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Effects of supplemental progesterone using a CIDR insert on pregnancy per embryo transfer of dairy heifer recipients of embryos produced *in vitro*

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ABSTRACT

The objective of this experiment was to evaluate effects of supplemental progesterone immediately following transfer of frozen-thawed, IVP embryos on P/ET. Holstein heifers (n = 452), allocated to nine transfer groups over time, were assigned to be embryo recipients in a completely randomized study from December 2016 to April 2017. All heifers were randomly assigned to one of two treatments: 1) control (CON; n = 212) with no further treatment, or 2) received a CIDR insert containing progesterone for 12 d, beginning on the day of transfer (D 7) and removed 12 d later on Day 19 (CIDR; n = 228). A subset of heifers were subjected to blood sampling on Day 7 (ET) and Day 19 (CIDR removal) to determine circulating concentrations of progesterone. Pregnancy was initially determined using a serum assay for pregnancy specific protein-B at approximately Day 40 after ET and confirmed a month later using trans-rectal ultrasonography. Overall, P/ET did not differ (P = 0.941) between treatment groups. At the initial pregnancy determination, P/ET differed (P = 0.007) among transfer groups. Concentrations of progesterone tended to be less (P = 0.064) in heifers in the CON group compared to heifers treated with the CIDR (3.6 ± 0.27 compared with 4.4 ± 0.27 ng/mL), and differed between transfer groups (P < 0.001) and days post-estrus (P = 0.019) of the recipients. In summary, while treatment with supplemental progesterone at the time of transfer of IVP embryos using a CIDR increased circulating progesterone, there was no influence on P/ET.

1. Introduction

The production of embryos *in vitro* (IVP) had exponential growth since the 1990s (Blondin, 2017). In 2014, 500,000 IVP embryos were produced and these accounted for almost 50% of global embryo production (Blondin, 2017), but pregnancy rates following transfer of IVP embryos are still typically less than those of *in vivo* derived embryos (19.8 compared with 55.9%, respectively (Souza et al., 2017). Compared to the *in vivo* counterparts, IVP embryos are less cryotolerant and have less developmental competence post-transfer (Hasler et al., 1995).

Early embryonic loss accounts for 75–80% of pregnancy loss in natural pregnancies of cattle (Sreenan and Diskin, 1983), and approximately 40% of embryonic loss is estimated to occur between Day 8 and 16 of pregnancy (Lonergan, 2011). Factors contributing to this pregnancy loss include poor embryo quality (Lussier et al., 1987), inefficient "cross-talk" between endometrial cells and the conceptus (Ulbrich et al., 2010), as well as asynchrony of the stage of uterine development post-estrus between donor and

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recipient females (Ferraz et al., 2016). In early gestation, endometrial secretions of growth factors, nutrients, steroids, ions, enzymes, and immunosuppressive agents that are imperative for embryonic development are markedly affected by progesterone (Graham and Clarke, 1997). Manning and Lamming (2001) reported that there was an association between lesser maternal concentrations of progesterone and lesser embryo development coupled with a delayed post-ovulatory increase in plasma progesterone and reduced concentrations of progesterone. When there are lesser circulating concentrations of progesterone between Days 3–8 post-ovulation, there are smaller Day 16 embryos, which can negatively affect production of interferon-tau and interfere with maternal recognition of pregnancy processes (Mann and Lamming, 2001). Conversely, when there are increased concentrations of progesterone in the first week post-ovulation there is an increase in production of interferon-tau (Mann and Lamming, 1999), enhanced endometrial gene expression leading to a constituent concentration in the uterine histotroph that is more conducive for pregnancy establishment (Forde et al., 2009), and an accelerated conceptus growth (Garrett et al., 1988).

