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ORIGINAL ARTICLE

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The effects of ketoprofen on ovarian function in dairy cows

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Abstract Two experiments were conducted to investigate the effects of ketoprofen on ovarian function in dairy cows. In experiment I, eight non-milking dairy cows were administered a 0.9% saline solution daily from day 8 (day −3) of ensuing synchronized estrous cycle at 24-h intervals over 4 days (control observation). After an estrous cycle rest, the cows were given the recommended daily therapeutic dose (3 mg/kg, i.m.) of ketoprofen (Ketofen 100; 10%, Merieux/Webster, Australia) from day 8 (day −3) of the synchronized estrous cycle at 24-h intervals over 4 days. All cows received an intramuscular injection of prostaglandin $F_{2\alpha}$ (30 mg, Lutalyse) either 6 h before the first treatment of saline solution or 6 h before the first injection of ketoprofen. Ultrasonography of the ovaries was performed daily from the day before (day −4) experimental treatments until day 2 after induced estrus, to monitor the growth of the ovulatory follicle and ovulation, and then on day 9 after estrus to determine the presence and the size of the corpus luteum. In experiment II, five non-milking dairy cows were used in two successive observations. The plasma preovulatory estradiol-17β peak and progesterone concentrations were determined, and ovarian ultrasonography was performed to determine ovulation and corpus luteum development. Results obtained from experiment I showed that the diameter of dominant ovulatory follicle on day of estrus was significantly ($p<0.05$) higher in saline-treated estrous cycle compared to that of ketoprofen-treated cycles (13.8±1.3 vs 10.9±0.4 mm). Furthermore, by 48 h after standing estrus, ovulation had taken place in seven of eight saline-treated estrous cycles, whereas only three ketoprofen-treated cows had ovulated by 48 h after standing estrus ($p<0.05$). A significantly ($p<0.05$) higher mean estradiol-17β peak was observed in ketoprofen-treated estrous cycles at estrus compared to that of the control estrous cycles (experiment II). Additionally, a significant ($p<0.05$) daily increase in the mean plasma progesterone concentration was observed after ketoprofen treatment beginning from day 0 to 6 of the subsequent estrous cycle ($p<0.05$). The results of the present study demonstrate that administration of ketoprofen during the pre- and periovulatory period of the estrous cycle in dairy cows impairs final growth of ovulatory follicle, leading to a disturbance in the normal process of ovulation.

Keywords Ketoprofen · Ovarian function · Ovulation · Dairy cows

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Introduction

Final growth of the dominant ovulatory follicle and ovulation are regarded as two classic examples of physiologic inflammatory reactions (Espey 1994; Richards et al. 2002). Studies have shown that a surge in gonadotrophins induces an acute inflammatory reaction in the ovulatory follicle (Brannstorm and Norman 1993; Pate 1995; Robker et al. 2000), leading to the rupture of the follicle surface. Administration of dexamethasone, a synthetic glucocorticoid (Vighio and Liptrap 1990), and aspirin, a nonsteroidal anti-inflammatory agent (NSAIA) (Stahringer et al. 1999), during the development of the ovulatory follicle has been shown to delay or interfere with ovulation in the cow. Odensvik et al. (1998) reported that intensive oral administration of flunixin meglumine is able to postpone luteolysis and prolong the estrous cycle in heifers. Salhab et al. (2001) also showed the inhibitory effect of meloxicam on ovulation in the rabbit.

Ketoprofen is a propionic acid NSAIA with a strong anti-inflammatory property, reducing the biosynthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) through the inhibition of cyclo-oxygenase enzymes in the arachidonic acid cascade. It is licensed for administration to food animals such as cattle (Adams 2001). Studies have confirmed that NSAIAs are increasingly used for the treatment of food-producing animals, particularly in cattle (Kopcha et al. 1992; Semrad 1993; Amiridis et al. 2001). Administration of NSAIAs is based on antipyretic, analgesic, and anti-inflammatory

properties. Nonsteroidal anti-inflammatory agents have been used in therapy for acute and peracute bovine inflammatory conditions, such as puerperal metritis (Amiridis et al. 2001), locomotor dysfunction (Fenwick and Daniel 1996; Whay et al. 2005), and mastitis (Anderson et al. 1986; Shpigel et al. 1994).

