Effects of Exogenous Oxytocin on Embryonic Survival in Cows

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Abstract

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The aim of this study was to evaluate the effect of oxytocin on embryonic survival in dairy cows. Pregnancy was verified using the early pregnancy factor (EPF) activity on Day 4 after artificial insemination (AI). Pregnant cows were randomly allotted to two groups: treated (n = 8) and control (n = 8). Oxytocin (100 IU, 5 ml, DIF Turkey) was administered twice daily by intravenous injections to treated cows and sterile saline (5 ml) to control cows immediately before milking on days 4 to 7 after AI. Blood samples were taken via jugular vein every day from day 4 to 8 and every other day until Day 20 following insemination to evaluate the effect of oxytocin on embryonic survival. The embryonic loss was diagnosed in 3 of the 8 cows treated with oxytocin, and embryonic survival rate was 62.5% in this group versus 87.5% in controls. Short cycles occurred in 37.5% of oxytocin-treated cows. At the same time their serum progesterone concentrations rose more slowly than in controls. It was concluded that cows administered oxytocin on days 4 to 7 after insemination are at a higher risk of pregnancy loss.

Conception rate, embryonic mortality, short cycle, progesterone

Reproductive performance in the cattle industry is arguably lower than desired. Early embryonic mortality is one of the major problems in animal breeding. Conception rates among cattle have been reported to be as high as 90%, yet calving rates are in the range of 50 to 60% (Sreenan and Diskin 1983) or lower, and much of this decline can be attributed to embryonic mortality. Progesterone synthesis by the corpus luteum (CL) is necessary for maintenance of early pregnancy in the cow (Stewart 1990). Premature regression of the CL and the subsequent reduction in progesterone secretion results in loss of pregnancy (Thatcher et al. 1994). Exogenous oxytocin is often used by dairies to increase milk letdown (Farin and Estill 1993; Lemaster et al. 1999). Oxytocin leads to luteal regression by driving the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium (Balaguer 1999). It may induce release of $PGF_{2\alpha}$ in pregnant cows and ewes (Burgess et al. 1990) albeit at a level much lower than that observed in cycling animals (La France and Goff 1985). Several researchers have demonstrated an increased release of $PGF_{2\alpha}$ after injections of oxytocin, which resulted in shortened luteal phases in cows (Newcomb et al. 1977; Milvae and Hansel 1980; Fuchs et al. 1996). Regression of the corpus luteum leads to a decline in circulating progesterone and deprivation of the fetus of nutrient support. This may result in abortion. Some studies reported that oxytocin influenced progesterone production from corpus luteum (Lemaster et al. 1999; Tan et al. 1982). However, other authors (Mares and Casida 1963; Richardson and Masson 1985) have not observed an effect of oxytocin on progesterone production. To our knowledge, the effect of oxytocin on embryonic loss has not been studied yet. Therefore, the objective of this work was to evaluate the effect of oxytocin on embryonic survival in dairy cows.

Materials and Methods

Holstein cows aged 3 - 5 years were used in this experiment. All animals were clinically healthy. The estrus of cows was synchronized with two intramuscular injections of 526 μg Cloprostenol (Estrumate, DIF Turkey) eleven

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days apart (Day 0). All cows were artificially inseminated (AI) with semen from a single Holstein bull of known high fertility at 12 h past detection of estrus. The experiment was conducted in accordance with the guidelines on animal welfare under the protocol approved by Ethical Committee of Veterinary Faculty of Firat University, Turkey.