Progesterone has been exogenously supplemented in numerous studies through various methods, including daily injections (Garrett et al., 1988; Pugliesi et al., 2013) and vaginally via insertion of a controlled internal drug releasing (CIDR) insert (VanCleeff et al., 1996) or progesterone-releasing insert (PRID; O'Hara et al., 2014). Endogenous concentrations of progesterone can also be increased following administration of human chorionic gonadotropin (hCG) to induce formations of accessory corpora lutea (CL; Kerbler et al., 1997; Wallace et al., 2011). Pregnancy per embryo transfer (P/ET) of *in vivo* derived embryos has also increased in recipient females administered 1000 IU hCG at time of transfer compared to control animals (Wallace et al., 2011). Monteiro et al. (2015) studied the effect of supplemental progesterone by inserting a CIDR during either the 4 days prior to a recipient receiving an IVP embryo or from 4 d prior through 10 d after transfer of an embryo. In both treatment groups, pregnancy rates were less (21.3 and 15.2%, respectively, compared with 39.7% in control cows) compared to cows not treated with supplemental progesterone (Monteiro et al., 2015). Treatment with progesterone within 2 d of insemination has resulted in a decreased fertility (Van Cleeff et al., 1996). It, therefore, is unclear whether administering supplemental progesterone exclusively after transfer will affect pregnancy rates. The objective of the present study was to determine whether supplemental progesterone administered using a CIDR, immediately after ET (Day 7) through Day 19 would affect circulating concentrations of progesterone and increase conception rates in dairy heifer recipients into which a frozen-thawed, IVP embryo was transferred. It was hypothesized that supplemental progesterone after ET would increase P/ET. Twelve days of exogenous progesterone treatment was selected to maximize the potential benefits of supplemental progesterone (while not applying it too early in the luteal phase) but to avoid extending the luteal phase and causing delay in the timing of the next ovulation and conception if pregnancy was not established as a result of embryo transfer.

2. Materials and methods

2.1. Animals

Nulliparous Holstein heifers (n = 452; 479 ± 33 d of age) from a commercial dairy in southern Alabama were used. Due to restrictions by the producer on certain dates, a subset of heifers were used for some sample collections. Body condition was assessed and scored (BCS) in a subset of 368 females as previously described (1 to 5 scale; Wildman et al., 1982; Edmonsen et al., 1989) at the time of embryo transfer by two trained individuals. These individuals were not present on two dates of embryo transfer, thus, there was no collection of BCS data on these days. Heifers were maintained on pasture and supplemented with a concentrate to meet or exceed nutritional requirements.

2.2. Embryo transfer

As heifers became approximately 15 months of age, they were assigned to a transfer group (nine total) which were scheduled as the embryo technician and producer could mutually schedule. Each group had at least 25 but no more than 84 heifers. The first transfer group received embryos in early December 2016 and the last group in the study received embryos in April 2017. For heifers in each group, ovulation was synchronized among animals using a CO-Synch + CIDR protocol (Lamb et al., 2001). Estrotect patches (Western Point, Inc., Apple Valley, MN) and tailhead paint (Paintstik; LA-CO Industries, Inc., Elks Grove Village, IL) were used as estrous detection aids in addition to daily, visual inspection for estrous behavior. The embryo transfer technician was scheduled to perform transfers 7 d after the expected day of estrus. There is variability in when heifers express behavioral estrus after synchronization, thus, the actual day of estrus ranged from 6 to 8 d before the day of transfer. The time of standing estrus was considered to be Day 0 for each heifer, therefore, the day of transfer was between Day 6 (n = 22) and 8 (n = 85) after estrus (mean of 7.2 ± 0.48 d; referred to as days post-estrus (dpe) subsequently in this manuscript). Heifers observed to be in estrus (Day 0) were evaluated on the day of transfer (same date for all heifers in a transfer group) for suitability for inclusion in the present study. At the time of embryo transfer, trans-rectal ultrasonography was used to examine ovarian structures by a single, experienced technician using a 7.5 MHz transducing probe (Ibex Pro; E.I. Medical Imaging, Loveland, CO). Animals with cystic structures, CL smaller than 15 mm in diameter, or anatomical tract abnormalities were excluded from the study (n = 12). Cavernous CL as well as location (right or left ovary) of CL were recorded. Only heifers that were detected in estrus and that received an embryo were assigned to a treatment group (n = 440).