No information is available regarding the effect of ketoprofen on ovarian function in dairy cows. Therefore, the following experiments were designed to determine (1) the effects of administration of ketoprofen on the estrous behavior, the growth of ovulatory follicle, and ovulation (experiment I), and (2) to evaluate the preovulatory estradiol-17β peak and progesterone concentrations, as well as corpus luteum development in the estrous cycle of dairy cows that were given ketoprofen (experiment II).

Materials and methods

Animals

Animals in experiments I and II were all selected from the Dairy Cattle Unit at the Veterinary School of Shiraz University. The age of the cows was from 3 to 7 years. The reproductive tracts of all cows were examined per rectum to confirm that they were free from any palpable abnormalities. In addition, records of estrous detection and palpation per rectum confirmed that all animals were cycling at the commencement of the experiments. All animals were managed as one group and housed in a dry lot with access to shelter and water. During the trial, daily feeding consisted of 2 kg grain and 7 kg of corn silage per head as fed, and free choice of alfalfa hay, containing 13% crude protein.

Experimental design

The study was performed so that each animal acted as its own control. In experiment I, eight non-milking multiparous dairy cows (345±37 kg LW; X±SD) were used in two successive observations. The first estrous cycle was a control cycle, and after a cycle of rest, the second estrous cycle was used as the treatment cycle. Daily growth of dominant ovulatory follicle, ovulation, and corpus luteum (CL) development were monitored via daily transrectal ultrasonography using a real-time, B mode ultrasound scanner (500 V, Ami, Canada) equipped with a 5 MHz linear array transducer. Cows were synchronized using two administrations of $PGF_{2\alpha}$ (30 mg i.m., Lutalyse, Pharmacia, Belgium) 14 days apart. The ovaries of each female were systematically scanned daily commencing on day 6 of the presynchronized estrous cycle, to determine the presence of a growing dominant follicle, to the expected day of ovulation, and then on day 9 after estrus, to evaluate the development of the corpus luteum. The presence of a growing dominant follicle (≥9 mm; more than 1 mm growth per day) on days 6, 7, and 8 of the estrous cycle was confirmed by a serial daily ultrasonography. At each examination, the diameter of follicles was determined and then the location of the follicles was sketched relative to each other and to the CL. This was compared with that of the previous day. To determine the growth rate of the dominant follicle, its diameter for each day was subtracted from the previous day's diameter. Ovulation was determined by the disappearance of the ovulatory follicle and subsequent development of a CL at the same site. In control observations, cows were administered a 0.9% saline solution intramuscularly as a placebo daily from day 8 (day −3) of the ensuing synchronized estrous cycle at 24-h intervals over 4 days. After one estrous cycle rest, cows were given the recommended daily therapeutic dose (3 mg/kg, i.m.) of ketoprofen (Ketofen 100, Merieux/Webster, Australia) starting at day 8 (day −3) of the estrous cycle at 24-h intervals over 4 days. All cows received an i.m. injection of $PGF_{2\alpha}$ (30 mg, Lutalyse) either 6 h before the first treatment of saline solution or the first administration of ketoprofen.

Experiment II involved five non-milking multiparous dairy cows (360±36.6 kg LW, X±SD), in which they were used in two successive observations. The estrous cycle of the cows was synchronized, as previously described in Experiment I. The schedule for ketoprofen administration was also carried out similarly to experiment I. Aside from monitoring for the presence of the dominant follicle at the commencement of the experiment, estrous behavior, diameter of ovulatory follicle, and ovulation as conducted in Experiment I, the plasma preovulatory estradiol surge and the progesterone concentrations of the control and ketoprofen-treated cycles were also examined. To monitor corpus luteum development, ovarian ultrasonography was continued every second day through to day 9 after estrus.

