

# Effect of the administration of flunixin meglumine on pregnancy rates in Holstein heifers

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**Fifty-two 15-month-old Holstein heifers were synchronised with single or double injections of prostaglandin  $F_{2\alpha}$ , followed by an injection of gonadotrophin-releasing hormone (GnRH) 48 hours later, and inseminated 12 to 14 hours after the injection of GnRH (day 0). Half of them were then injected twice intramuscularly with 1.1 mg/kg flunixin meglumine 12 hours apart, on the evening of day 15 and the morning of day 16, and the other 26 were not treated. Pregnancy was diagnosed by ultrasound 29 and 65 days after they were inseminated. On day 29, 20 of the treated heifers were pregnant compared with 13 of the control heifers ( $P<0.05$ ); on day 65, 18 of the treated heifers were still pregnant compared with 12 of the control heifers ( $P<0.10$ ).**

IN ruminants, a positive feedback loop between endometrial prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) and luteal oxytocin causes of the corpus luteum to regress (Flint and others 1992), but the maintenance of the corpus luteum and the secretion of progesterone is essential for maintaining pregnancy (Mann and Lamming 1995). The anti-luteolytic mechanism is established through a complex interaction between the conceptus and the maternal environment, including the uterus and the corpus luteum. A trophoblastic protein, interferon-tau ( $IFN-\tau$ ), drives this interaction; in cattle and sheep the  $IFN-\tau$  inhibits the expression of the oxytocin receptor in the luminal epithelium (Farin and others 1990, Wathes and Lamming 1995), in sheep by suppressing the expression of the oestradiol receptor- $\alpha$  ( $ER\alpha$ ) (Spencer and Bazer 1995). As a result,  $IFN-\tau$  reduces the oxytocin-dependent pulsatile release of  $PGF_{2\alpha}$  from the uterine endometrium, thus extending the lifespan of the corpus luteum (Thatcher and others 1995, Bazer and others 1997).

Up to 40 per cent of the total embryonic losses occur between days 8 and 17 of pregnancy (Thatcher and others 1994), during which the conceptus lengthens and secretes  $IFN-\tau$ , which is critical for the maintenance of the corpus luteum and pregnancy. Thatcher and others (2001) suggested that these losses may occur because the conceptus may not be able to inhibit the luteolytic secretion of  $PGF_{2\alpha}$ . Mann and Lamming (2001) demonstrated that poorly developed conceptuses produce small or undetectable amounts of  $IFN-\tau$ , when the corpus luteum should be being maintained. Slowly developing embryos may not be able to secrete sufficient  $IFN-\tau$  at the critical time to reduce the secretion of  $PGF_{2\alpha}$ . In lactating dairy cows the positive effect of bovine somatotropin on pregnancy rate at first service postpartum appears to be mediated by stimulating the growth of the conceptus and the secretion of  $IFN-\tau$  (Bilby and others 2004). An alternative strategy to increase embryo survival would be to inhibit or delay the luteolytic process in cattle that are bearing conceptuses that are slightly behind in development.

Flunixin meglumine is a strong non-steroidal anti-inflammatory drug (NSAID) that inhibits the activity of the enzyme prostaglandin H synthase-2 ( $PGHS-2$ ) and the conversion of arachidonic acid to  $PGF_{2\alpha}$  (Anderson and others 1990). Guilbault and others (1987) reported that giving cows twice-daily intramuscular injections of flunixin meglumine during the first six days postpartum inhibited the secretion of  $PGF_{2\alpha}$ , as indicated by a decrease in the concentration of 13, 14-dihydro-15-keto- $PGF_{2\alpha}$  ( $PGFM$ ) in the peripheral blood. Injections four times daily between days 15 and 22 of the oestrous cycle prevented the secretion of  $PGF_{2\alpha}$  and delayed luteolysis until day 22 and, on the basis of a decrease in the concentrations

of progesterone in the blood, luteolysis occurred on day 23 (Aiumlamai and others 1990).

Oxytocin, by binding to its receptors on endometrial epithelial cells, induces the endometrium to secrete  $PGF_{2\alpha}$  (Mann and Lamming 1995). When cows were given injections of oxytocin at eight hour intervals between days five and eight after mating, embryonic survival decreased in association with an increase in the concentration of  $PGFM$  on day 5 (Lemaster and others 1999). However, simultaneous injections of flunixin meglumine with the oxytocin restored embryonic survival. In vitro,  $PGF_{2\alpha}$  has a direct negative effect on embryonic development (Scenna and others 2004), and it is therefore likely that the  $PGF_{2\alpha}$  induced by the oxytocin had a detrimental effect on embryonic development and the inhibition of the secretion of  $PGF_{2\alpha}$  by flunixin meglumine prevented embryonic death. The drug had a similarly beneficial effect when it was administered just before a stress that would have induced embryonic death at 14 days after mating (Merrill and others 2003). The results of these studies support the hypothesis that flunixin meglumine should inhibit the uterine synthesis of  $PGF_{2\alpha}$  and increase the survival of early embryos.

