ABSTRACT

Aarabi N, Hovareshri P, Ghasemzadeh-Nava H, Mohammadsadegh M., Effect of progesterone supplementation on late embryonic survival in Holstein dairy cows, Online J Vet Res., 16 (4):182-, 2012 Embryonic mortalities after maternal recognition of pregnancy and placentation are a significant problem in dairy cows. Effects of progesterone supplementation at day 28 of pregnancy on late embryonic death in dairy cows is reported. Eighty lactating dairy cows were given 1.9 mg progesterone CIDR for 14 days and another 80 untreated were used as controls. Pregnancy and serum progesterone levels were determined on Days 28-30 and 45, respectively, and estrogen concentrations were assayed on Days 28-30 after Artificial insemination(AI) in both groups. Serum progesterone concentrations increased (P < 0.01) on Day 45 after AI in cows treated with CIDR (8.69ng/ml ± 1), compared with controls (8.2±ng/ml±1) but there was no relationship between estrogen levels on Days 28-30 and pregnancy loss on Day 45 in any group. Cows with two CLs did not have greater concentrations of progesterone than cows with one CL. Milk production and size of CL did not affect pregnancy retention. Pregnancy rate was not significantly different between Days 28-30 and 45 (73% and 85% in control and CIDR groups respectively), although in the treatment group, serum progesterone concentrations increased significantly 14 days after starting the treatment, compared to the control group. It was concluded that progesterone supplementation on days 28-30 of pregnancy might reduce late
embryonic death in dairy cows.

Keywords: CIDR; Corpus luteum; Progesterone; Estrogen; Pregnancy loss; Cattle

RAW DATA file provided

INTRODUCTION

Embryonic and fetal mortalities decline reproductive efficiency, leading to economical losses in dairy cattle. Late embryonic losses of cows can be defined when losses occur between Days 25 and 45 of gestation (1). Fetal loss and abortions are used to describe pregnancy losses between Days 45 and 260 of gestation, respectively (2). The embryonic losses between Days 27 and 31 and Days 38 and 50 of gestation have been estimated in lactating dairy cows (2, 4). It has been shown that late embryonic mortality averaged 12.8% for different studies. In previous studies, fetal loss was estimated to be 10.7% between Days 38 and 90, also fetal mortality decreases during the third trimester of pregnancy (4). Another study indicated that pregnancy loss was 8.3% between Days 45 and 90 and 9.6% between Days 41 and 120–150 of gestation (6). Late embryonic death after day 27 of gestation reported from 3.2% in dairy cows producing 6000–8000 kg of milk per year in Ireland (7) to up to 42.7% in high producing cows under heat stress (8, 9). It was shown that the percentage of late embryonic death was 28% (10). Several studies have shown that abnormal hormonal profiles including progesterone and estrogen can be affected on embryonic mortality (12, 13). High-producing dairy cows may suffered from low progesterone concentrations during the luteal phase of the estrous cycle, owing to an increment blood flow to the liver which can increase the rate of steroid metabolism, and so may participate to higher pregnancy losses(14).

There is a correlation between negative energy balance and fertility which may be relevant to blood progesterone concentrations (15). Cows with the most negative energy status during the postpartum period had lower serum P4 levels during their third estrous cycles postpartum (16). NEB during early stages of follicular growth and development could also lead to lower P4 secretion (17). The level of P4 secretion may partially explain the pattern of serum P4 concentrations in lactating cows. Lactating cows had lower progesterone concentration and ovulated larger follicles more frequently than virgin heifers (18).

Two logical possibilities to explain why progesterone is lower in the lactating dairy cows than in the heifer are: 1) Reduction of progesterone secretion by the corpus luteum and, 2) Increased progesterone metabolism. Feed intake, milk yield, and route of administration of exogenous progesterone influenced metabolism and excretion of progesterone in some studies in lactating dairy cows (16, 19, 25). Decrement Progesterone concentration usually occurs between 28 to 37 days of pregnancy(61).

