

## Full Length Articles

# Supplementation of fish meal and exogenous administration of COX-2 inhibitor on conception rate in repeat breeding Jersey crossbred cows

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### ABSTRACT

The study was conducted on repeat breeding cows at Large Animal Gynaecology Ward, Department of Clinics, Madras Veterinary College, Chennai and some private cattle farms in and around Koduvalli region, Tiruvallur district, Tamil Nadu. In experiment I, Jersey crossbred cows (n=31) synchronized by PGF<sub>2</sub>α and inseminated on exhibited estrum. The cows were randomly divided into five groups viz. Group I (control, n=6), group II (n=7, 100mg), group III (n=6, 150mg), group IV (n=6, 200mg) and group V (n=6, 250mg) and supplemented with fish meal on day 13, 14 and 15 of post insemination in addition to their routine feed. Pregnancy diagnosis was carried out by rectal palpation on day 60 of post insemination. In experiment II, twelve repeat breeding Jersey crossbred cows were selected and synchronized using PGF<sub>2</sub>α followed by timed insemination. On day 10 of post estrus, rectal examination was performed to identify the corpus luteum and randomly divided into two group viz. Group I and Group II (control). Group I cows were injected with Meloxicam @ 0.5 mg/ kg body weight through intramuscularly on day 13 to 15 post-insemination. The conception rate in experiment I as in Group I, group II, group III, group IV and group V are 16.67%, 71.43%, 66.67%, 66.67% and 50.0%, respectively. The conception rate in meloxicam treated animal was 66.7% and in control animal was 33.3%. The fish meal treatment and Cox-2 inhibitor in repeat breeding crossbred cows had improved the conception rate. Further, the supplementation of 100mg of fish meal in repeat breeding cows has shown higher conception rate as compared to other groups in experiment II. Hence, it may be used in field condition for enhancement of conception rate, however, further studies in larger population is warranted.

**Key Words:** Cattle, Fish meal, COX2 inhibitor, Conception rate

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## INTRODUCTION

Though the fertilization rates are as high as 80-95%, the embryonic mortality is one of the major causes for the poor reproductive efficiency since 40-53% of embryos were lost—between 8 and 16 days of pregnancy in cattle (Binelli *et al.*, 2009). Recently,  $PF_2\alpha$  synthesis inhibitors have been used to modulate endometrial  $PGF_{2\alpha}$  production either by manipulating oxytocin receptor system or subsequent  $PGF_{2\alpha}$  synthesizing enzymatic machinery especially COX-2 and  $PGF$  Synthase pathway to improve the fertility rate in bovine. Administration of oxytocin receptor antagonist between days 12 and 20 of the cycle has been reported to suppress the increase in PGFM concentration and delays luteal regression in cyclic goats (Homeida and Khalafalla 1987). Similarly, nonspecific COX inhibitor (Flunixin and meloxicam) has been shown to inhibit luteolysis and extended the estrous cycle in cycling heifers (Aiumlamai *et al.*, 1990). Administration of COX inhibitors viz. flunixin meglumine and ibuprofen prior to embryo transfer has been reported to increase pregnancy rate (10 to 25%) through inhibition of uterine  $PGF_{2\alpha}$  release in cattle (McNaughtan *et al.*, 2002). It also decreased embryonic loss caused by stress on day 14 post-insemination in beef cows (Merrill *et al.*, 2003). Recently, administration of flunixin on day 15 and 16 post-insemination increased conception rate as compared to control (69.2% vs. 46.2%) in Holstein heifers (Guzeloglu *et al.*, 2007). COX-2 is the primary isoenzyme involved in the endometrial production of  $PGF_{2\alpha}$  rather than COX-1. Moreover, COX-1 is a constitutive enzyme involved in normal physiological process and its inhibition may

affect the normal physiological functions in the body. Hence, selective COX-2 inhibitor would be more effective in preventing  $PGF_{2\alpha}$  synthesis and enhancing fertility as compared to non-specific COX inhibitors.

