# EFFECT OF FLUNIXIN MEGLUMINE ON CONCEPTION RATE FOLLOWING EMBRYO TRANSFER IN CROSSBRED CATTLE

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## ABSTRACT

The present study was designed to study the effect of flunixin meglumine following embryo transfer during luteal phase of estrous cycle on Prostaglandin fetal metabolite (PGFM) and progesterone in embryo recipient cows. Eighteen crossbred cows were selected and divided into three groups; Control (n=6): NSS, T-I (n=6): flunixin meglumine @ dose rate of 1.1 mg/kg half-hour before transfer, T-II (n=6): flunixin meglumine @ dose rate of 1.1 mg/kg half-hour before transfer and on Day 16 and Day 17 of oestrous cycle. Blood samples were collected on 7½ day of oestrous cycle without any treatment i.e. before embryo transfer and then half an hour after treatment. Again blood samples were taken at 15 minute, 50 minute and 150 minute following embryo transfer, On day 16, 17 and 18 of estrous cycle, blood sampling was done every 12 hour and for progesterone estimation blood samples were taken before treatment/ transfer, 150 min after transfer/treatment then on 16$^{th}$ , 17$^{th}$ , 18$^{th}$ , and 32$^{nd}$ day following estrus. Results of the study showed that the PGFM level and progesterone level differ non-significantly ($p>0.05$) between the groups but after treatment, there was marked decrease in PGFM level and simultaneously increased progesterone concentration. Out of three pregnancies, one animal aborted on day 76 of gestation. On the basis of above findings, it may be concluded that administration of flunixin meglumine during luteal phase of estrous cycle may be beneficial in improving the conception following embryo transfer in crossbred cattle as evident by decreased level of PGFM and increased level of progesterone during critical days of maternal recognition of pregnancy.

**Keywords:** Flunixin meglumine; PGFM; Progesterone; Embryo recipient

## INTRODUCTION

Superovulation and embryo transfer are efficient tools for producing large number of high quality seed stock and to maximize the number of young born from females of high genetic quality. The present study reports the effect of flunixin meglumine, an potent anti-inflammatory drug on embryo recipient cattle with the hypothesis that prostaglandin released during uterine manipulation before and during embryo transfer which is detrimental to normal embryonic development would be reduced. Flunixin meglumine, a COX inhibitor will help in reducing $PGF_{2á}$ during critical development of embryo. Thus, may be helpful in improving conception following embryo transfer in cattle. Interestingly it is seen that production of PGF2a during luteolysis and after the uterine manipulation followed a similar pulsatile release of PGF2a (Flint and Sheldrick, 1982). The endometrium of bovine contains comparatively large amount of arachidonic acid that can be readily converted to different products such as PGF2a (Salamonsen and Findlay, 1990). Thus, if the endometrial production of PGF2a is minimised specially during early development of embryo, administration of Flunixin meglumine could be beneficial for embryonic development.

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## MATERIALS AND METHODS

The present study was conducted on Cross-bred cattle at Instructional Dairy Farm (I.D.F.), Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar-263145, District – Udham Singh Nagar (Uttarakhand). All the animals were kept at I.D.F. under uniform feeding and managemental conditions throughout the experiment period. Normal cyclic cows and heifers (n=18) that attained 250 kg body weight, having functional reproductive organs, normal reproductive cycle, absence of history of any pathological reproductive condition like pyometra, metritis, endometritis and free from systemic diseases were selected.

Sixty days prior to starting of experiment, deworming was done with 90 ml oxyclozanide and levamisol HCL suspension. Mineral mixture (Supplivite M, Zydus AH) at the dose rate of 50 gm per animal per day was fed 30 days prior to experiment. White side test was performed in both donor and recipient animals during estrus to rule out sub clinical endometritis. The infected animals were treated with intrauterine antibiotic (Lenovo-AP, Intas pharmaceuticals) for 3 days to ensure sterile uterine environment. Crossbred cows (n=18) were selected and divided into three groups; control, Control (n=6): NSS, T-I (n=6): flunixin meglumine @ dose rate of 1.1 mg/kg half an hour before transfer, T-II (n=6): flunixin meglumine

@ dose rate of 1.1 mg/kg half an hour before transfer and on 16th and 17th day of estrous cycle.