Before embryo transfer, all heifers were administered epidural anesthesia (4 mL of 2% Lidocaine; MWI Animal Health, Boise, ID). In all heifers that satisfied all criteria, there were transfers by the same experienced technician of a frozen-thawed, IVP embryo (TransOva Genetics, Boonsboro, MD) into the uterine horn ipsilateral to the CL. Embryos were derived as a result of 48 different matings of 35 Holstein donor females with use of sex-sorted semen from 12 Holstein bulls. Prior to freezing, all embryos were assigned a developmental stage and quality grade in accordance with the guidelines of the International Embryo Technology Society (Stringfellow and Givens, 2010) standards. Embryos ranged in the developmental stage from 4 to 7 and quality grades of 1 or 2. In heifers of the CON group, there was transfer of 11, 94, 55, and 52 embryos that were in developmental stages 4, 5, 6, and 7, respectively. Of those embryos, 158 had a quality grade of 1 and 70 a grade of 2. In heifers of the CIDR group, there was transfer of 5, 96, 67, and 60 embryos that were in developmental stages 4, 5, 6, and 7, respectively. Of those embryos, 134 had a quality grade of 1 and 78 a grade of 2. To ensure embryos from the same batch (sire, dam, and IVF batch) were equally divided among heifers in the CON and CIDR groups, at the time of transfer, embryos that were odd-numbered were transferred into heifers of the CIDR group and embryos that were even-numbered were transferred into heifer of the CON group. While this ensured that each batch of embryos were equally allocated this approach also did result in there being more heifers in the CIDR than CON group. As heifers in the chute were deemed acceptable to receive an embryo, the treatment and embryo (odd or even number) assignments were made and applied. Information about heifer suitability was not available before the time of transfer and this allowed for the most expedited treatment assignment without extending the duration of time needed for transfers. Heifers deemed suitable for embryo transfer were randomly assigned to one of two treatment groups at the time of transfer: 1) control (CON; $n = 212$) with no further treatment, or 2) treated with a CIDR insert containing 1.38 g of progesterone for 12 d (CIDR; $n = 228$; Eazi Breed CIDR; Zoetis Animal Health, Parsippany, NJ). This timing and duration of treatment with progesterone was selected to coincide with animal handling at the time of ET and to coincide with when regression of the CL would occur if the recipient did not get pregnant to allow for subsequent breeding in a timely manner.

2.3. Blood collection and analysis

Blood samples were collected from a subset of heifers (due to time constraints, blood samples from all heifers of three transfer groups were collected; $n = 186$) using the coccygeal venipuncture approach into 10 mL evacuated tubes (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was collected at Day 7 (time of transfer) and 19 (CIDR removal). After collection, blood was refrigerated at 4 °C for 24 h before being centrifuged at 4 °C at 1500 × g for 15 min. Serum was removed and stored at −20 °C until analysis. Samples were later analyzed for concentrations of progesterone using an immunoassay (Immulite 1000; Siemens, Malvern, PA) according to manufacturer's instructions. Briefly, 200 μL of thawed plasma was pipetted into a sample cup containing a bead coated with polyclonal rabbit anti-progesterone, and an alkaline phosphatase conjugated to progesterone, and loaded into the machine. The sample and reagents were incubated for 30 min to determine the concentration of progesterone. After the incubation period there was automatic flushing separation of the bound from the unbound sample and reagent, addition of the chemiluminescent substrate, and determination of the signal using a photomultiplier tube. The signal generated is proportional to the bound enzyme attached to the bead.

2.4. Pregnancy determination

Pregnancy was determined using a serum assay for pregnancy-specific protein B (PSPB; Bio Tracking, Inc., Moscow, ID) and confirmed using real-time ultrasonography or rectal palpation by an experienced technician. Pregnancy was initially determined between Day 36 and 57 of gestation depending on transfer group and confirmed approximately 1 month later.

2.5. Statistical analyses

Some continuous variables were grouped for data analysis and care was taken so these were evenly distributed between treatment groups. To analyze the continuous variable of age in days, heifers were categorized into five age groups representing a 45 d period for each group: 1 (403–449 d; $n = 76$), 2 (450–495 d; $n = 248$), 3 (496–541 d; $n = 80$), 4 (542–587 days; $n = 16$), and 5 (588–631 d; $n = 5$). Heifers were also categorized based on concentrations of progesterone on Day 7 and 19 into three groups: low (≤1.0 ng/mL; animals most likely without a CL), medium (1.1–5.0 ng/mL; animals most likely with a growing CL), and high (≥5.1 ng/mL; animals most likely with a fully functional CL). For Day 7 samples, this equaled 3, 127, and 31 heifers in low, medium, and high groups, respectively. For Day 19 samples, this equaled 48, 64, and 49 heifers in low, medium, and high groups, respectively.

Pregnancy per embryo transfer was analyzed using the GLIMMIX procedure of SAS (9.4; SAS Institute, Inc., Cary, NC) and the initial model included fixed effects of treatment, age of heifer, BCS, days post-estrus (DPE; 6 to 7 and 8) at the time of transfer, date of transfer (transfer group), embryo quality grade and developmental stage, and serum concentrations of progesterone. Terms with the greatest P-value were removed individually from the model in a backwards, stepwise manner to derive the final model for each dependent variable. Two way interactions of independent variables in the final models were included when $P < 0.10$. Observations from 440 heifers were included in the final model for P/ET at initial and confirmation pregnancy determinations. The final model for P/ET at initial pregnancy determination included the effects of treatment and embryo transfer group. The final model for P/ET at confirmation included the effects of treatment.