This reduction could be due to decreased secretion of progesterone from the corpus luteum or progesterone metabolism in the liver which causes death of embryo/fetus between days 30 to 60 of pregnancy. Several studies have described the effect of exogenous progesterone supplementation on development of the embryo. They noticed progesterone concentrations can
be increased by exogenous supplementation (21, 22, 23). In cows, the corpus luteum as a source of progesterone plays an important role to maintain pregnancy at least during the first 200 days of pregnancy (20, 29, 30).

There is a positive correlation between plasma concentrations of progesterone and pregnancy maintenance on Week 5 of gestation (24). As demonstrated by Lopez et al, progesterone supplementation in high producing dairy cows during the early fetal period reduces pregnancy losses. They also indicated the presence of an additional corpus luteum can prevent late embryo death (4, 34, 35, 36).

Poor placentation has been suggested as the leading cause of late embryonic losses between Days 30 and 45 of gestation. Most pregnancy loss after Day 30 in dairy cows and heifers occurred by Day 42, and losses increased as circulating concentrations of progesterone around Day 30 of pregnancy decreased (24). There is a negative relationship between feed intake and serum progesterone concentration during luteal phase or treatment with exogenous progesterone (25, 26). One of the consequences of high milk production is an increased metabolic rate linked to a greater intake of dry matter. This process reduces plasma concentrations of steroid hormones such as progesterone and estradiol-17β (27).

In one study Bridges showed that there was a tendency for more pregnancies to continue when concentrations of estradiol were lower during the week 5 (60). Another study indicated that pregnancies tended to maintain When concentrations of estradiol were higher during the week 5(51).

The objective of the present study was to investigate the effect of CIDR on late embryonic losses in lactating dairy cows.

**MATERIALS AND METHODS**

This study was conducted on a lactating Holstein dairy cows belonged to a dairy farm with 1100 lactating cows and 3.5% fat-corrected milk- 305-d. and the Rolling Herd Average of 9800 kgs. Cows were kept in open stalls and dry lots and fed a total mixed ration (TMR) three times daily. The TMR was formulated to meet or exceed requirements for lactating cows (31). Cows were milked three times a day. Voluntary waiting Period was around 50-60 days postpartum, the parity of cows ranged from 2 to 4 and the Body Condition Score was determined on the basis of scale1–5 (32). Cows showing health disorders such as mastitis, left displacement abomasum, lameness, and respiratory diseases were not included in the study. One-hundred and sixty dairy cows which were detected pregnant on 28-30 after AI by ultrasonography (7.5MHz transrectal probe, siui, cts 900v, China) enrolled in this study. Uterine horns of the cows were scanned 3 times weekly by ultrasonography for detecting of embryonic loss until day 45.

A real-time transrectal ultrasonographic examination was performed on Days 28-30 and 45 to record the presence of a normal embryo (heartbeats), fluid filled cavity representing the allantoic cavity in the uterine lumen as described by Pierson and Ginther (33), and measuring corpus
luteum diameter on days 28 and 45 after AI. Animals found to be pregnant, were randomly assigned to the control (untreated cows, n=80) or treatment (n=80) groups. Cows in treatment group received Controlled Internal Drug-Releasing device containing 1.9 g progesterone (CIDR; Pfizer Animal Health, New York, NY, USA) on day 28-30 after AI and were removed 14 days after administration. On Days 45, pregnancy status was determined by ultrasonographic examination. Blood samples were collected into vacuum tubes, by puncture of the median coccygeal vein or artery on Days 28-30 and 45 of gestation. Upon collection, samples were immediately placed on ice, and serum was separated by centrifugation (for 10 min at 3000 × g) and stored at -22 °C until assayed.

Serum P4 concentrations on days 28-30 and 45 after AI and also serum estrogen concentrations on day 28-30 after AI were determined by using a validated commercial Radio Immuno Assay kit (Immunotech kit, Marseille France). The intra and inter-assay coefficients of variation (CVs) of progesterone were 6.5 and 9.0%, and estrogen were 5.1 and 8.2% respectively. The sensitivity of the test was 0.05 ng/mL and the recovery rate of the assay ranged from 85 to 110%.