Five recipient cows were synchronised for estrus for each donor. They were palpated for presence or absence of CL on the ovaries. Cloprostenol @ 500 µg i/m (VetmateTM, Vetcare) was injected for cows having CL. Then all the 5 recipient cows were given second injection of Cloprostenol @ 500 µg i/m (VetmateTM, Vetcare) after 10 days, for synchronous estrus induction.

Using sterile condition, the transferable embryos were loaded in Embryo transfer gun and were transferred at the tip of uterine horn, epsilateral to ovary bearing corpus luteum as per standard protocol.

For estimation of serum PGFM profile of recipient, blood samples were taken before embryo transfer i.e. on 7½ day of estrous cycle without giving any treatment, than half an hour after treatment. Other samples were collected at 15 minute, 50 minute and 150 minute post transfer. On 16th, 17th and 18th day of estrous the sampling was done after every 12 hour interval. The serum progesterone profile of crossbred recipient was analysed at different interval during embryo transfer. Before transfer i.e. on 7½ day of estrous cycle without giving any treatment followed by150 minute post transfer. Again blood was collected on 16th, 17th,18th day and 32nd days from estrus. Blood serum was separated, using centrifuge machine at 3000 rpm for 15 minutes and was transferred into sterilized serum vials and stored at -200 C in deep freezer till analysis.

The serum PGFM was estimated in duplicate using ELISA kit (Kit Bioassay Technology Laboratory, Shanghai Korain Biotech CO., LTD., Junjiang, China. CV(%) = SD/meanX100; Intra-Assay: CV< 10%; Inter-Assay: CV< 12%). Progesterone was estimated using RIA kit ((M/ S Beckman Coulter IM 1188). The data generated were analyzed statistically (Snedecor and Cochran, 1989) using analysis of variance (ANOVA) to test the significant difference of mean.

**RESULTS AND DISCUSSION**

The mean serum PGFM concentration in Control, T-I and T-II group before transfer at zero hour and half an hour after treatment was 454.63±90.09, 304.36±89.46; 294.88±59.32 and 441.62±103.38, 376.44±102.78 and 324.49±94.84 pg/ml, respectively. There was no significant difference between the groups.

The mean serum PGFM concentration at 15 minutes, 50 minutes and 150 minutes after transfer in Control, T-I and T-II group did not differ significantly. At 15 minutes there was increase in PGFM in all the groups, at 50 minutes there was decrease in level of PGFM and then at 150 minutes there was again rise in PGFM concentration which might be due to uterine manipulation during embryo transfer, but in T-I and T-II the rise was lower as compared to control.

There was no significant difference in the mean PGFM concentration on 16th, 17th and 18th day of estrous cycle at 0 (zero) hour and 12th hour in Control, T-I and T-II. In group T-II, there was marked decrease in PGFM concentration after flunixin meglumine injection, this decrease in PGFM concentration was not observed in Control and T-I group on 16th, 17th, and 18th day (Table 1).

The mean serum PGFM concentration recorded in the present study was in agreement with Smith et al. (1979) and Mason et al. (2014) but higher than Parkinson et al. (1990). Administration of flunixin meglumine might have exerted an inhibitory effect on PGFM and decreased its level.

In present study, the mean serum progesterone concentration in Control, T-I and T-II before transfer at zero hour and 150 minutes after transfer/treatment was 1.17±0.68, 1.17±0.31 and 1.65±0.53 and 1.98±1.40, 1.98±0.58 and 1.65±0.41 ng/ml respectively and they differ non-significantly.

There is no significant difference between the groups in the mean serum progesterone concentration on treatment day at 16th and 17th day of estrous cycle. However, there is increase in progesterone concentration in T-II group. In T-II group, there was marked increase in progesterone level on 18th day. On day 32nd the mean serum concentration of progesterone was higher in T-II group i.e. 4.13±2.23 ng/ml as compared to Control and T-I 2.69±1.82 and 1.15±0.70 ng/ml, respectively (Table 2).

One cow has been excluded from control group due to cystic ovarian degeneration and only five cows were taken in control group for statistical analysis.