Shukla (2006) reported that administration of COX-2 inhibitor (meloxicam) on day 15 and 16 of estrous cycle increased the cycle length as compared to control in buffalo. Therefore, administration of drugs like selective COX-2 inhibitor and PGFS inhibitor may inhibit  $PGF_{2\alpha}$  production from the endometrium and enhance embryo survival thus fertility. Recently, emphasis has been given on the COX-2 inhibitor, feeding of polyunsaturated fatty acid for the enhancement of embryonic survival and thus augmentation of fertility. Information pertaining to these drugs and inhibitors on embryonic survival and fertility are as such meager in bovine. The attainment of the objectives proposed in the present study would, therefore, be helpful to develop package of practices for augmentation of fertility and thus productivity of bovine.

## MATERIALS AND METHODS

**Place of study:** The study was conducted on repeat breeding cows at Large Animal Clinic Gyneacology Ward, Department of Clinics, Madras Veterinary College, Chennai and some private cattle farms in and around Koduvalli region, Tiruvallur district, Tamil Nadu.

**Experimental Design:** In Experiment I, the repeat breeding cows were selected through infertility camp near Koduvalli and Red Hills region, Tiruvallur district,

Tamil Nadu. Forty repeat breeding cows were selected for this study and screened for subclinical endometritis. Nine repeat breeding crossbred animal out of 40 were identified as positive for subclinical endometritis through White side test and those animals were not included for the study and remaining 31 animals were selected for the study. Experimental cow subjected to detection of estrus after synchronization by using PGF2 $\alpha$  by visual observations and rectal palpation. The cross-bred Jersey cows detected in estrus were inseminated using frozen semen straws and randomly divided into five group viz. Group I (control, n=6), group II (n=7, fish meal treatment, 100mg), group III (n=6, fish meal treatment, 150mg), group IV (n=6, fish meal treatment, 200mg) and group V (n=6, fish meal treatment, 250mg). All experimental cows except control were supplemented fish meal with 50g of jaggery on 13, 14 and 15 of post insemination in addition to their routine feed. In experiment II, twelve repeat breeding cows were selected for the study. Experimental cows were subjected to detection of estrus after synchronization by using PGF2 $\alpha$  by visual observations and rectal palpation. Cows were detected in estrus inseminated using frozen semen straws. Each animal was palpated per rectum on day 10 post estrus for the presence of corpus luteum and randomly divided into two group viz. Group I (Inj. Meloxicam treatment) and group II (control). Animals of group I were injected with Meloxicam (Melonex TM, Intas Pharmaceuticals Ltd., Ahmedabad, India) @ 0.5 mg per kg body weight per day intramuscularly on day 13 to 15 post-insemination. The animals of group II were

untreated and kept as control. Blood samples were collected from all experimental animals by jugular venipuncture on day 13, 15, 18, 21 and 24 post-insemination. The serum was separated and stored at -20° C until progesterone estimation. The pregnancy diagnosis was carried out through per-rectum on 60 days post insemination. The concentration of progesterone was estimated using the diagnostic I125 kits supplied by Immunotech, France. For progesterone analytical sensitivity of the kit was 0.05ng/ml; the intra-assay and inter-assay coefficient of variation 5.8% and 9.0%, respectively. The pregnancy diagnosis was carried out through per-rectum on 60 days post insemination.

**Statistical analysis:** The statistical analysis was carried out using SPSS version 13.0 for windows. The data for progesterone profile were analyzed within groups by one-way ANOVA.

## RESULTS AND DISCUSSION

The conception rate in experiment I as in Groups I, II, III, IV and V are 16.67, 71.43, 66.67, 66.67 and 50.0%, respectively (Table 1). Among that feeding of fish meal about 100g gives more conception rate. The results indicate that the feeding of PUFA during critical period can be used to inhibit secretion of endometrial PGF2 $\alpha$  which occurs due to delay or insufficient IFN- $\tau$  secretion by embryo or decrease in response of endometrium to the IFN- $\tau$ ; resulting in failure of Maternal Recognition of Pregnancy (MRP). *In vitro* studies revealed uterine tissues when incubated in medium only (M) or media supplemented with fatty acids viz. eicosapentaenoic

(20:5omega3; EPA), docosahexaenoic acids (22:6omega3; DHA) or linoleic acid (C18:2omega6; LIN) revealed luteotrophic PGE<sub>2</sub> release from pregnant endometria was higher than from non pregnant endometria, while PGF<sub>2</sub>α concentrations were similar. Polyunsaturated fatty acids like EPA or DHA inhibit secretion of PGF<sub>2</sub>α from bovine endometrial cells in vitro. EPA and DHA exert their regulatory

effects as alternative substitutes that reduce the lipid pool of arachidonic acid in the endometrium. High concentrations of EPA and DHA has been reported in Menhaden fish meal and supplementation of lactating dairy cows with Menhaden fish meal for 25 days reduced PGFM plasma concentrations in response to an estrogen injection followed by an oxytocin injection on day 15 of a synchronized estrous cycle.