The objective of this study was to investigate the effect of administering flunixin meglumine just before luteolysis on the pregnancy rate in heifers.

## MATERIALS AND METHODS

Fifty-two Holstein heifers on a private dairy farm were housed in a closed barn and fed the same diet. They were recruited over a period of two months and were 15 months of age when they were inseminated. They were all examined per rectum to evaluate the normality of their reproductive tract, and synchronised with either two injections 14 days apart or a single injection of 25 mg  $PGF_{2\alpha}$  (Dinolytic; Pfizer) administered intramuscularly. The injections were made after a corpus luteum had been detected by ultrasonographic examination and rectal palpation. Exactly 48 hours after the single or the second injection of  $PGF_{2\alpha}$ , 10  $\mu$ g of an analogue of gonadotrophin-releasing hormone ( $GnRH$ ) (Receptal; Intervet) was administered intramuscularly, followed by artificial insemination 12 to 16 hours later. All the inseminations were made by the same veterinarian, and semen from sires with proven fertility was used. The day of insemination was designated as day 0. The heifers were assigned randomly to a treatment group and a control group. The treatment group was injected intramuscularly twice with 1.1 mg/kg bodyweight flunixin meglumine (Fulimed; Alke Ilac Sanayi) on day 15 at 21.00

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**TABLE 1: Numbers pregnant and pregnancy rates (%) on days 29 and 65 for 26 control heifers and the 26 heifers treated with flunixin meglumine**

Day	Control group	Treated group	P
29	13/26 (50)	20/26 (76.9)	0.04
65	12/26 (46)	18/26 (69)	0.09

and on day 16 at 09.00; the control group did not receive any treatment. Pregnancy was diagnosed between days 28 and 32 (mean 29 days) by ultrasonography with a 6 to 8 MHz, linear-array transrectal real-time ultrasonography (Falco Pie Medical). Pregnancy rate was defined as the percentage of heifers diagnosed pregnant to the single insemination. Pregnancy was confirmed by ultrasonography and rectal palpation at about day 65 to calculate a 65-day pregnancy rate and to assess embryonic losses.

### Statistical analysis

The effect of the treatment on pregnancy rates and embryonic losses was analysed by chi-squared test (PRCO FREQ) and logistic regression analysis (PROC LOGISTIC), using the programs of SAS/STAT (1989).

## RESULTS

On day 29, 20 of the treated heifers (76.9 per cent) were pregnant, compared with 13 (50 per cent) of the control heifers ( $P < 0.04$ ) (Table 1). On day 65, 18 (69.2 per cent) of the treated group remained pregnant, compared with 12 (46.2 per cent) of the control group ( $P < 0.09$ ). The rates of embryonic loss (two in the treated group and one in the control group) were not significantly different.

## DISCUSSION

In cattle, early embryonic mortality has been considered one of the major problems affecting the dairy industry. The fertilisation rate in dairy cattle ranges between 55 and 98 per cent depending on the status of the cow (Santos and others 2004). In dairy heifers, the fertilisation rate approaches 100 per cent with the percentage of viable embryos decreasing to about 70 per cent by six days after insemination. Approximately 40 per cent of embryonic losses are likely to occur by between eight and 17 days of pregnancy (Thatcher and others 1994).

Any attempt to prevent or reduce embryonic mortality in cattle needs to be founded on the underlying mechanisms that regulate the development of the embryo and the maintenance of pregnancy. In this study, half the heifers were given flunixin meglumine to inhibit the activity of PGHS-2 and so reduce the synthesis of  $PGF_{2\alpha}$  by the uterus or ovary and contribute to the antiluteolytic process during early pregnancy. On day 29, 20 of these 26 heifers were pregnant, compared with 13 of the control heifers, an increase presumably attributable to an increase in embryonic survival.

In previous studies pregnancy rates in the range of 35 to 55 per cent have been observed when heifers were inseminated at a fixed time after a variety of synchronisation protocols (Pursley and others 1997, Stevenson and others 2000, Tenhagen and others 2005). The pregnancy rates achieved in this study were quite reasonable after a synchronisation system centred on the presence of a corpus luteum when  $PGF_{2\alpha}$  was administered before the ovulatory injection of GnRH.