Monitoring of estrous behavior

Twenty-four hours after the $PGF_{2\alpha}$ administration, all cows were observed six times a day (1 h each time) for signs of estrus. Standing estrus was considered to be the primary and most reliable sign of estrus. The number of standing estrus and mountings were recorded as a quantitative measure of estrous activity. The occurrence of secondary signs of estrus, including vaginal mucus discharge and swelling of the vulva, was also recorded.

Blood sampling

In experiment II, an intensive blood sampling was carried out to determine plasma concentrations of estradiol-17β and progesterone. Blood samples were collected from the coccygeal vein into heparinized tubes from all cows once a day commencing 1 day before initiation of either saline or ketoprofen administration through to 24 h after administration of $PGF_{2\alpha}$, then every 4 h for 48 h, then every 6 h for a further 24 h, and then every second day until day 10 after estrus. Samples were centrifuged at 1,500×g for

15 min, and plasma was collected and stored at −30°C until hormonal analyses were carried out.

Hormone analyses

Plasma progesterone concentrations were determined using a radioimmunoassay kit (Coat-a-Count, Progesterone, Diagnostic Products Corporation, Los Angeles, USA). The sensitivity of the assay was 0.1 ng/ml, and the intra- and interassay coefficients of variation were 10 and 14%, respectively. Plasma estradiol-17β concentrations were assayed using a commercially available enzyme immunoassay kit (Specra, Orion Diagnostic, Finland). The sensitivity of the assay was 3 pg/ml and the intra- and interassay coefficients of variation averaged 9.2 and 11.5%, respectively. The oestradiol-17β peak was defined as the highest estradiol-17β concentration measured during the period of intensive blood sampling.

Statistical analysis

General linear models procedure for repeated measures analysis of variance (ANOVA) was used to determine the growth of the dominant ovulatory follicle (experiment I), corpus luteum development, and changes in plasma progesterone concentrations (experiment II) between the control and ketoprofen-treated estrous cycles. Wilcoxon assigned rank test was used to statistically analyze the difference in the diameter of ovulatory follicle and the estradiol-17β peak at estrus between the control and ketoprofen-treated estrous cycles. Differences in the proportion of the number of cows showed standing estrus and ovulation after each treatment were assessed using Fischer exact test. Data were analyzed using the computer-based software SPSS (Version 10). A value of $p<0.05$ was considered significant.

Results

Clinical observations

Of eight cows used in experiment I, seven cows (87.5%) showed standing estrus after saline administration, whereas only five cows (62.5%) showed standing estrus after receiving ketoprofen. The mean number of mountings per hour was higher in saline-treated cycles compared to that of ketoprofen-treated cycles (5.6±1.3 vs 3.6±0.7; $p=0.06$). A significantly higher mean number of standing estruses per hour was observed in saline-treated cycles compared to that of the ketoprofen-treated cycles (9.9±1.7 vs 4.4±1.3, $p<0.05$). Furthermore, saline-treated cycles had vaginal mucus discharge with normal amount on the day of estrus. In contrast, a lower amount of vaginal mucus discharge was observed in four ketoprofen-treated cycles on the day of estrus. Severe swelling of the vulva was observed in three ketoprofen-treated cows, while a normal degree of swelling of the vulva was observed in all saline-treated cycles. In experiment II, standing estrus was observed in all five saline-treated estrous cycles, whereas only three out of five ketoprofen-treated cycles showed standing estrus. The vulva of these three-ketoprofen treated cows was severely swollen at estrus.