The Early Conception Factor (ECF) is a glycoprotein and occurs in blood 48 h after conception. Early pregnancy factor (EPF) is a protein called specific gamma interferon and a secreted substance with growth regulatory and immunomodulatory properties that is required for successful establishment of pregnancy. EPF has important characteristics that render it a superior applicant for an early chemical signal between the embryo and mother (Morton 1998). The detection of EPF in pregnant animals has thus important implications for the study of embryonic-maternal signaling in mammals (Cruz et al. 2001). EPF is needed for survival of the embryo during the pre and peri-implantation stages of pregnancy (Igarashi 1987; Athanasas et al. 1989; Athanasas-Platsis et al. 1991, 2000). It is detectable in maternal serum within 24 h of fertilization in all species studied so far (Morton 1998). Its values are higher in pregnant than in non-pregnant cows 2 days after AI (Ko 1998). Although it is not a certain indicator of pregnancy, EPF activity can be used as a method for early pregnancy diagnosis in cattle (Sakonju et al. 1993). In our experiment, serum EPF activity for confirmation of pregnancy on Day 4 following insemination was used. Non-pregnant cows were removed from the trial. Pregnant cows were randomly allotted to two groups as treated (n = 8) and control (n = 8). Oxytocin (100 IU,5 ml, DIF Turkey) was given twice daily by IV injections to treated group and sterile saline (5 ml) to control group immediately before milking on days 4 - 7 after AI. Serum EPF activity was repeatedly used to confirm whether pregnancy was sustained throughout the study along with progesterone to evaluate the effect of oxytocin. Rectal palpation was also performed in cows not seen in estrus on day 45 after AI to confirm pregnancy. Blood samples were taken via jugular vein every day from day 4 to 8 and every other day until Day 20 post insemination. Blood samples were allowed to clot; the serum was removed, incubated at 56 °C for 30 minutes, and then stored at -20 °C until assayed.

Serum was assayed for progesterone by radioimmunoassay (A commercial assay kit, Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA) as described in detail by Yildiz (1999). The inter- and intra-assay coefficients of variation were 10.8 and 9.5%, respectively. The sensitivity of the assay was 15 pg/tube.

Serum EPF activity was assayed using the rosette inhibition test (RIT). The test is well documented (Morton et al. 1974, 1976; Rolfe et al. 1984; Cavanagh and Morton 1996). This test is based on the capacity of lymphocytes, in the presence of guinea-pig serum as a source of complement, to bind heterologous erythrocytes to their cell surface (rosette formation) (Bach and Antoine 1968). The rosette inhibition test does not give a 100% reliable result but it is so far the only applicable method to detect the presence of EPF at concentrations at which it normally appears *in vivo* (Morton 1998). The assay was carried out as described by Sakonju et al. (1993) and Yildiz (1999). In summary, the lymphocyte suspension (0.1 ml), previously incubated with diluted test serum, was incubated at 37 °C for 90 min with absorbed diluted guinea-pig complement (0.05 ml) and anti-lymphocyte serum, serially double-diluted over a range of 1 in 128 × 10^3. Rosettes were formed by adding a suspension (0.1 ml) of sheep red blood cells, centrifuging the mixture at 120 *g* for 5 min, and re-suspending it gently by rotation and pipetting. The suspension was then spread on a Rosenthal counting chamber and the numbers of rosettes in all divisions were counted. The rosette inhibition titer (RIT) was defined as the highest dilution of anti-lymphocyte serum causing a reduction of at least 25% in the rosette number compared to the proportion of rosettes in the two tubes without antiserum. This titer was multiplied by 1/500 and recorded as the logarithm to base 2.

Data analysis

Females were declared non-pregnant when serum RIT was < 5 (Yildiz and Deveci 2000). Verification of short cycle was determined by progesterone concentration (P4 < 1.0 ng/ml). At each stage after AI, data were analyzed by T-test (SPSS 1999).

Results

The effect of oxytocin on embryonic survival is shown in Fig. 1. Embryonic loss occurred in 3 of 8 cows on days 7 (in 2 of 8 cows) and 12 (in 1 of 8 cows) following the oxytocin injection. However, pregnancy was not maintained in 1 of the 8 pregnant cows in control group as diagnosed on Day 10. Thus the embryonic survival rate was 62.5% in oxytocin group versus 87.5% in controls on Day 15 after insemination.

