified according to morphological forms (Lindner and Wright,

Table 1: Superovulatory response and embryo recovery (Mean ± SD) with FSH-P and PMSG treatment in Sahiwal cows

| Hormones | Attempt | Numbers of CLS palpated on ovaries | | | Number of embryos recovered from uterine horns | | Recovery rate |
|---|---|---|---|---|---|---|---|
| | | Right | Left | Total | Right | Left | Total | % |
| FSH-P | I | 5.00±0.82 (4-6) | 4.00±1.63 (2-4) | 9.00±1.63a (9-11) (3-5) | 4.00±0.82 2-4) | 3.00±0.82 6-8) | 7.00±0.82a | 77.77 |
| | II | 4.50±0.71 (4-5) | 4.00±1.41 (3-5) | 8.50±2.12a (7-10) (2-3) | 2.5±0.71 (2-4) | 3.00±1.41 (4-7) | 5.50±2.12a | 69.71 |
| | Mean | 4.75±0.35 | 4.00±0.12 | 8.75±0.35a | 3.25±1.06 | 3.00±0.42 | 6.25±1.06a | 73.41 |
| PMSG | I | 4.00±0.82 (3-5) | 2.00±0.82 (1-3) | 6.00±0.82b (5-7) (2-4) | 3.00±0.82 (1-1) | 1.00±0.00 3-5) | 4.00±0.80b | 66.66 |
| | II | 2.00±2.83 (0-4) | 1.50±2.12 (0-3) | 3.56±3.33b (0-7) (0-2) | 1.00±1.41 (0-2) | 1.00±1.41 (0-4) | 2.00±2.83b | 51.81 |
| | Mean | 3.00±1.41 | 1.75±0.35 | 4.93±1.51b | 2.00±1.41 | 1.00±0.25 | 3.00±1.41b | 59.24 |

Values in parenthesis indicate range. Values with different letters in the same column differ significanlty ($P<0.05$)

1983). Micrometery of collected embryos was performed according to Linares and King (1980).

Mean values with standard deviation were calculated for the number of CLs formed and embryos recovered. The superovulatory response and rates of recovered embryos by the two regimens were compared through paired T-test.

## RESULTS AND DISCUSSION

The FSH-P and PMSG treated Sahiwal cows came into oestrus 42.6 ± 1.5 and 46 ± 1.8 hours after the cloprostenol treatment, respectively. Samad *et al.* (1993) reported oestrus in cows after 40 and 42 hours in superovulatory regimens of FSH-P at one month interval after prostaglandin treatment. Similarly, Vlahove *et al.* (1985) reported that FSH and PMSG treated buffaloes came into oestrus within 42.8 ± 1.5 and 45.3 ± 1.5 hours after prostaglandin administration, respectively.

Superovulatory response and embryo recovery in Sahiwal cows with these hormones is given in Table 1. With FSH-P the number of CLs formed in first and second superovulatory attempts averaged 9.00 ± 1.63 and 8.50 ± 2.12 whereas the number of embryos recovered was 7.00 ± 0.82 and 5.50 ± 2.12, respectively. Mean superovulatory response and embryo recovery with PMSG was 4.93 ± 1.51 and 3.00 ± 1.41. In the first attempt the mean CLs formed was 6.00 ± 0.82 and the number of embryos recovered was 4.00 ± 0.80. In the second attempt the corresponding values were 3.56 ± 3.33 and 2.00 ± 2.83.

In the present study, the number of CLs formed on the ovaries with FSH-P (8.75 ± 0.35) was significantly ($P<0.05$) higher than PMSG (4.93 ± 1.51). Significantly higher ovulation rates have been reported in cows initiated with FSH treatment on 9th day of the cycle than PMSG (Goulding *et al.*, 1990). In the present study ovaries of one cow superovulated with PMSG became cystic. Formation of ovarian cysts and presence of anovulatory follicles alongwith corpus luteum in gonadotrophin stimulated ovaries has previously been reported in buffaloes (Ullah *et al.*, 1992). Such endocrine abnormalities following superovulation with PMSG attributed to long half life of this hormone may be overcome by the administration of specific antibodies against PMSG (Lauria *et al.*, 1982).

In the present study, 6.25 ± 1.06 (73.74%) and 3.00 ± 1.41 (59.24%) embryos were recovered from cows treated with FSH-P and PMSG, respectively, the difference being significant ($P<0.05$). Reported recovery of embryos from superovulated cow ranged from 25 to 75% of the ovulations (Avery *et al.*, 1962, Hafez *et al.*, 1963; Rowson and Moore, 1966; Rowe *et al.*, 1977). Recovery of embryos is mainly influenced by endocrine disturbances created by superovulatory treatment, which alter the normal development of embryos and make the flushing difficult. In superovulated donors at day 6 after oestrus, there was 10 times more estrogen in the peripheral plasma as in the normal cow at oestrus (Booth *et al.*, 1975). Fertilization failure is another factor and often been attributed to abnormalities in oocyte maturation (Moore *et al.*, 1985). Lower superovulation and embryo recovery rate with PMSG than FSH-P observed in this study might be due to its long half life that adversely affects on gamete migration and early stage of embryonic development (Bouters *et al.*, 1983).

A total of 32 embryos were recovered with FSH-P. Out of these, 12 and 11, (37.50 and 34.37%) were in compact morulae and early blastocyst stage, while 4(12.5%), 3 (9.37%), 1(3.12%) and 1(3.12) were in

morulae, degenerated, retarded and unfertilized forms, respectively. With PMSG treatment, out of 16 recovered embryos, 5 (31.25%), 4 (25%), 2 (12.50%), 3 (18.75%), 1 (6.25%) and 1 (6.25%) were in compact morulae, early blastocyst, morulae, degenerated, retarded and unfertilized forms, respectively.

Previously, Alcivar *et al.* (1985) reported blastocyst, compact morulae, retarded and unfertilized embryos as 36, 28, 25 and 11 percent, respectively, after superovulation treatment with FSH in cow heifer. Donaldson (1986) reported several stages of embryonic development in the same collection and stated that all ova were not ovulated and fertilized simultaneously. Even if two ova were fertilized at the same time, the rate of cleavage for each may differ. Morphological characteristics of the bovine embryos have been described by Linares and King (1980). A great deal of variability exists in morphological development and embryo quality within and among donors. In the light of above findings it may be concluded that FSH-P is better both for superovulation response as well as in embryo recovery rate than PMSG hormone.

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