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Comparison of artificial insemination versus embryo transfer in lactating dairy cows

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Abstract

Conception rates (CR) are low in dairy cows and previous research suggests that this could be due to impaired early embryonic development. Therefore, we hypothesized that CR could be improved by embryo transfer (ET) compared with AI. During 365 days, 550 potential breedings were used from 243 lactating Holstein cows (average milk production, 35 kg/day). Cows had their ovulation synchronized (GnRH-7d-PGF$_{2\alpha}$-3d-GnRH) and they were randomly assigned for AI immediately after the second GnRH injection (Day 0) or for transfer of one embryo 7 days later. Circulating progesterone concentrations and follicular and luteal size were determined on Days 0 and 7. Pregnancy diagnosis was performed on Days 25 or 32 and pregnant cows were reevaluated on Days 60–66. Single-ovulating cows with synchronized ovarian status had similar CR on Days 25–32 with ET ($n = 176$; 40.3%) and AI ($n = 160$; 35.6%). Pregnancy loss between Days 25–32 and 60–66 also did not differ ($P = 0.38$) between ET (26.2%) and AI (18.6%). When single ($n = 334$) and multiple ($n = 57$) ovulators were compared, independent of treatment, multiple ovulators had greater ($P < 0.001$) circulating progesterone concentrations on Day 7 (2.7 ng/ml versus 1.9 ng/ml) and there was a tendency ($P = 0.10$) for a greater CR in multiple ovulators (50.9% versus 38.1%). However, there was no difference in CR between AI and ET cows with multiple ovulations (50.0% versus 51.7%). In single-ovulating cows, CR tended to be lower for AI than ET in cows ovulating smaller follicles (diameter ≤ 15 mm; 23.7% versus 42.3%; $P = 0.06$) but not average-diameter follicles (16–19 mm; 41.2% versus 37.3%; $P = 0.81$) or larger (≥ 20 mm; 34.3 versus 51.0%; $P = 0.36$) follicles.

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Thus, although ET did not improve overall CR in lactating cows, follicle diameter and number of ovulating follicles may determine success with these procedures.

Keywords: Embryo transfer; Dairy cattle; Pregnancy; Artificial insemination; Synchronization

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1. Introduction

Several reports have described an association between high milk production and low reproductive efficiency in dairy cows [1–4]. At least three studies [5–7] have shown that lactating dairy cows have poor early embryonic development. Sartori et al. [6] reported a very high percentage of non-viable embryos (~70% during summer and ~50% during winter) by Day 6 after estrus, as compared to heifers during summer (~30% non-viable embryos) and non-lactating cows during winter (~20% non-viable embryos). Moreover, although fertilization of the oocyte in lactating cows appeared to be decreased by summer heat stress, fertilization rate for lactating cows during winter was very high (~90%). Therefore, low conception rates (CR) in lactating dairy cows appear to be at least partly due to compromised early embryonic development, which can be augmented by fertilization failure and more profoundly impaired early embryonic development during heat stress.

A number of studies from Florida compared embryo transfer (ET) to AI in order to improve CR of lactating dairy cows during summer heat stress [8–11]. In all these studies, CR was greater for ET than AI when fresh or frozen in vivo produced embryos were transferred, or when fresh in vitro produced embryos were used. To the best of our knowledge, there is no published study in which ET was compared to AI in dairy cows during cooler times of the year. Therefore, it is not known whether transfer of an embryo would improve CR and reduce pregnancy loss in lactating dairy cows during non-heat stress times of the year. The present experiment tested the hypothesis that CR can be improved by ET compared with AI, not only during summer (as demonstrated by other studies), but also during other seasons of the year. In addition, we hypothesized that the high pregnancy loss (Day 25 and later) after AI of lactating dairy cows could be overcome by ET, consistent with the idea that events in the oocyte or during early embryonic development are responsible problems in pregnancy.

2. Materials and methods

During a 1-year interval, (December 2001–December 2002), 550 potential breedings from 243 lactating Holstein cows were used. Every week, cows that were ≥60 days postpartum, or cows detected non-pregnant after breeding were assigned to the experiment. At the time of assignment, cows averaged 142.1 ± 3.3 days postpartum, yielding 34.9 ± 0.3 kg of milk/day, with an average lactation number of 2.4 ± 0.1 and had been bred 1.7 ± 0.1 times. Cows were housed in stanchion barns or free-stalls at the University of Wisconsin–Madison, Madison, WI, USA. They were milked twice daily and fed a TMR that consisted of corn silage and alfalfa silage as forage supplemented with concentrates of corn and soybean meal. The TMR was balanced to meet or exceed minimum nutritional requirements for lactating dairy cows. Daily milk yield for each cow was recorded on Dairy Comp 305 (Valley Agricultural Software, Tulare, CA, USA).