The GLM procedure of SAS (9.4) was used to analyze concentrations of progesterone and the initial model included the fixed effects of treatment, age of heifer, BCS, DPE, embryo transfer group, embryo quality grade and stage of development, and pregnancy status. Observations from 175 heifers were included in the final model for concentrations of progesterone at Day 7. Observations from 172 heifers were included in the final model for concentrations of progesterone at Day 19. The final model for concentrations of progesterone at Day 7 included DPE, embryo transfer group, and the respective two-way interaction. The final model for concentrations of progesterone at Day 19 included treatment, embryo transfer group, pregnancy status at initial determination, and the

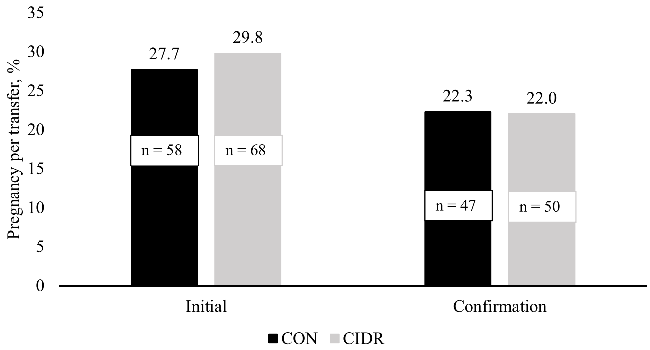

Fig. 1. Pregnancy per transfer between treatment groups at initial pregnancy determination and pregnancy confirmation.

interactions of transfer group × initial pregnancy determination status and treatment × initial pregnancy determination status. Each mating (batch) was distributed equally between treatment groups, so donor and batch were excluded from final analyses. In all cases, statistical significance was considered at $P \leq 0.05$.

3. Results

3.1. Pregnancy per embryo transfer

Overall pregnancy rates at the initial pregnancy detection were 27.5% (121 of 440 heifers) and rates at pregnancy confirmation were 23.3% (103 of 440 heifers). The P/ET were similar for heifers when there was transfer of quality grade 1 and 2 embryos at both the initial pregnancy determination ($P = 0.852$; 30.5 vs. 22.2%) and at confirmation, ($P = 0.979$; 23.6 vs. 16.5%). There were no differences ($P \geq 0.962$) in P/ET at either pregnancy determination among the four embryonic developmental stages of transferred embryos, regardless of quality score (initial: 7.1 (n = 14), 31.8 (n = 192), 22.3 (n = 121), and 29.5% (n = 112)) (confirmation: 8.3 (n = 12), 21.9 (n = 169), 17.9 (n = 106), and 25% (n = 104)) for scores 4, 5, 6, and 7, respectively. Similarly, heifer age, BCS, and concentrations of progesterone at either sampling day had no effect ($P > 0.10$) on P/ET at either pregnancy determination.

The P/ET were similar between treatment groups at the initial pregnancy determination and at confirmation (Fig. 1). At the initial pregnancy determination, there was an effect of transfer group (Table 1) on P/ET, however, there was no effect of transfer group on P/ET at the time of pregnancy confirmation. This study was not designed with enough statistical power for detection of differences in embryonic mortality between the CON (15.5%) and CIDR (25.0%) group. The total number of heifers in which there was pregnancy loss between the two times of pregnancy determinations was 41 heifers.

3.2. Concentrations of progesterone

On Day 7, concentrations of progesterone in serum were similar between treatment groups ($P = 0.869$). Serum concentrations of progesterone on the day of ET were affected by the interaction between DPE and transfer group (Table 2). Concentrations of progesterone on Day 7 were not affected ($P > 0.10$) by heifer age, BCS, embryo stage of development, or embryo quality grade.

On Day 19, as expected, heifers that were pregnant at initial pregnancy determination had greater ($P < 0.001$) concentrations of progesterone, independent of treatment group, when compared to non-pregnant animals (5.2 ± 0.32 [n = 121] compared with 2.8 ± 0.20 ng/mL [n = 103], respectively). Also as expected, heifers in the CON group had lesser ($P = 0.055$) circulating concentrations of progesterone at Day 19 compared to heifers in the CIDR group (3.6 ± 0.27 compared with 4.4 ± 0.27 ng/mL, respectively). There were interactions for treatment × initial P/ET (Table 3) and transfer group × initial P/ET (Table 3) on concentrations of progesterone on Day 19. Concentrations of progesterone on Day 19 were not affected ($P > 0.10$) by heifer age, BCS, embryo stage of development, or embryo quality grade.