Eighteen crossbred embryos were transferred to eighteen healthy crossbred cows. Cows were divided into three groups i.e. control (n=6), T-I (n=6) and T-II (n=6). In control no conception was found, in T-I one cow conceived but aborted after 76 days and in T-II two pregnancies were confirmed. The average serum PGFM concentration in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before transfer at zero hour and half an hour after treatment was 419.63±90.09, 346.22±108.33 and 243.53±16.95 and 441.62±103.38, 395.98±191.62 and 280.08±57.72 pg/ml respectively.

At 15 minutes there was an increase in PGFM in all the groups, at 50 minutes there was decrease in level of PGFM but a very slight increase was observed in Treated pregnant/expected pregnancy (T-II) group and then at 150 minutes there was again rise in PGFM concentration

which might be due to uterine manipulation during embryo transfer.

The average PGFM concentration on 16th, 17th and 18th day of estrus at 0 (zero) hour and 12th hour in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II), there was observable difference in serum level between the group. In Treated non-pregnant (T-II) and Treated pregnant/ expected pregnancy (T-II) there was marked decrease in PGFM concentration after flunixin meglumine injection (Table 3).

In present study it was observed that giving flunixin meglumine at the dose rate of 1.1 mg/kg on 16th and 17th day effectively reduces the PGFM level and simultaneously increased progesterone concentration which would be beneficial for embryo survival and help in improving conception after embryo transfer. The decrease in PGFM concentration may provide an extra time for less viable embryos to secrete sufficient amount of IFN-t to inhibit the luteolytic secretion of PGF2a. It may be possible that flunixin meglumine may only delay luteolysis and increases the survivability of poor quality embryos that would die later during the pregnancy. Maternal factors may be responsible to inhibit the luteolytic releases of PGF2a.

The average serum progesterone concentration in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) before transfer at zero hour and 150 minutes after transfer/ treatment was 1.17±0.68, 1.92±0.85 and 1.33±0.57 and 1.98±1.40, 1.58±0.71and 1.71±0.44 ng/ml, respectively.

The average progesterone concentration on treatment day at 16th and 17th day of estrus in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) was 2.21±0.76, 1.15±0.37 and 2.61±0.41 and 3.67±0.90, 1.25±1.07 and 4.24±0.72 ng/ml, respectively.

On 18th day, the average progesterone level in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/expected pregnancy (T-II) was 2.70±1.14, 1.98±1.83 and 5.25±0.72 respectively. On day 32nd the average progesterone level was higher in Treated pregnant/expected pregnancy (T-II) group i.e. 8.12±2.19 as compared to Control non-pregnant and Treated non-pregnant (T-II) 2.69±1.82 and 0.13±0.01ng/ ml, respectively (Table 4)

On the basis of above findings, it may be concluded that administration of flunixin meglumine during luteal phase at the time of maternal recognition of pregnancy decreases PGFM level and boost progesterone level which may be beneficial for the conception of embryo following embryo transfer. From the present study it can be concluded that Flunixin meglumine administration during luteal phase of estrous cycle following embryo transfer may be beneficial in improving conception.

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**Table 1: Mean (±SE) serum PGFM concentration (pg/ml) of different groups on day of transfer (day 7), 16th, 17th and 18th day of estrus in crossbred recipients**

| Particulars | | | PGFM (pg/ml) | | |
|---|---|---|---|---|---|
| | | | Control | T1 | T2 |
| Before transfer | Before treatment | 0 hr | 419.63±90.09 | 304.36±89.46 | 294.88±59.32 |
| | After treatment | 1/2 hr | 441.62±103.38 | 376.44±102.78 | 324.49±94.84 |
| After transfer | Day of transfer | 15 min | 488.41±113.83 | 402.09±120.56 | 329.11±108.62 |
| | | 50 min | 457.35±134.86 | 390.54±142.28 | 327.88±93.94 |
| | | 150 min | 479.42±147.11 | 382.38±89.18 | 320.94±112.64 |
| | 16th day | 0 hr | 289.44±67.56 | 308.80±71.62 | 317.67±59.68 |
| | | 12 hr | 242.32±56.42 | 307.69±44.91 | 213.02±48.86 |
| | 17th day | 0 hr | 232.76±38.77 | 328.20±76.51 | 307.61±61.06 |
| | | 12 hr | 251.66±66.65 | 388.08±153.42 | 243.07±41.20 |
| | 18th day | 0 hr | 336.68±69.59 | 351.34±92.58 | 367.01±108.15 |
| | | 12 hr | 376.74±20.47 | 371.34±62.45 | 289.66±54.22 |

