**Effect of post-insemination progesterone supplementation on pregnancy rate in dairy cows**

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**Abstract**

Progesterone plays an important role in maintenance of pregnancy. It is hypothesized that insufficient progesterone early in pregnancy may result in embryonic loss, and that supplemental progesterone would decrease pregnancy loss in dairy cows. In Experiment 1, 84 cows and 16 heifers from a single dairy operation were selected randomly. Within each age category, controlled internal drug release (CIDR) devices were inserted into the vagina of every other female on Day 4 post-insemination and removed on Day 18 post-insemination. Transrectal ultrasonography was performed to determine pregnancy at 4 time periods [days 30 to 37 (week 5), days 44 to 51 (week 7), days 58 to 65 (week 9), and days 86 to 93 (week 13)]. Progesterone supplementation had no effect on pregnancy rate. In Experiment 2, there were no differences in progesterone concentrations between cows that did and did not receive a CIDR. Further, cows receiving CIDR devices did not have an increase in circulating progesterone concentrations 30 min or 1 h after CIDR insertion. It appears that progesterone supplementation does not increase circulating levels of progesterone in the early pregnant lactating dairy cow. Alternative methods to influence progesterone concentrations and/or early embryonic loss need to be investigated.

**Résumé**

La progestérone joue un rôle important dans le maintien de la gestation. Une hypothèse a été émise à l’effet qu’une insuffisance de progestérone en début de gestation pourrait entraîner la perte de l’embryon, et qu’un supplément de progestérone diminuerait les pertes de gestation chez les bovins laitiers. Dans la première expérience, 84 vaches et 16 taures provenant d’une seule opération laitière ont été sélectionnées au hasard. À l’intérieur de chaque catégorie d’âge, un dispositif interne de libération contrôlée de médicament (CIDR) était inséré dans le vagin d’une femelle sur deux au jour 4 suivant l’insémination et retiré au jour 18 suivant l’insémination. Une échographie trans-rectale a été réalisée à quatre périodes afin de déterminer s’il y avait gestation [jours 30 à 37 (semaine 5), jours 44 à 51 (semaine 7), jours 58 à 65 (semaine 9) et jours 86 à 93 (semaine 13)]. Un supplément en progestérone n’a pas eu d’effet sur le taux de gestation. Lors de la 2e expérience, il n’y avait pas différence dans les concentrations de progestérone entre les vaches ayant reçu ou non un dispositif CIDR. De plus, les vaches ayant eu un dispositif CIDR n’ont pas présenté d’augmentation des concentrations de progestérone en circulation 30 min ou 1 h après l’insertion du CIDR. Il semble qu’un supplément en progestérone n’augmente pas les niveaux de progestérone en circulation tôt dans la gestation des vaches laitières. Des méthodes alternatives pour influencer les concentrations de progestérone et/ou les pertes embryonnaires tôt en début de gestation doivent être examinées.

(Traduit par Docteur Serge Messier)
days 4 to 18 of pregnancy in dairy cattle would decrease pregnancy loss and to determine how CIDR devices would influence circulating concentrations of progesterone in lactating dairy cows.

Materials and methods

This project was approved by the Institutional Animal Use and Care Committee at North Dakota State University.

Experiment 1

This study was conducted on a 420-Holstein cow dairy operation located in central North Dakota. Females (84 cows; 16 heifers) included in the study were those bred by artificial insemination after the Ovsynch protocol (first service after a 50-day voluntary waiting period). Within an age category (heifer and cow), CIDR devices containing 1.38 g of progesterone (Pfizer Animal Health, New York, New York, USA) were inserted in every other female on day 4 after insemination (insemination = day 0) and removed on day 18. Females receiving CIDR devices were denoted “CIDR” and those that did not were denoted “No CIDR.” Day 4 was chosen as the insertion day because this is when circulating levels of progesterone diverge (11) and day 18 shortly follows the time of maternal recognition of pregnancy (2). On Tuesdays of each week, cows that ranged between days 30 to 37 (week 5), days 44 to 51 (week 7), days 58 to 65 (week 9), and days 86 to 93 (week 13) after insemination had their state of pregnancy recorded through the use of transrectal ultrasonography (Bantam Linear Ultrasound Scanner; EI Medical, Loveland, Colorado, USA). Females were recorded as pregnant when a viable conceptus, as noted by a detectable heart beat, was visualized.

Experiment 2

In order to determine progesterone changes with CIDR treatment during early pregnancy, 18 multiparous Holstein dairy cows from the NDSU dairy herd were inseminated (Day 0) by artificial insemination. On day 4 after breeding, blood samples were collected via coccygeal venipuncture. Thereafter, a CIDR device was inserted into the vagina of 9 cows. Blood samples of the cows that received CIDR devices were collected 30 min, 1 h, and 1 d after CIDR insertion. On day 18, blood samples were collected prior to the removal of the CIDR, and 1 h and 1 d after the CIDR was removed. Cows that did not receive CIDR devices had blood samples collected on days 4, 5, 18, and 19 after breeding. Immediately after collection, blood samples were placed on ice, allowed to clot and centrifuged at 2000 × g for 20 min. Serum was frozen at -20°C until assayed for progesterone via Immulite progesterone kits (DPC, Los Angeles, California, USA).