Table 1
Pregnancy per embryo transfer (P/ET) percentage for each transfer group at initial pregnancy determination (approximately 40 d); Mean percentage pregnant with number pregnant out of total heifers in the transfer group in parentheses.

	Transfer group								
	1	2	3	4	5	6	7	8	9
P/ET, %	34.0[bc] (18/53)	25.5[abc] (13/51)	31.0[abc] (26/84)	28.6[abc] (12/42)	44.0[c] (11/25)	25.8[abc] (17/66)	16.7[ab] (7/42)	12.5[a] (5/40)	35.1[bc] (13/37)

[abc] Lack of a common superscript indicates a difference ($P \leq 0.05$).

Table 2
Mean concentrations of progesterone in serum at time of embryo transfer (7 d after expected estrus) between heifers in which there was transfer of an embryo on Day 6 or 7 (≤ 7) or 8 post-estrus; Least-squares means and standard errors are presented.

Group[1]	Concentrations of progesterone, ng/mL		P-value		
	Days post-estrus				
	≤ 7[2] n = 130	8 n = 33	Group	Days post-estrus	Interaction
1	4.2 ± 0.26[b]	5.8 ± 0.42[c]	0.016	0.128	0.054
3	4.3 ± 0.25[b]	4.4 ± 0.33[b]	–	–	–
6	2.7 ± 0.19[a]	3.4 ± 1.51[abc]	–	–	–

[abc] Lack of a common superscript indicates a difference ($P \le 0.05$).
[1] For heifers in embryo transfer groups 1, 3, and 6, there were blood samples collected, whereas there were no collections form heifers in the other transfer groups.
[2] Only five heifers in group 3 had a days post-estrus of 6, therefore, these were grouped with Day 7 post-estrus heifers.

Table 3
Mean concentrations of progesterone in serum of heifers at 19 d post-estrus (treatment cessation) by treatment group (CON; no treatment and CIDR; received a CIDR insert Days 7–19) or embryo transfer group and pregnancy status at initial pregnancy determination (approximately Day 40); Least-squares means and standard errors are presented.

Item	Concentrations of progesterone, ng/mL		P-value		
Treatment	Pregnant	Not pregnant	Treatment	Pregnancy status	Interaction
CON	5.2 ± 0.46[c] n = 58	2.0 ± 0.28[a] n = 154	0.064	< 0.001	0.033
CIDR	5.2 ± 0.45[c] n = 68	3.6 ± 0.29[b] n = 160	–	–	–
Transfer Group[1]			Transfer group	Pregnancy status	Interaction
1	4.1 ± 0.54[b] n = 18	1.9 ± 0.39[a] n = 35	0.001	< 0.001	0.096
3	5.5 ± 0.56[c] n = 26	4.0 ± 0.35[b] n = 59	–	–	–
6	6.0 ± 0.62[c] n = 17	2.5 ± 0.33[a] n = 51	–	–	–

[abc]Lack of a common superscript, within treatment and within embryo transfer group, indicates a statistical difference ($P \le 0.05$).
[1] For heifers in embryo transfer groups 1, 3, and 6, there was collection of blood samples, whereas for heifers in other transfer groups there were not collections.

4. Discussion

The overall P/ET rate in the present study was similar to some previously reported (28.3% (Vieira et al., 2014); 27.7% (Block et al., 2010); 26.7% (Sousa de et al., 2017)), but less than others (45% (Thompson et al., 1998; 45% (Kruip and den Daas, 1997); 36% (Pontes et al., 2010). When compared with IVP embryos fertilized using sex-sorted sperm, P/ET in the present study were less than the 40.9% that Zu et al. (2006) reported. Further, Hasler (2014) reported that heifer recipients had 10–23% greater pregnancy rates compared to cows. Thus the P/ET in the present study were somewhat less.

There were no differences in P/ET when transferred embryo developmental stage or quality grades were evaluated. This finding is inconsistent with those of Hasler (2012) where the survival rate was less for embryos in a developmental stage 7 post-transfer when compared to embryos in developmental stages 5 and 6 post-transfer (43.8 compared with 55.3% compared with 52.1%, respectively). The aim of the present study was not to evaluate mechanisms resulting in embryonic loss, however, there have been previous reports where there was a lesser survival of IVP embryos in recipients as a result of the failure of placental membrane development and lesser placental blood vessel development (Farin et al., 2006).