Follicular development and ovulation

A composite summary of the results obtained from experiment I is shown in Table 1. Repeated measures analysis of variance showed a significant ($p<0.05$) increase in the mean(±SEM) diameter of the dominant ovulatory follicle commencing from day of induced luteolysis to the day of estrus in saline-treated cycles, whereas no significant increase was observed in ketoprofen treated cycles. By 48 h after standing estrus, ovulation had taken place in seven of eight saline treated cycles; however, when the cows were treated with ketoprofen only, three cows had ovulated within 48 h after standing heat. Ovulation either did not take place or was delayed in the remaining five ketoprofen-treated cows (experiment I).

Results obtained from experiment II showed that the diameter of dominant ovulatory follicle on day of estrus was significantly ($p<0.05$) higher in saline-treated estrous cycles compared to that of the ketoprofen-treated cycles (11.3±0.3 vs 8.9±1.1 mm). Ultrasonography revealed that ovulation had taken place in saline treated estrous cycles within 58±12.2 h. after the estradiol-17β peak, while the dominant ovulatory follicles remained unovulated by 72±9.8 h in ketoprofen-treated cycles. The follicles then luteinized and formed a luteal structure in ketoprofen-treated estrous cycles. The mean(±SEM) diameter of the corpus luteum on day 9 of the estrous cycles was 18.9±1.5 and 12.2±1.5 mm in the control and ketoprofen-treated cycles, respectively ($p<0.05$). Repeated measures ANOVA demonstrated that the increase in mean diameter of corpora lutea in the ketoprofen-treated estrous cycles was

Table 1 The effects of administration of ketoprofen on the growth of ovulatory follicle and ovulation in eight cows

	Control cycle	Treated cycle
Diameter of DF (mm) on day 8 (day −3)	9.5±0.6	9.3±0.3
Daily growth rate of OF (mm/day)	1.0±0.1[a]	0.5±0.4[b]
Diameter of OF (mm) on day of estrus (day 0)	13.8±1.3[a]	10.9±0.4[b]
No. of standing estruses observed per hour	9.9±1.7[c]	4.4±1.3[d]
CL size (mm) on day 9 after estrus	14.8±0.9[a]	9.2±0.6[b]

In experiment I, data are expressed as mean(±SEM)
Values followed by superscripted a and b are statistically significant at $p<0.05$. Values followed by superscripted c and d are statistically significant at $p=0.07$
DF Dominant follicle (≥9 mm), *OF* ovulatory follicle

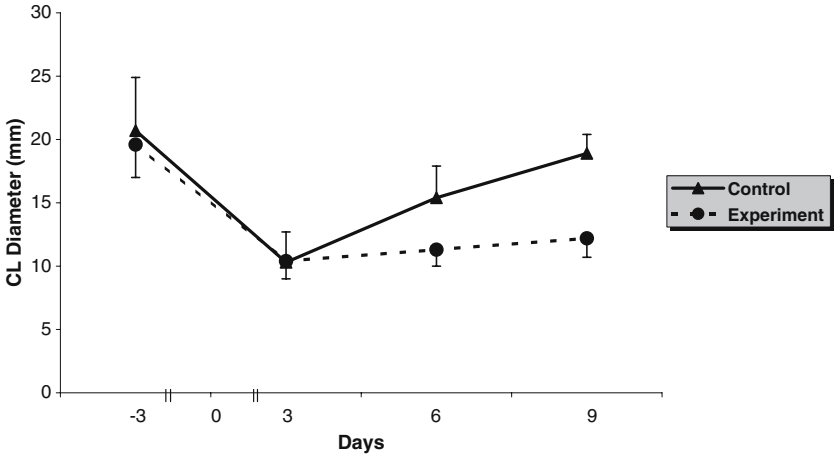

Fig. 1 The mean (±SEM) diameter of corpus luteum in control and ketoprofen-treated estrous cycles (Experiment II)

significantly ($p<0.05$) lower than that observed in the saline-treated cycles between days 0 and 9 after estrus (Fig. 1).