**Table 1. The effect of selective COX-2 inhibitor (fish meal) on conception rate in repeat breeding crossbred cows**

S. No	Attributes	Group I (Control)	Group II	Group III	Group IV	Group V
1.	Animals treated	6	7	6	6	6
2.	Animal Responded for estrus synchronization (PGF <sub>2</sub> α)	6	7	6	6	6
3.	Onset of estrus following estrus synchronization (h)	58.00	58.43	60.67	58.34	61.00
4.	Duration of estrus	19.42	20.57	22.57	18.57	18.00
5.	Duration of treatment (days)	--	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)	13,14 and 15 Post AI (3 days)
6.	Conception rate (%)	1/6 (16.67)	5/7 (71.43)	4/6 (66.67)	4/6 (66.67)	3/6 (50.00)

The analysis of experiment II has shown that the meloxicam treated animals are having higher conception rate 66.7% as compared to control group 33.3% (Table 2). Among COX inhibitors potential candidates capable of inhibiting uterine PGF<sub>2</sub>α release are non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs act by competitive inhibition of COX enzyme either non-selectively through both COX-1 and COX-2 isoforms *i.e.* flunixin, ibuprofen or specifically through COX-2 isoform *i.e.* celecoxib, rofecoxib, valdecoxib, and also through preferentially inhibiting COX-2

*viz.* meloxicam, nimusulide etc. Research has shown that intensive parenteral administration of flunixin is able to postpone luteolysis and prolong the estrous cycle in heifers. The present study was in agreement with studies by Rajkumar *et al.*, (2010) who observed meloxicam as COX-2 inhibitor improved pregnancy rates in buffaloes post-Artificial Insemination. Further, Ladol *et al.* (2010) who also reported 60 per cent conception rate in repeat breeding buffaloes treated with meloxicam @ 0.5 mg/kg, body weight, intramuscularly on days 13, 14 and 15 post AI whereas it was 25 per cent only in control.

**Table 2. The effect of selective COX-2 inhibitor (inj. Meloxicam) on conception rate in repeat breeding crossbred cows**

S. No	Attributes	Group I (Inj. Meloxicam, n=6)	Group II (Control, n=6)
1.	Animals treated	6	6
2.	Duration of treatment (days)	3	3
3.	Animal Responded for estrus synchronization (PGF <sub>2</sub> α)	6	6
4.	Onset of estrus following estrus synchronization (h)	59.67	60.34
5.	Duration of estrus	18.34	20.33
6.	Conception rate (%)	<b>4/6 (66.7)</b>	<b>2/6 (33.3)</b>

The average progesterone concentration estimated on 13, 15, 18, 21 and 24 post-insemination on individual pregnant Jersey crossbred cow basis varied from 5.32±0.04 to 6.41±1.02; 4.87±.20 to 6.72±0.05; 3.87±0.25 to 5.93±0.60; 5.04±0.31 to 6.41±0.10 and 5.32±0.20 to 6.43±0.14 for groups I, II, III, IV and V, respectively. The present results indicate that the progesterone values are getting increased significantly ( $P>0.5$ ) after day 20 of pregnancy and shows increasing trend as the gestation advances in pregnant cows. However, the value of progesterone in non- pregnant cows is getting decreased on day 15 onwards and reaches in basal level on day 20 of post cycle. These results also support the establishment of pregnancy after feeding of fish meal to the cows which are affected with repeat breeding syndrome. As a conclusion the selective COX-2 inhibitor and fish meal treatment in repeat breeding cross-bred cows had improved the conception rate. Hence, it may be used in field condition for improvement of conception rate, however, further studies in larger population is warranted.

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