In the current study, there was an effect of embryo transfer group on P/ET at the time of initial pregnancy determination, where embryos transferred into recipients in March had the least P/ET percentage compared to groups where there was transfer in December, January, February, and April. This finding is not surprising because there are many factors that change over time that can contribute to pregnancy establishment, however, it would be speculation to try to pinpoint one reason. Although there are no data from the present study to support that heat stress was or was not the cause, this factor cannot be discounted as a contributing factor in results from the present study. Lussier et al. (1987) observed that an interval of 40 to 50 d, equivocal to two estrous cycles, is required for follicles to develop from the antral stage (0.13 mm) to the preovulatory size, indicating the oocyte and follicular cells in the Graafian follicle may be affected by adverse effects for an extended period of time prior to ovulation. Heat stress negatively affected oocyte quality and increased the abundance of degenerate theca and granulosal cells (Roth et al., 2001), which could affect embryonic development and quality. The conditions that the oocytes in this project were collected are unknown. The quality grades of embryos were, however, known and P/ET percentages when there was transfer of embryos of the two quality grades in the present study did not differ. This finding is inconsistent with those from previous studies where the transfer of embryos assigned a quality grade 1 resulted in 5 to 10% greater pregnancy rates compared with those assigned to a quality grade 2 (Vieira et al., 2014; Ferraz et al., 2016). In addition, BCS of heifers did not affect pregnancy outcomes in the present study. Ambrose et al. (1999), however, projected that pregnancy rates of lactating Holstein cows that were ET recipients would be projected to increase by 37% for each whole unit increase in BCS.

Concentrations of progesterone at cessation of treatment for all pregnant animals, and pregnant animals that received a CIDR insert, were similar to those described previously (Pandey et al., 2016). Previously, BCS has been positively associated with plasma concentrations of progesterone (Ambrose et al., 1999). Authors predicted that with every whole unit increase of BCS in lactating Holstein ET recipients, there would be an increase in circulating progesterone of 2.9 ng/mL (Ambrose et al., 1999). *In vitro* fertilization rates and blastocyst development (Palma et al., 2008; Mikkola and Taponen, 2017), and pregnancy rates (Tonello et al., 2005) can vary depending of the bull semen that was used for fertilization of ova. The effects of sire, however, were not evaluated in the present study because semen from each bull was evenly represented between treatment groups.

Palma et al. (2008) reported that IVP embryos fertilized with sex-sorted sperm have ultrastructural differences compared to those fertilized with non-sexed sperm. Even though there were these differences in this previous study, Rasmussen et al., (2012) did not detect differences in pregnancy rates between lactating cow recipients into which there was transfer of IVP embryos derived from use of sex-sorted sperm compared to IVP embryos derived from fertilization with non-sorted sperm cells (d 32: 28 compared with 24%; d 60: 18 compared with 21%, respectively). There are often differences in pregnancy rates following transfer of frozen-thawed, *in vivo* derived embryos or either frozen-thawed or fresh IVP embryos. Hasler (2001) reported pregnancy rates of 56.1% and Lopatarova et al. (2010) 50.7% when there was transfer of frozen-thawed, *in vivo* derived embryos. These rates are greater than those when there is transfer of IVP embryos. Ambrose et al. (1999) reported pregnancy rates when there was transfer of fresh IVP embryos of 14.3% while with transfer of frozen-thawed embryos there was only a 4.8% pregnancy rate. Block et al. (2010) documented a pregnancy rate after transfer of fresh IVP embryos of 56.3% while with transfer of frozen-thawed IVP embryos there was only a pregnancy rate of 33.1%. Although in the current study there was only assessments with frozen-thawed IVP embryos, it may be a culmination of insults that led to the lesser than expected pregnancy rates.

Pregnancy rates per embryo transfer can be influenced by donor-recipient uterine development stage synchrony, and embryo developmental stage and embryo quality grade (Hasler, 2001), but there were no such effects in the present study. Heifer P/ET percentages were unaffected by DPE of the recipient at ET, however, it has been reported previously that animals that are only 6 DPE at the time of embryo transfer are less likely to become pregnant than those at 7 and 8 DPE (38.4 compared with 44.3 and 43%, respectively; Ferraz et al., 2016).

In conclusion, administration of supplemental progesterone with use of a CIDR insert for 12 days beginning at the time of transfer of IVP frozen-thawed embryos resulted in greater circulating concentrations of progesterone in treated heifers which were later detected to be non-pregnant. Perhaps more importantly, however, treatment had no effect on P/ET percentage of dairy heifer recipients. The use of IVP embryos clearly allows for advantages in genetic improvements, however, further research to improve pregnancy outcome is necessary to increase the applicability of this assisted reproductive technology in beef and dairy production enterprises.

