



Effect of COX-2 modulator on estrous cycle and prostaglandins secretion in buffalo (*Bubalus bubalis*)

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The non-steroidal anti-inflammatory drugs (NSAIDs) have an ability to inhibit the prostaglandins synthesis acting by competitive inhibition of cyclooxygenase enzyme either selectively or non-selectively. Application of COX inhibitors in animal reproduction is a fairly recent development (Rajkumar *et al.* 2010). Non-selective COX inhibitor postponed luteolysis and prolonged the estrous cycle in heifers (Odensvik *et al.* 1998), Llama (Aba *et al.* 2000) and ewes (Ake-Lopez *et al.* 2005). The COX-2 selective inhibitors show minimal side effects as compared to non-selective one, but their role in reproduction has not been well studied. Modulation of COX-2 pathway with respect to prostaglandin secretion and its mediated luteal functions will be helpful to study regulation of estrous cycle and enhancing embryonic survival thus maintenance of pregnancy in farm animals. Meloxicam [4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1, 1-dioxide] is a NSAID of the oxycam class which shows preferential inhibition of COX-2 (Davies and Skjodt 1999). In view of the facts cited above, a pilot study was designed to study the effect of selective COX-2 modulator-meloxicam on estrous cycle length, secretion of prostaglandins (PGE₂ and PGF_{2α}) and nitric oxide in buffalo.

Healthy, parous, cyclic water buffaloes (5) having no gross abnormality of reproductive organs on gynaecological examination and maintained under standard managerial practices at Indian Veterinary Research Institute, Izatnagar, were used. All the buffaloes were examined per rectally on

alternate day to monitor the ovarian and uterine changes for ascertaining estrus and development of corpus luteum. Ovarian structures were confirmed by ultrasonography using real time B- mode ultrasound equipped with 5.0–7.5 MHz transrectal transducer.

Initially, the experimental animals (5) were not administered any drug and served as control. However, same animals in the next cycle were administered selective COX-2 inhibitor-meloxicam @ 0.5 mg/kg body weight intramuscular, once daily on 15th and 16th day of cycle (day 0 = estrus) and served as treatment group.

The uteri of both control and treated animals were flushed non-surgically using two way Foley's catheter with normal saline on day 13, 15 and 17 of cycle. Each uterine flushing was centrifuged immediately to remove cell debris and supernatant were stored at –20°C until assay. Blood samples were also collected on these days. Serum was separated and stored at –20°C till assay.

PGE₂ and PGF_{2α} were estimated in uterine flushing using ELISA kits, where as progesterone was estimated in serum using RIA kit. Nitric oxide in serum as well as in uterine flushing was also estimated using modified method of Sastry *et al.* (2002). The data was subjected to statistical analysis using one way ANOVA and independent T- test as per Snedecor and Cochran (1994).

Estrous cycle was significantly ($P < 0.05$) longer by approximately 2 days observed in meloxicam treated group (Table 1), indicating luteo-protective action of meloxicam. Non-specific COX inhibitors also affected the luteal function and estrous cycle length in heifers (Odensvik *et al.* 1998), Llama (Aba *et al.* 2000), and ewes (Ake-Lopez *et al.* 2005). The extension of estrous length in present study was less as compared to findings of Odensvik *et al.* (1998) which might be due to shorter duration of treatment (2 vs 9 days). The parenteral administration of flunixin meglumine (FM), a non-selective COX inhibitor postponed luteolysis and prolonged the estrous cycle in heifers (Aiumlamai *et al.* 1990). Information on use of COX-2 inhibitor for modulation of reproduction in animals is meager (Rajkumar *et al.* 2010).

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Table 1. Effect of selective COX-2 inhibitor-meloxicam on estrous cycle length

Groups	Length of estrous cycle (days)
Control	21.2±0.255 ^a
Treatment	23.1±0.400 ^b

a, b, means±SE with different superscripts in a column differ significantly ($P<0.05$).