Ovarian hormone profiles

Ovarian ultrasonography and plasma progesterone assay showed that all cows had a functional corpus luteum on the day before commencement of ketoprofen administration. In experiment II, mean plasma progesterone concentration on the day before commencement of the experiment in the saline- and ketoprofen-treated estrous cycle was 6.5±2.4 and 5.8±3.2 ng/ml, respectively. Mean plasma progesterone concentrations at estrus was higher ($p=0.06$) in ketoprofen-treated estrous cycles (1.9±0.7 ng/ml) compared to that of the saline-treated cycles (0.6±0.6 ng/ml) in experiment II. Repeated measure ANOVA demonstrated that the increase in plasma progesterone concentration in the ketoprofen-treated estrous cycles was significantly ($p<0.05$) greater than that observed in the control cycles between days 0 and 6 after estrus (Fig. 2). The estradiol-17β peak was defined as the highest estradiol-17β concentration measured during the surge. A distinct peak in estradiol-17β concentration coinciding with estrus was detected in all control estrous cycles. The mean estradiol-17β peak for the control estrous cycles was 23.7±5.9 pg/ml. However, a significantly ($p<0.05$) higher mean concentration in estradiol-17β peak (62.3±27.9 pg/ml) was detected at estrus in ketoprofen-treated cycles.

Discussion

The most notable observation, as a result of administering ketoprofen during the pro-estrus phase of the bovine estrous cycle in the present study, was the occurrence of abnormal profiles in progesterone and estradiol-17β secretions. This, in turn, resulted in disturbances in estrus activity and ovulation in the ketoprofen-treated cows. The treatment regimes used in the current study, administration of ketoprofen once a day for 4 days, was according to its recommended clinical application in cattle (Damian et al. 1997). In bovine medicine, the recommended dose is 3 mg of ketoprofen per kilogram body weight once a day for 3 to 4 days, administered intravenously or intramuscularly.

A higher mean progesterone concentration on day of estrus was observed in the ketoprofen-treated estrous

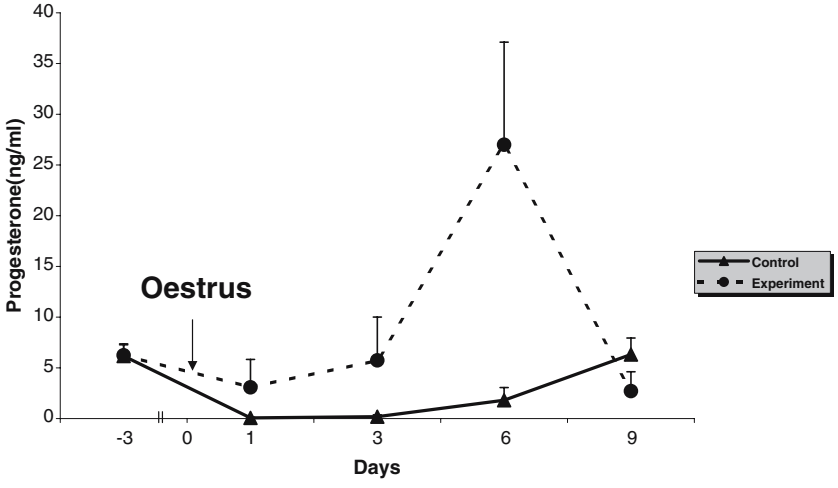

Fig. 2 The mean (±SEM) concentration of progesterone in control and ketoprofen-treated estrous cycles (Experiment II)

cycles comparing to that of the control cycles in experiment II of the present study. This implies that luteal regression was delayed in the estrous cycle of the cows that received ketoprofen. The higher mean progesterone concentration observed on day of estrus in ketoprofen-treated estrous cycles could explain the significantly smaller mean diameter of the ovulatory follicles observed in ketoprofen-treated cycles in experiments I and II. Impairment of luteolysis has been previously reported in cows intensively (four times daily) administered flunixin meglumine (Odensvik and Gustafsson 1994; Odensvik et al. 1998). Final growth of the dominant ovulatory follicle and ovulation are accompanied by a decline in plasma progesterone concentration to less than 1 ng/ml in cows (Vailes et al. 1992).