The incidence of short luteal phases was observed during this experiment (Fig. 2). Progesterone concentration in 3 of the 8 cows on days 10 (in 2 of cows 8) and 14 (in 1 of cows 8) was below 1.0 ng·ml^{-1}. Pregnant cows in the oxytocin group had lower progesterone (P4) levels compared to pregnant cows in the control group on days 8 ($P < 0.05$), 12 ($P < 0.05$), 14 ($P < 0.01$), 16 ($P < 0.05$), 18 ($P < 0.05$) and 20 ($P < 0.01$). Short cycles were shown in oxytocin group (3/8) in comparison with the control (0/8). Short cycles occurred in 37.5% of those cows that received oxytocin. However, only 1 of the 8 cows in control group returned to estrus during the trial period.

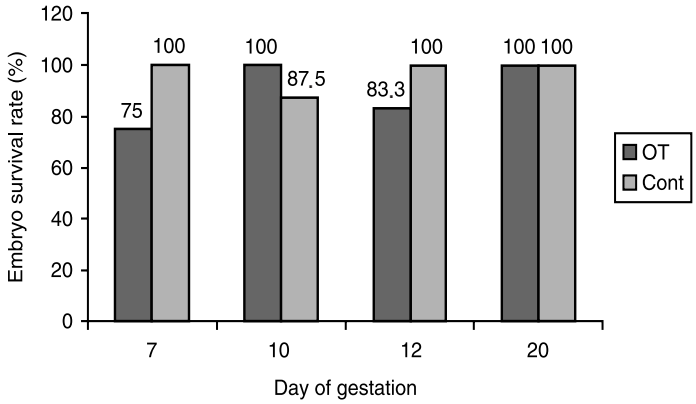

Fig. 1. Embryo survival rate in control cows and in oxytocin infused cows

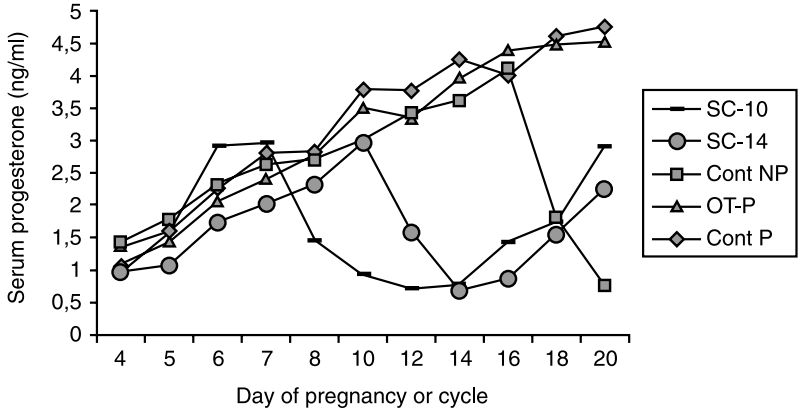

Fig. 2. Progesterone concentration of pregnant (Cont P) and non-pregnant (Cont NP) in control cows, and short cycle of days 10 (SC-10) and 14 (SC-14), and pregnant (OT-P) in oxytocin infused cows. Data are means, with standard errors omitted for clarity

Discussion

The results of our study have shown negative effects of oxytocin on the embryonic survival. Overall, all pregnant cows responded to the oxytocin injection with a reduction in serum P4. Percentage of pregnancy was significantly lower in the experimental group given oxytocin in comparison with the control group. These results are in agreement with previous studies reporting responsiveness to an oxytocin bolus of the early pregnant cows (Lemaster et al. 1999; Balaguer 1999). Generally the effects of oxytocin on pregnancy are insufficiently described. Oxytocin infusion can block the establishment of pregnancy (Wathes et al. 1991). Interaction with oxytocin receptors in the oviduct (Ayad et al. 1990) can influence embryo transport. The indirect effects of oxytocin by stimulation of uterine $PGF_{2\alpha}$ on establishment of pregnancy in cows seem reasonable (Lemaster et al 1999). Oxytocin stimulates the secretion of $PGF_{2\alpha}$ from the endometrium (Burns et al. 1997; Balaguer 1999). $PGF_{2\alpha}$ plays a detrimental role in oxytocin-induced embryonic

loss, aside from luteal regression (Balaguer 1999; Fuchs et al. 1996; Lemaster et al. 1999).