The higher pregnancy rate in the treated group indicate that two injections of flunixin meglumine on days 15 and 16 effectively decreased early embryonic losses, possibly as a result of an inhibition of the secretion of  $PGF_{2\alpha}$  from the

uterus or within the ovary that would otherwise have triggered luteolysis. Lemaster and others (1999) reported that oxytocin-induced increased in  $PGFM$  concentrations in cattle on day 5 after insemination were inhibited by the injection of flunixin meglumine. Oxytocin secreted by the corpus luteum and/or the pituitary is a potential activator of  $PGF_{2\alpha}$  synthesis and is apparently blocked by treatment with flunixin meglumine. Similarly, extensive feeding of flunixin meglumine granules to heifers for nine days, starting on day 15 of the oestrus cycle, decreased the number of  $PGFM$  pulses, delayed luteolysis and significantly extended the length of the cycle (Odensvik and others 1998).

The higher pregnancy rate at day 29 in the treated group was probably due to a reduction in the embryonic losses that occur in a short period of time when luteolysis is initiated. Apparently a large number of conceptuses are not developed sufficiently to suppress the release of luteolytic pulses of  $PGF_{2\alpha}$  from the endometrium effectively.

Mann and Lamming (2001) demonstrated that some embryos are so poorly developed that the concentration of  $IFN-\tau$  was either very low or undetectable in uterine flushings on day 16 after insemination. The cows with undetectable  $IFN-\tau$  activity had a similar  $PGFM$  response to oxytocin as cyclic cows, whereas the cows with measurable  $IFN-\tau$  activity had an attenuated  $PGFM$  response to oxytocin. It was concluded that the maternal recognition of pregnancy associated with the maintenance of a corpus luteum is successful when the embryo is well developed and secretes appreciable amounts of  $IFN-\tau$ .

Poorly developed embryos may be viable but slower to develop, so that the processes of maternal recognition of pregnancy induced by  $IFN-\tau$  are not initiated at the appropriate time. In this study, the flunixin meglumine may have exerted an inhibitory effect on the synthesis of  $PGF_{2\alpha}$  that delayed its pulsatile secretion. The intensive use of the drug delays luteolysis and increases the length of the oestrous cycle in heifers (Aiumlamai and others 1990, Odensvik and others 1998). In heifers, a single oral dose of flunixin meglumine decreased the concentration of  $PGFM$  and it remained low for 10 to 30 hours (Odensvik 1995); this suggests that giving two doses of the drug 12 hours apart to the heifers in this study may have resulted in lower concentrations of  $PGFM$  for more than 40 hours. At luteolysis, the secretion of  $PGF_{2\alpha}$  continues for about two to three days (Basu and Kindahl 1987) and it is therefore likely that the present dosing regimen delayed and/or inhibited the synthesis of  $PGF_{2\alpha}$  and delayed potential luteolysis. Such a delay would provide extra time for a slowly developing but viable conceptus to secrete sufficient  $IFN-\tau$  to inhibit a luteolytic secretion of  $PGF_{2\alpha}$  after the clearance of the flunixin meglumine.

It is possible that flunixin meglumine might just delay luteolysis and extend the survival of possibly poor quality embryos that would die later in pregnancy. However, between day 29 and day 65 only two of the 20 embryos in the treated group were lost, compared with one of the 13 control embryos, and at day 65 the pregnancy rate remained higher in the treated group, although not significantly so. The conceptus secretes appreciable amounts of prostaglandins (Lewis and others 1982). However, the acute treatment with flunixin meglumine did not appear to affect the conceptus adversely or the processes of early communication between mother and conceptus, and thus compromise the development of the fetus to day 65.

The failure to inhibit the luteolytic releases of  $PGF_{2\alpha}$  on time could also be related to maternal factors. Mann and Lamming (2001) demonstrated a link between the maternal endocrine environment and the development of the conceptus, in that more developed conceptuses with a high potential to secrete  $IFN-\tau$  were associated with higher concentrations of progesterone in the maternal circulation. Progesterone was

not measured in this study, but it is possible that the conceptuses that maintained pregnancy owing to the treatment with flunixin meglumine may have developed in a lower progesterone environment that predisposed them to a slower rate of development.

Although flunixin meglumine is relatively expensive, the improvement in the pregnancy rate, and the potential for an earlier start to lactation, and a reduction in the expenses for resynchronisation and additional inseminations should justify the cost.

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