Ultra measurements of CL dimensions (length (L) and width (W)) were used to calculate luteal tissue volume. First, the radius (R) was calculated by the formula \( R = (L/2+W/2)/2 \), and then volume (V) was calculated using the formula \( V = \frac{4}{3} \pi R^3 \).

**RESULTS**

Chi square test showed that there was no significant difference between the pregnancy rates on day 45 in the treatment (91%) and the control (85%) groups (P= 0.2) (Figure1).

Figure 1.Pregnancy rate on day 45 in control and treatment groups.
Shapiro-Wilk and Kolmogorov-Smirnov tests showed that the distribution of serum progesterone concentration was not normal (p=0.00). Mann-Whitney test showed that there was no significant difference between progesterone levels of the treatment (8.6±1, median=9) and the control (8.2±1.9, median=9) groups on day 45 (P = 0.6). Kruskal-Wallis test indicated that there were no discrepancies in progesterone levels in four groups (p=0.6). The difference of Progesterone levels between day 28 (8.26±1.2, median=9) and day 45 (8.69±1, median =9) in the treatment group was statistically significant (p=0.01) while there was no significant difference in progesterone levels between these two days in the control group (8.67±7.6, median=9 and 8.2±1.9, median=9, respectively) (p=0.7), indicating the positive effect of CIDR supplementation to increase serum concentrations of P4, supporting the embryonic survival. Kolmogorov-Smirnov and Shapiro-Wilk tests showed the distribution of corpus luteum volume in different days in two groups were not normal. The differences between the corpus luteum volume on days 28 and 45 in control (9190.7 ± 13515.21, Median=6703.8 and 8289.7± 9279.6, Median=7396.3) and treatment (7130.5±1588.8, Median=7039.9 and 7931.2 ±1910.5, Median=8575.8) groups were significant (P<0.05) (table1). The difference of the corpus luteum volume on day 45 between the control and treatment groups was not significant (p=0.06). Spearman test showed that there was no relationship between the number of CLs and serum progesterone concentrations on day 28 (p=0.3) as well as on day 45 (p=0.1).

Kolmogorov-Smirnov and Shapiro-Wilk tests showed that the distribution of estradiol concentrations was not normal (p=0.02). The difference of serum estradiol concentrations on day 28 between two groups was not significant (p=0.6). Spearman test showed that there was no relationship between the serum estradiol concentrations on day 28 and pregnancy survival on day 45 (P=0.9). Pierson test showed that there was no correlation between BCS and progesterone concentrations on day 28 (p=0.5) and day 45(p=0.1) in both groups. Pierson test indicated that there was no relationship between milk production and progesterone concentration on days 28 and 42 (p=0.4). Fisher Exact test showed that there was no difference between the number of CLs and pregnancy loss on day 42.

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<th>Median</th>
<th>Grouped Median</th>
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<th>Std. Deviation</th>
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**DISCUSSION**

This study was conducted to determine the effect of progesterone supplementation of CIDR on the survival rate of late embryos on lactating cows. High frequencies of late embryonic and early...
fetal losses have been observed in lactating dairy cows during attachment of the embryo in the uterus and placentation, between days 28 and 45 of pregnancy (57). It has been reported that almost half (47.5%) of the total embryonic loss occurred between days 28 and 42 of gestation (59). Some observations in beef cows led to consideration of the possibility that follicular size in timed-inseminated cows after GnRH might affect late embryonic losses (3, 58). It is accepted that progesterone plays a major role in controlling the maternal secretion of nutrients, growth factors and immunosuppressive agents required for successful embryo/fetal development and pregnancy recognition (20, 43). Intra-vaginal progesterone supplementation during the late embryonic period gave rise to an increase in plasma progesterone concentrations during the first week of treatment. Commercially available devices for progesterone supplementation for cattle have been tested in ovariectomized (37), anestrous postpartum (38, 39), cyclic cows (41), and during the early embryonic period (41,42) which lead to increased milk production and plasma progesterone concentrations. It has been reported that intra-vaginal progesterone supplementation has the potential to reduce the incidence of pregnancy loss during the early fetal period (4).