Monitoring of plasma P4 following oxytocin injection on days 4 to 7 of gestation suggested that luteal function in three cows was impaired after the injection of oxytocin but remained functional in five of the eight cows. The regression of the CL occurred in 3 of 8 cows given oxytocin on days 4 to 7 after insemination. These results are similar to those of Balaguer (1999) and Lemaster et al. (1999) and Tallam et al. (2000). Oxytocin can stimulate the release of sufficient $PGF_{2\alpha}$ to initiate luteolysis, even though uterine oxytocin receptor concentration is very low in early pregnancy (Sheldrick and Flint 1985). Fuchs et al. (1996) indicated that oxytocin caused luteal regression by stimulating endometrial secretion of $PGF_{2\alpha}$. $PGF_{2\alpha}$ disrupts luteal secretion of progesterone, resulting in loss of pregnancy.

Serum concentrations of progesterone declined after oxytocin injection. In the oxytocin-infused animals, the serum progesterone concentrations rose more slowly than in the controls. These results agree with the earlier studies of Lemaster et al. (1999) and Milvae and Hansel (1980). Schams (1987) reported that sensitivity to oxytocin might differ with the stage of pregnancy. Wathes et al. (1991) suggested that oxytocin infusions could inhibit luteal development. Pitzel et al. (1990) reported that oxytocin inhibited progesterone secretion *in vitro*. Tan et al. (1982) observed that high concentrations of oxytocin inhibited gonadotrophin-stimulated secretion of progesterone from bovine corpus luteum of early pregnancy *in vitro*. Sernia et al. (1991) observed that progesterone secretion was inhibited by a direct action of oxytocin. Low progesterone concentrations in the early luteal phase are detrimental to embryo survival, probably via an effect on the uterine environment (Wilmut et al. 1985; Ashworth et al. 1989).

In conclusion, using oxytocin twice daily immediately before milking on days 4 to 7 in cows induced luteal regression in 3 of 8 cows and decreased the pregnancy rate. For this reason it seems likely that cows given oxytocin during this period, are at a higher risk of pregnancy loss.

Vliv exogenního oxytocinu na životnost embryí krav

Účelem studie bylo vyhodnotit vliv oxytocinu na životnost bovinních embryí. Březost byla ověřována pomocí aktivity faktoru časné březosti (early pregnancy factor, EPF) čtvrtý den po umělé inseminaci. Březí krávy byly náhodně rozděleny do skupiny pokusné (n = 8) a kontrolní (n = 8). Čtvrtý až sedmý den po umělé inseminaci byl bezprostředně před dojením pokusné skupině podáván dvakrát denně intravenózně (5 ml, DIF, Turkey) oxytocin (100 IU) a kontrolní skupině fyziologický roztok (5 ml). K zjištění účinků oxytocinu na životnost embryí byly z v. jugularis 4. až 8. den a dále každý druhý den až do 20. dne po inseminaci odebírány krevní vzorky. Ztráta embrya byla zjištěna u 3 z 8 krav, jimž byl podáván oxytocin. U těchto krav tedy embrya přežila v 62,5% případů, naproti tomu u kontrolní skupiny přežilo 87,5% embryí. U 37,5% krav, kterým byl podán oxytocin, se vyskytly krátké cykly. Koncentrace progesteronu v séru se zvyšovaly ve srovnání s kontrolní skupinou pomaleji u krav, jimž byl aplikován oxytocin. Z práce vyplývá, že u krav, kterým je podáván v období mezi 4.–7. dnem oxytocin, je zvýšené riziko ztráty embrya.

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