Impairment in luteolysis can result in elevated progesterone concentrations, leading to disturbance in the occurrence of preovulatory estradiol-17β surge (Adashi 1994). This, in turn, negatively affect luteinizing hormone secretion, either causing a delay in ovulation with respect to onset of estrus or failure of ovulation with resulting luteinization or atresia of the ovulatory follicle(s) (Wathes et al. 2003). Of particular interest was the observation that the estradiol-17β peak was significantly higher in ketoprofen-treated estrus cycles compared to that of the control cycle in experiment II. This effect has not been previously reported in cattle administered NSAIAs. No clear explanation can be presented for this observation. Engelhardt et al. (1989) showed that experimental administration of a high dose of estradiol valerate to nonlactating Holstein cows in the pro-estrus phase of the estrous cycle prevented ovulation and resulted in atresia of the dominant ovulatory follicle. Taking this observation together with the well-documented relationship between the level of plasma progesterone concentration and the occurrence of ovulation (Vailes et al. 1992) could explain the ovulatory failure in ketoprofen-treated estrous cycles of dairy cows in the current study. Alternatively, ketoprofen, as a strong inhibitor of cyclooxygenase (Adams 2001; Norman 2001), may have impaired the inflammatory reactions taking place during the final growth of ovulatory follicles and ovulation.

A slight swelling of the vulva is regarded as a secondary sign of estrus in dairy cattle (Coe and Allrich 1989; Stevenson 2000). The increase in plasma concentration of estradiol-17β at estrus is responsible for a higher blood supply to the reproductive tissues, which in turn causes swelling of the vulva (Diskin and Sreenan 2000). Thus, the severe swelling of the vulva observed at estrus in most ketoprofen-treated estrous cycles of the cows in the current study could be ascribed to the high peak of estradiol-17β at estrus.

Results obtained from experiment II showed that mean plasma progesterone concentration was significantly higher on days 0, 3, and 6 of the ketoprofen-treated estrous cycles compared to that of the control cycles. Repeated measure ANOVA demonstrated that the increase in progesterone concentration was significantly greater after ketoprofen administration between days 0 and 6 of the estrous cycle compared to that of the corresponding days of the control cycles. This occurred despite the fact the mean diameter of the corpus luteum was significantly lower in ketoprofen-treated cycles compared to that of the control cycles between days 3 and 9 of the estrous cycle. It is difficult to explain why the ketoprofen administration evoked an increasing plasma progesterone concentration during the subsequent estrous cycle. Stahringer et al. (1999) reported an increasing concentration of progesterone in aspirin-treated cows between day 6 and 14 of their estrous cycle. The smaller sized corpora lutea, accompanied by a higher mean plasma progesterone concentration in ketoprofen-treated cows, may reflect some alteration in the cellular mechanisms controlling the production and/or secretion of the hormone.

The results of the present study demonstrate that administration of clinically recommended dose of ketoprofen during the pre- and periovulatory period in dairy cows is able to delay luteal regression and impair final growth of ovulatory follicles, leading to a disturbance in the normal process of ovulation and corpus luteum development in dairy cows.

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References

Adams HR (ed) (2001) Autacoids and anti-inflammatory drugs: the analgesic, antipyretic, anti-inflammatory drugs. In: Veterinary pharmacology and therapeutics, 8th edn. Iowa State University Press, pp 413–446

Adashi EY (1994) Endocrinology of the ovary. Hum Reprod 9:815–827

Amiridis GS, Leontides L, Tassos E et al (2001) Flunixin meglumine accelerates uterine involution and shortens the calving to first oestrous interval in cows with puerperal metritis. J Vet Pharmacol Ther 24:365–370