The serum progesterone concentration was more than 1.0 ng/ml between days 13–17 in both the groups, indicating presence of corpus luteum (Fig 1). Though, progesterone concentration in the present study did not differ significantly between the groups but showed a declining trends towards day 17 in control animals which was not observed in treatment group. Odensvik *et al.* (1998) also could not observe any significant difference in serum progesterone concentration but recorded increased estrous cycle length in heifers while using non-selective COX inhibitor. Extension of estrous cycle length in treated animals would further support the effect of meloxicam on luteal function.

PGF_{2α} concentration in uterine flushing increased significantly ($P<0.05$) from day 13 to 17 of the estrous cycle in animals of both the control and treatment groups (Table 2), however, the trend of PGE₂ concentration was in decreasing order though non-significant. PGF_{2α} as observed

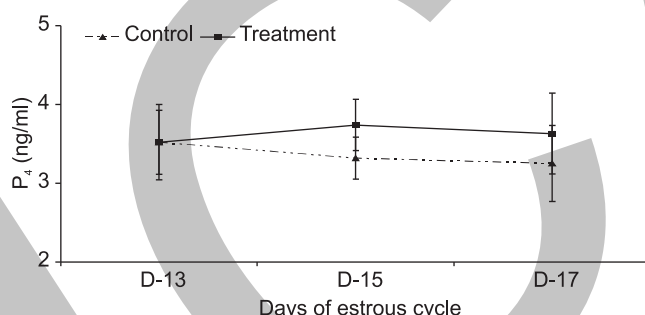


Fig 1. Serum progesterone profile.

in present study was also reported to be present in extremely small quantities in uterine flushing (0.3–0.6 ng/ml) and non-detectable level of PGE₂ (<10 pg/ml) on day 13, 15 and 17 of estrous cycle in ewes (Ellinwood *et al.* 1979). This may be due to short half life of prostaglandins especially in the presence of various luminal cells and cell debris in the uterus.

Table 2. Concentration of PGF_{2α} (ng/ml) in uterine flushing

Groups	Day-13	Day-15	Day-17
Control	0.065±0.006 ^b	0.079±0.005 ^a	0.089±0.005 ^a
Treatment	0.060±0.008 ^B	0.080±0.002 ^A	0.085±0.007 ^A

a, b, means±SE with different superscripts in a row differ significantly ($P<0.05$).

PGE₂ concentration in uterine flushing recorded in the present study was slightly higher but comparable to the finding of Ellinwood *et al.* (1979).

The respective decreasing and increasing trend of PGE₂ and PGF_{2α} concentration in this study may be an indication for initiation of process of luteolysis somewhere on day 15 to 17 of the cycle. The data as such on prostaglandin concentration in uterine flushing is meager which makes difficult to compare the results.

The present study could not find any significant difference in the PGF_{2α} concentration in uterine flushing between the control and treatment group. PGF_{2α} in uterine venous plasma of pregnant ewes were higher (Wilson *et al.* 1972, Ellinwood *et al.* 1979) or not different (Pexton *et al.* 1975, Nett *et al.* 1976 and Levis *et al.* 1977) than concentration in the cycling ewes on days 13–17 post estrus. Nett *et al.* (1976) suggested that the frequency of peak concentration of PGF_{2α} in utero-ovarian venous plasma may be important in luteolysis rather than its mean concentration. The concentration of PGF_{2α} was higher on day 15 and tended to be more on day 17 of pregnancy than on day 15 or 17 of the estrous cycle (Ellinwood *et al.* 1979).

It has been suggested that PGF_{2α} is most effective as a luteolytic factor when it reaches the corpus luteum through blood vessels (Hayashi and Miyamoto 1999). Higher PGF_{2α} on day 15 and 17 of cycle as observed in the present study are comparable to the findings of Ellinwood *et al.* (1979). Manipulation of the reproductive tract during embryo transfer increases release of PGF_{2α} from the uterine endometrium and administration of PGF_{2α} synthesis inhibitor at the time of embryo transfer improved pregnancy rates in cows (Scenna *et al.* 2005). This may be the reason of slight increase in PGF_{2α} concentration in successive flushings on day 15 and 17 of cycle as observed in the present study.