Author contributions

M.M.S. and J.E.L. participated in the conceptualization of the study, analysis of data, and writing of the manuscript. M.M.S. participated in data collection. All authors reviewed the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality in conducting the experiment and publishing the manuscript.

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References

Ambrose, J.D., Drost, M., Monson, R.L., Rutledge, J.J., Leibfried-Rutledge, M.L., Thatcher, M.J., Kassa, T., Binelli, M., Hansen, P.J., Chenoweth, P.J., Thatcher, W.W., 1999. Efficacy of timed embryo transfer with fresh and frozen in vitro produced embryos to increase pregnancy rates in heat-stressed dairy cattle. J. Dairy Sci. 82, 2369–2376.

Block, J., Bonilla, L., Hansen, P.J., 2010. Efficacy of in vitro embryo transfer in lactating dairy cows using fresh or vitrified embryos produced in a novel embryo culture medium. J. Dairy Sci. 93, 5234–5242.
Blondin, P., 2017. Logistics of large scale commercial IVP embryo production. Reprod. Fert. Dev. 29, 32–36.
Edmonsen, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72 (1), 68–78.
Farin, P.W., Piedrahita, J.A., Farin, C.E., 2006. Errors in development of fetuses and placentas from in vitro-produced embryos. Theriogenology 65 (1), 178–191.
Ferraz, P.A., Burnley, C., Karanja, J., Viera-Neto, A., Santos, J.E., Chebel, R.C., Galvão, K.N., 2016. Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States. Theriogenology 86 (7), 1834–1841.
Forde, N., Carter, F., Fair, T., Crowe, M.A., 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. Biol. Reprod. 81 (4), 784–794.
Garrett, J.E., Geisert, R.D., Zavy, M.T., Morgan, G.L., 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fert. 84, 437–446.
Graham, J.D., Clarke, C.L., 1997. Physiological actions of progesterone in target tissues. Endocr. Rev. 18 (4), 502–519.
Hasler, J.F., 2001. Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. Theriogenology 56 (9), 1401–1415.
Hasler, J.F., 2012. Effects of embryo stage on pregnancy rate following direct transfer of bovine embryos frozen in ethylene glycol. Reprod. Fertil. Dev. 24, 131 Abstr.
Hasler, J.F., 2014. Forty years of embryo transfer in cattle: A review focusing on the journal of Theriogenology, the growth of the industry in North America, and personal reminisces. Theriogenology 81 (1), 152–169.
Hasler, J.F., Henderson, W.B., Hurtgen, P.J., Jin, Z.Q., McCauley, A.D., Mower, S.A., Neely, B., Shuey, L.S., Stokes, J.E., Trimmer, S.A., 1995. Production, freezing, and transfer of bovine IVF embryos and subsequent calving results. Theriogenology 43 (1), 141–152.
Kerbler, T.L., Buhr, M.M., Jordan, L.T., Leslie, K.E., Walton, J.S., 1997. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. Theriogenology 47 (3), 703–714.
Kruip, T.A.M., den Daas, J.H.G., 1997. In vitro produced and cloned embryos: effects on pregnancy, parturition, and offspring. Theriogenology 47 (1), 43–53.
Lamb, G.C., Stevenson, J.S., Kesler, D.J., Garverick, H.A., Brown, D.R., Salfen, B.E., 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79 (1), 2253–2259.
Lonergan, P., 2011. Influence of progesterone on oocyte quality and embryo development in the cow. Theriogenology 76 (9), 1594–1601.
Lopatarova, M., Cech, S., Krontorad, P., Holy, L., Lalova, H., Dolezel, R., 2010. Conception rate after sex determination and cryopreservation of D7 bovine embryos. Vet. Med. 55, 10–18.
Lussier, J.G., Matton, P., Dufour, J.J., 1987. Growth rates of follicles in the ovary of the cow. Reproduction 81, 301–307.
Mann, G.E., Lamming, G.E., 1999. The influence of progesterone during early pregnancy in cattle. Reprod. Domest. Anim. 34 (3-4), 269–274.
Mann, G.E., Lamming, G.E., 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 121, 175–180.
Mikkola, M., Taponen, J., 2017. Quality and developmental rate of embryos produced with sex-sorted and conventional semen from superovulated dairy cattle. Theriogenology 87, 135–140.