Anderson KL, Smith AR, Shanks RD et al (1986) Efficacy of flunixin meglumine for the treatment of endotoxin-induced bovine mastitis. Am J Vet Res 47:1366–1371

Brannstorm M, Norman RJ (1993) Involvement of leukocytes and cytokines in the ovultory process and corpus luteum formation. Hum Reprod 8:1762–1775

Coe BL, Allrich RD (1989) Relationship between endogenous oestradiol-17β and estrous behavior in heifers. J Anim Sci 67:1546–1551

Damian P, Craigmill AL, Riviere JE (1997) Extralabel use of nonsteroidal anti-inflammatory drugs. J Am Vet Med Assoc 211:860–861

Diskin MG, Sreenan JM (2000) Expression and detection of oestrus in cattle. Reprod Nutr Dev 40:481–491

Engelhardt H, King GJ, Miller RB et al (1989) Estradiol-induced blockade of ovulation in the cow: effects on luteinizing hormone release and follicular fluid steroids. Biol Reprod 40:1287–1297

Espey L (1994) Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol Reprod 50:233–238

Fenwick DC, Daniel RCW (1996) Evaluation of the effect of ketoprofen on experimentally induced ephemeral fever in dairy heifers. Aust Vet J 74:37–41

Kopcha M, Kaneene J, Shea ME et al (1992) Use of nonsteroidal ant-inflammatory drugs in food animal practice. J Am Vet Med Assoc 201:1868–1872

Norman RJ (2001) Reproductive consequences of COX-2 inhibition. The Lancet 358:1287–1288

Odensvik K, Gustafsson H (1994) Effect of flunixin during asynchronous embryo transfer in the heifer. Anim Reprod Sci 36:13–24

Odensvik K, Gustafsson H, Kindahl H (1998) The effect on luteolysis by intensive oral administration of flunixin granules in heifers. Anim Reprod Sci 50:35–44

Pate JL (1995) Involvement of immune cells in regulation of ovarian function. J Reprod Fertil Suppl 49:365–377

Richards JS, Russell DL, Ochsner S, Espey LL (2002) Ovulation: new dimensions and new regulators of the inflammatory-like response.Annu Rev Physiol 64:69–92

Robker R, Russel DL, Yoshioka S et al (2000) Ovulation: a multi-gene, multi-step process. Steroids 65:559–570

Salhab AS, Gharaibeh MN, Shomaf MS et al (2001) Meloxicam inhibits rabbit ovulation. Contraception 63:329–333

Semrad SD (1993) Comparative efficacy of flunixin, ketoprofen, and ketorolac for treating endotoxemic neonatal calves. Am J Vet Res 54:1511–1516

Shpigel NY, Chen R, Winkler M (1994) Anti-inflammatory ketoprofen in the treatment of field cases of bovine mastitis. Res Vet Sci 56:62–68

Stahringer RC, Neuendorff DA, Randel RD (1999) The effect of aspirin administration and parity on plasma salicylate concentrations and postpartum reproductive parameters in Brahman cows. Prostaglandins Other Lipid Mediat 58:125–138

Stevenson JS (2000) A review of oestrous behaviour and detection in dairy cows. BSAS Occas Publ 26:43–62

Vailes LD, Washburn SP, Britt JH (1992) Effects of various steroid milieus or physiological states on sexual behavior of Holstein cows. J Anim Sci 70:2094–2103

Vighio GH, Liptrap RM (1990) Plasma hormone concentrations after administration of dexamethasone during the middle of the luteal phase in cows. Am J Vet Res 51:1711–1714

Wathes, DC, Taylor VJ, Cheng Z, Mann GE (2003) Follicle growth, corpus luteum function and their effects on embryo development in postpartum dairy cows. Reproduction 61:219–237

Whay HR, Webster AJ, Waterman-Pearson AE (2005) Role of ketoprofen in the modulation of hyperalgesia associated with lameness in dairy cattle. Vet Rec 157(23):729–733