Statistical analysis

Statistical analysis of pregnancy rate was performed using the CATMOD procedure of SAS (version 9.1; SAS Institute, Cary, North Carolina, USA) for the effects of progesterone supplementation (CIDR versus No CIDR), age (cow or heifer), week after insemination, and their 2-way interactions. Significance was determined using a chi-squared test at \( P < 0.05 \). In Experiment 2, data were analyzed using the general linear model procedure of SAS. Class statement included CIDR treatment and time. Model included effect of CIDR, time, and their interaction on progesterone concentrations and percentage change from day 4 concentrations of progesterone. Cow was used as a covariate. Effect of time or CIDR was considered significant if \( P < 0.05 \).

Results

The results from Experiment 1 are based on 98 dairy animals; 2 animals were removed from the study due to death or culling. There were no interactions between age, CIDR, or week after insemination \( (P \geq 0.67) \). Progesterone supplementation had no effect \( (P = 0.35) \) on conceptus loss during early pregnancy in dairy cows and heifers supplemented with CIDR devices from days 4 to 18 after insemination (Figure 1). There was an age effect: cows had a higher pregnancy rate (31.7%) compared with heifers (18.8%). Initial overall pregnancy rate was 34% at time of the first ultrasound (week 5 post-insemination). By the fourth ultrasound (week 13 after inseminating) the overall pregnancy rate had decreased to 31%.

In Experiment 2, there was no effect of CIDR insertion on progesterone concentrations, or percentage change per individual cow, on days 4, 5, 18, or 19 (Figure 2). In cows that received CIDR devices, average circulating progesterone concentrations, or percentage change of circulating progesterone per individual cow, after 30 min or 1 h after CIDR insertion did not differ from pre-CIDR insertion progesterone concentrations (Figure 3). Further, on day 18, progesterone concentrations did not differ just prior to CIDR removal and 1 h after CIDR removal. As expected, progesterone concentrations were increased on days 18 and 19 after breeding, compared with day 4.

Discussion

Progesterone supplementation with a CIDR device had no impact on pregnancy rate or circulating progesterone concentrations in dairy cows. Villarroel et al (12) demonstrated that progesterone-releasing intravaginal devices (PRIDs) inserted in repeat-breeder cows (3 to 6 unsuccessful inseminations) from day 14 to day 19 improved the...
pregnancy rate. Robinson et al (8) measured increased pregnancy rates in dairy cows when PRIDs were placed into the vagina on day 5 post-insemination and removed on day 12. This same laboratory further demonstrated that cows receiving injections of progesterone on days 5, 7, 9, and 11 post-insemination had increased pregnancy rates compared with the control and cows receiving a PRID [days 5 to 12 (9)]. Interestingly, concentrations of progesterone did not differ between PRID, progesterone injected, or control cows during the first 22 days post-insemination (9). It has been demonstrated that when a CIDR is placed into ovariectomized cow or female in the early follicular phase of the estrous cycle (13,14), progesterone concentrations will increase 500% to 600% within 1 h after insertion. Furthermore, progesterone will not be detectable 7 h after removal. Robinson et al (8) demonstrated that PRID supplementation increased progesterone concentrations in lactating dairy cows. However, there was no increase in circulating progesterone in the study herein. Unfortunately, feed intake, milk production, or feed analyses, during the time that cows received progesterone supplementation, were not collected in either study. The ration that was fed to the dairy cows used in Experiment 2 was 54% forage and 46% concentrate. The reason that an increase in progesterone was not observed in this study, could have resulted from differences in caloric intake, either from differences in dietary energy concentration and/or feed intake from that of Robinson et al (8). The lack of progesterone increase in this study may likely be the result of increased metabolism of progesterone by the liver. Increased feed intake, which is necessary for increased milk production, results in an increase in blood flow to the liver and hepatic progesterone clearance (15). Therefore, providing supplemental progesterone to high producing dairy cows may not exhibit any effect on embryonic survival simply due to high metabolism of progesterone.

Insufficient circulating progesterone concentrations have been shown to be associated with embryonic loss during early pregnancy (11). Furthermore, early progesterone supplementation in beef cows results in advanced conceptus development by day 14 of pregnancy (10). This enhanced conceptus development may not persist, as progesterone supplementation with CIDR devices on days 3 to 9 post-insemination in ewes did not impact pregnancy rate, birth weight, or numbers of lambs born (16).

Providing progesterone supplementation as early as day 4 did not result in embryonic loss in dairy cattle. Pregnancy rate remained unchanged (29.1%) for dairy cows and heifers receiving progesterone supplementation from week 5 through week 13 after insemination. Pregnancy rates for the group not receiving supplements decreased with each ultrasound period (39.5% > 37.5% > 35.4% > 33%), although differences were not significant. This is important, as there...
appears to be no negative effect of the growing corpus luteum, which is necessary for maintenance of pregnancy. Further, in Experiment 2, no decline in concentrations of progesterone occurred when CIDR devices were removed. Others have shown that plasma progesterone returns to pre-supplementation levels during subsequent estrous cycles upon removal of progesterone supplementation; indicating endogenous progesterone secretion was not affected (17).

In summary, the present study fails to support the hypothesis that supplemental progesterone in dairy cows from day 4 to day 18 after breeding will decrease pregnancy loss and increase calving rate. Even with additional progesterone being administered, circulating progesterone values were relatively unchanged. This observation is indicative of pregnancy loss being more associated with progesterone metabolism compared with progesterone production and secretion in high producing dairy cows.

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**References**