Monteiro, P.L.J., Nascimento, A.B., Pontes, G.C.S., Fernandes, G.O., Melo, L.F., Wiltbank, M.C., Sartori, R., 2015. Progesterone supplementation after ovulation: effects on corpus luteum function and on fertility of dairy cows subjected to AI or ET. Theriogenology 84 (7), 1215–1224.
O'Hara, L., Forde, N., Kelly, A.K., Lonergan, P., 2014. Effect of bovine blastocyst size at embryo transfer on day 7 on conceptus length on day 14: Can supplementary progesterone rescue small embryos? Theriogenology 81 (8), 1123–1128.
Palma, G.A., Olivier, N.S., Neumueller, C.H., Sinowatz, F., 2008. Effects of sex-sorted spermatozoa on the efficiency of in vitro fertilization and ultrastructure of in vitro produced bovine blastocysts. Anat. Histol. Embryol. 37 (1), 67–73.
Pandey, N.K.J., Gupta, H.P., Prasad, S., Sheetal, S.K., 2016. Plasma progesterone profile and conception rate following exogenous supplementation of gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone releasing intra-vaginal device in repeat-breeder crossbred cows. Vet. World 9 (6), 559–562.
Pontes, J.H.F., Silva, K.C.F., Basso, A.C., Rigo, A.G., Ferreira, C.R., Santos, G.M.G., Sanches, B.V., Porcionato, J.P.F., Vieira, P.H.S., Faifer, F.S., Sterza, F.A.M., Schenk, J.L., Seneda, M.M., 2010. Large-scale in vitro embryo production and pregnancy rates from Bos taurus, Bos indicus, and indicus-taurus dairy cows using sexed sperm. Theriogenology 74 (8), 1349–1355.
Pugliesi, G., Oliveria, M.L., Scolari, S.C., Lopes, E., Pinaffi, F.V., Miagawa, B.T., Paiva, Y.N., Maio, J.R., Noqueira, G.P., Binelli, M., 2013. Corpus luteum development and function after supplementation of long-acting progesterone during the early luteal phase in beef cattle. Reprod. Domest. Anim. 49 (1), 85–91.
Roth, Z., Arav, A., Bor, A., Zeron, Y., Braw-Tel, R., Wolfenson, D., 2001. Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction 122, 737–744.
Sousa de, R.V., da Silva Cardoso, C.R., Butzke, G., Dode, M.A.N., Rumpf, R., Franco, M.M., 2017. Biopsy of bovine embryos produced in vivo and in vitro does not affect pregnancy rates. Theriogenology 90, 25–31.
Sreenan, J.M., Diskin, M.G., 1983. Early embryonic mortality in the cow: Its relationship with progesterone concentrations. Vet. Rec. 112 (22), 517–521.
Stringfellow, D.A., Givens, M.D., 2010. Manuel of the International Embryo Transfer Society (IETS), 4th ed. IETS, Champaign, IL.
Thompson, J.G., Allen, N.W., McGowan, L.T., Bell, A.C.S., Lambert, M.G., Tervit, H.R., 1998. Effect of delayed supplementation of fetal calf serum to culture medium on bovine embryo development in vitro and following transfer. Theriogenology 49 (6), 1239–1249.
Tonello, T.T.M., Accorsi, M.F., Ferraz, M.L., Watanabe, M.R., Meirelles, F.D.P., Meirelles, F.V., Watanabe, Y.F., 2005. Produçao in vitro de embriões bovinos a partir de sêmen sexado. Proceedings of the XIX Meeting of the Brazilian Society of Embryo Technology. 26–29 August. Acta Sci. Vet. 33 (Suppl. 1), 350.
Ulbrich, S.E., Zitta, K., Hiendleder, S., Wolf, E., 2010. In vitro systems for intercepting early embryo-maternal cross-talk in the bovine oviduct. Theriogenology 73 (6), 802–816.
VanCleeff, J., Macmillan, K.I., Drost, M., Lucy, M.C., Thatcher, W.W., 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of return to service. Theriogenology 46 (7), 1117–1130.
Vieira, I.M., Rodrigues, C.A., Mendanha, M.F., Sa Filho, M.F., Sales, J.N.S., Souza, A.H., Santos, J.E.P., Baruselli, P.S., 2014. Donor category and seasonal climate associated with embryo production and survival in multiple ovulation and embryo transfer programs in Holstein cattle. Theriogenology 82 (2), 204–212.
Wallace, L.D., Breiner, C.A., Spell, A.R., Carter, J.A., Lamb, G.C., Stevenson, J.S., 2011. Administration of human chorionic gonadotropin at embryo transfer induced ovulation of a first wave dominant follicle, and increased progesterone and transfer pregnancy rates. Theriogenology 75 (8), 1506–1515.
Wildman, E.E., Jones, G.M., Wagner, P.E., Bowman, R.L., Troutt, H.F., Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65 (3), 495–501.