The concentration of serum NO was nonsignificantly higher on day 15 as compared to day 13 and 17 of the estrous cycle in control animals, however, it was lower ($P<0.05$) on day 15 as compared to day 13 and 17 of the cycle in treatment group (Table 3).

The serum NO concentration decreased significantly ($P<0.01$) on day 15 of the estrous cycle i.e. just 4 h after the meloxicam treatment. The increase in serum NO on day 15 in control animals might be due to initiation of cascade of luteolysis which is also supported by Skarzynski *et al.* (2003) that NO inhibits luteal steroidogenesis and may be one of

Table 3. Concentration of nitric oxide (μM/L) in serum

Groups	Day-13	Day-15	Day-17
Control	14.07±1.694	16.49±0.772 ^a	15.40±1.034
Treatment	13.77±0.850	11.10±0.738 ^b	13.38±1.096

a, b means±SE with different superscripts in a column differ significantly ($P<0.01$).

Table 4. Concentration of nitric oxide ($\mu\text{M/L}$) in uterine flushing

Groups	Day-13	Day-15	Day-17
Control	15.10 $\pm$ 1.347	15.84 $\pm$ 0.831 ^A	16.19 $\pm$ 0.884
Treatment	14.72 $\pm$ 0.811 ^a	11.49 $\pm$ 0.602 ^{bB}	13.80 $\pm$ 1.027 ^{ab}

a, b, Means $\pm$ SE with different superscripts in a row differ significantly ($P<0.05$); A, B, means $\pm$ SE with different superscripts in a column differ significantly ($P<0.01$).

the components of an autocrine/paracrine luteolytic cascade induced by $\text{PGF}_{2\alpha}$. Intra-peritoneal injection of meloxicam stimulated NO production in rats (Cella *et al.* 2006). Both indomethacin and meloxicam stimulated NO production and reduced $\text{PGE}_2/\text{PGF}_{2\alpha}$ secretion. Reduced $\text{PGE}_2/\text{PGF}_{2\alpha}$ in uterine flushings after meloxicam treatment was also observed in this study. Indomethacin reduced COX-2 and iNOS expression (Ribeiro *et al.* 2004). The difference on nitric oxide production by the COX-2 inhibitors in the above studies might be due to different species used i.e. buffalo vs. rodents.

The concentration of NO in uterine flushing did not differ significantly on day 13, 15 and 17 of the estrous cycle in control animals (Table 4). However, it was significantly lower ($P<0.05$) on day 15 as compared to day 13 and 17 of the cycle in meloxicam treated group. The NO concentration in uterine flushing decreased after meloxicam treatment as compared to control group, which was statistically significant ($P<0.01$) on day 15 of the estrous cycle i.e. just 4 h of injection. The findings could not be compared due to lack of information on nitric oxide concentration in uterine flushing. The results of decreased NO concentration in serum and uterine flushing in treated animals supported our hypothesis of luteoprotective action of meloxicam.

The COX-2 selective inhibitors show minimal side effects as compared to non-selective one, but their role in reproduction has not been well studied. The COX-2 selective inhibitors show minimal side effects as compared to non-selective one, but their role in reproduction has not been well studied. The present study may be concluded that exogenous administration of selective COX-2 inhibitor-meloxicam exhibited luteotropic action through prostaglandins and nitric oxide synthesis pathways and causing extension of estrous cycle in buffaloes. Thus meloxicam may be an alternative for treatment of reproductive disorders due to luteal insufficiency.

SUMMARY

The present study was conducted on 5 healthy, parous, cyclic water buffaloes to study the effect of selective COX-2 inhibitor-meloxicam on cycle length and luteal functions in buffalo. The positive effect of meloxicam on extension of estrous cycle length was observed in treated as compared to control animals. $\text{PGF}_{2\alpha}$ concentration in uterine flushing

increased significantly from day 13 to 17 of the cycle in both groups. On the other hand, PGE_2 concentration showed decreasing trend. The nitric oxide concentration in serum and uterine flushing was significantly low on day 15 of the estrous cycle in treated as compared to control animals supported luteotropic action of selective COX-2 inhibitor. It is concluded that exogenous administration of meloxicam has luteotropic action regulating through prostaglandins and nitric oxide pathways.

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