The present findings indicate that there is slightly association between serum progesterone concentrations and embryo loss reduction. Cows treated with the CIDR on day 28 might reduce embryonic loss on Day 45 compared to control group, although CIDR insertion on Day 28-30 after AI increased serum progesterone concentration on Day 45, compared to control group.

Another research indicated supplementary progesterone reduced the incidence of pregnancy loss, is uncertain, the vaginally deposited progesterone could have effect to increase concentrations of the hormone in uterine arterial blood (44). Bartolomes observed that, administration of a 2.1 mg deslorelin implant on Day 27 of pregnancy in lactating dairy cows increased the number of corpora luteas, increased plasma progesterone concentrations, and reduced follicular development on Day 45, but failed to reduce pregnancy losses between Days 27 and 45 or Days 45 and 90 (48). It was shown that luteal size did not affect peripheral concentrations of progesterone, although ovarian effluent concentrations of progesterone were related to luteal content in cycling ewes (45). A previous study indicated that peripheral concentration was not related to luteal content in cycling dairy cows (46). Heifers and non-lactating dairy cows had equivalent or greater concentrations of peripheral progesterone despite having smaller CL than lactating dairy cows (47). Niswender reported luteal content and concentration were not associated with peripheral concentrations in these cows. Therefore, ovarian blood flow (49) and catabolism of progesterone, either jointly or independently, must be more important regulators of peripheral concentrations than secretion. When given exogenous progesterone after luteectomy, cows in the high progesterone group maintained greater concentration of progesterone than those in the Low progesterone group. Thus, systemic catabolic factors affected peripheral concentrations of progesterone more than either ovarian blood flow or luteal production (48).

In previous studies, it was suggested that concentrations of progesterone in late embryo period in high milk producing dairy cows can be correlated negatively with production (5, 59, 60), but in our study this relationship was not found. This finding was in agreement with previous study
which showed there was no significant relationship between milk production and embryonic loss (7, 50). It seems most of the embryonic loss occur within 2 weeks after AI rather than at the late embryo stage (7, ). Another article demonstrated neither feed intake nor metabolizable energy had an effect on plasma progesterone concentration (11). In a recent study by lopez, it was demonstrated that the pregnancy loss rate was unaffected by milk production, which ranged from 15 to 64kgs (4).

The present study findings indicated no positive association between additional corpora lutea and the pregnancy loss. Our data was opposite to lopez study, who suggested there is positive correlation between additional CL and maintenance of pregnancy (4). Our data was in agreement with bartolome study who reported additional CLs cannot affect any significant reduction to reduce late embryo death (48).

The ovary is the primary source of estrogens between days 20 and 60 of gestation, as the placenta contributes little until approximately day 50 (53, 54). However, size of the largest or of the two largest follicles on either ovary did not affect pregnancy status, although such an effect may have been expected (55, 56). Any association of retention pregnancy with estradiol was equivocal from one study to another. It was indicated that in 5wk pregnancy, more pregnancy to continue when concentrations of estradiol were higher (51). On the other hand in a previous study it was demonstrated there was a tendency for more pregnancies to continue when concentrations of estradiol were lower in 5wk pregnancy (52). We showed that there was no relationship between estrogen concentration on Days 28-30 and embryo death on Day 45 after AI.

In conclusion, our findings indicate that treatment with progesterone at the time of pregnancy Diagnosis on Day 28-30 in dairy cows, significantly increases serum progesterone concentrations 14 days after starting of treatment, compared to control group, and might reduce late embryonic death in dairy cows, but more studies is needed, because the number of cows in treatment group (n=80) was too small to establish a clear positive effect of CIDR treatment to reduce late embryonic loss.

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