**Table 2: Mean (±SE) serum progesterone concentration (ng/ml) of different groups on day of transfer (day 7), 16th, 17th, 18th and 32nd day of estrus in crossbred recipients**

| | | | Progesterone (ng/ml) | | |
|---|---|---|---|---|---|
| | | | Control | T-II | T-II |
| Before transfer | Before treatment | 0 hr | 1.17±0.68 | 1.17±0.31 | 1.65±0.53 |
| After transfer | After treatment/ Day of transfer | 150 min | 1.98±1.40 | 1.98±0.58 | 1.65±0.41 |
| | 16th day | 12 hour | 2.21±0.76 | 1.76±0.62 | 1.88±0.45 |
| | 17th day | 12 hour | 3.67±0.90 | 2.54±0.62 | 2.75±0.97 |
| | 18th day | 12 hour | 2.70±1.14 | 2.53±0.78 | 3.62±1.26 |
| | 32nd day | | 2.69±1.82 | 1.15±0.70 | 4.13±2.23 |

**Table 3: Average serum PGFM concentration (pg/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/ expected pregnancy (T-II) before and after transfer in crossbred recipient**

| Particulars | | | PGFM (pg/ml) | | |
|---|---|---|---|---|---|
| | | | Control non-pregnant | Treated non-pregnant (T-II) | Treated pregnant/ expected pregnancy (T-II) |
| Before transfer | Before treatment | 0 hr | 419.63±90.09 | 346.22±108.33 | 243.53±16.95 |
| | After treatment | 1/ 2 hr | 441.62±103.38 | 368.89±179.37 | 280.08±57.72 |
| After transfer | Day of transfer | 15 min | 488.41±113.83 | 395.98±191.62 | 262.23±89.23 |
| | | 50 min | 457.35±134.86 | 384.61±175.11 | 271.15±53.71 |
| | | 150 min | 479.42±147.11 | 349.51±120.87 | 292.36±77.89 |
| | 16th day | 0 hr | 289.44±67.56 | 313.03±103.68 | 322.30±63.77 |
| | | 12 hr | 242.32±56.42 | 206.13±96.26 | 219.90±25.13 |
| | 17th day | 0 hr | 232.76±38.77 | 299.26±106.67 | 315.95±63.93 |
| | | 12 hr | 251.66±66.65 | 209.88±43.03 | 276.25±64.18 |
| | 18th day | 0 hr | 336.68±69.59 | 450.66±178.15 | 283.36±100.01 |
| | | 12 hr | 219.74±20.47 | 284.00±46.21 | 295.32±100.40 |

**Table 4: Average serum progesterone concentration (ng/ml) in Control non-pregnant, Treated non-pregnant (T-II) and Treated pregnant/ expected pregnancy (T-II) before and after transfer in crossbred recipient.**

| Particulars | | | Progesterone (ng/ml) | | |
|---|---|---|---|---|---|
| | | | Control non-pregnant | Treated non-pregnant (T-II) | Treated pregnant/ expected pregnancy (T-II) |
| Before transfer | Before treatment | 0 hr | 1.17±0.68 | 1.92±0.85 | 1.33±0.57 |
| After transfer | After treatment/ Day of transfer | 150 min | 1.98±1.40 | 1.58±0.71 | 1.71±0.44 |
| | 16th day | 12 hour | 2.21±0.76 | 1.15±0.37 | 2.61±0.41 |
| | 17th day | 12 hour | 3.67±0.90 | 1.25±1.07 | 4.24±0.72 |
| | 18th day | 12 hour | 2.70±1.14 | 1.98±1.83 | 5.25±0.72 |
| | 32nd day | | 2.69±1.82 | 0.13±0.01 | 8.12±2.19 |

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