Cows had ovulation synchronized with a modified Ovsynch protocol [12,13] consisting of i.m. injections of 50 μg of Gonadorelin (Fertagyl; Intervet Inc., Millsboro, DE, USA) on Days −10 (GnRH1) and 0 (GnRH2), and a single i.m. injection of 25 mg of $PGF_{2\alpha}$ (Lutalyse; Pharmacia Animal Health, Peapack, NJ, USA) on Day −3. At the time of GnRH2, the ovaries were evaluated by transrectal ultrasonography using an Aloka 500-V equipped with a 7.5 MHz linear-array transducer (Corometrics Medical Systems Inc., Wallingford, CT, USA). Cows with at least one follicle $\geq$10 mm at the time of GnRH2 and with a regressing or no visible CL (495 out of 550), were selected and randomly assigned to the AI or ET treatments. The AI cows were inseminated with commercial frozen-thawed semen immediately after the GnRH2 injection and the ET cows received one embryo 7 days later into the uterine horn ipsilateral to the CL. When cows had a CL on both ovaries, a coin was tossed to determine the side of ET (heads and tails were assigned to left and right horns, respectively). The embryos transferred in the study (21.5% fresh and 78.5% frozen, 89.2% grade 1 and 10.8% grade 2 based on the IETS grading system) were collected from superovulated dairy cattle (heifers and cows) during all seasons except summer. All AI and ET were performed by one of two technicians (R.S. or J.N.G.). On Day 7, ovaries of cows from both groups were checked by ultrasonography for the presence and size of a CL. Cows that had been assigned for ET and did not have CL on Day 7 did not receive an embryo. Cows from both groups that did not have CL on Day 7 were considered not pregnant and reassigned to the experiment. For analysis of CL data, luteal tissue volume was estimated using the methodology described by Sartori et al. [14].

Blood samples were collected by coccygeal venipuncture on Days 0 and 7, and serum samples were stored at −20 °C until assayed for progesterone concentrations. Circulating progesterone was evaluated from unextracted sera using an antibody-coated-tube RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and inter-assay CVs were 6.2% and 7.9%, respectively.

Pregnancy diagnosis was performed by ultrasonography on Day 25. For cows with inconclusive results regarding pregnancy status (25% of the cows), another evaluation was performed 7 days later (Day 32). Pregnant cows were re-evaluated on Days 60–66 and 100–120, and calving data were recorded. Cows detected not pregnant at the ultrasound evaluation were resynchronized and randomly reassigned to the treatments.

For statistical analyses, the binomial distribution (procedure GENMOD of SAS) was assumed for the proportional variables, e.g. CR, synchronization rate and embryonic loss. The continuous variables, e.g. milk yield and serum progesterone concentration, were evaluated using a mixed effects model (procedure MIXED of SAS [15]) with cow treated as a random effect. Follicle size versus probability of conception was analyzed (macro GLIMMIX of SAS) with the binomial distribution assumed for the response variable "conception" ($y = 0$ for no conception and $y = 1$ for conception). One-way and two-way interactions with expected effects were included in the model. Using the estimates for the fixed effects obtained in the logistic regression analysis, the probability of conception was calculated for each follicular size in cows receiving AI or ET using the formula $P = 1/1 + \mathrm{e}^{-(b_0+b_1X_1+b_2X_2+\cdots+b_iX_i)}$.

3. Results

Out of 550 potential breedings, 55 cows (10.0%) were not selected for AI or ET because at the time of GnRH2, ovarian status had not been synchronized, as indicated by lack of a follicle(s) ≥10 mm and/or presence of a mature CL at this time. Overall, 27.5% (151/550) of the cows did not synchronize to the modified Ovsynch protocol. Cows with circulating progesterone concentrations ≥0.5 ng/ml on Day 0 ($n = 66$; 12.0%), <0.5 ng/ml on Day 7 ($n = 9$; 1.6%) and/or without a responsive follicle to GnRH on Day 0 ($n = 81$; 14.7%), were considered not synchronized. We used a circulating progesterone concentration of 0.5 ng/ml as the synchronization threshold, based on our results that 0.5 ng/ml was the lowest Day 7 progesterone concentration that was found to maintain a pregnancy in our study and because 12 cows in the present study with circulating progesterone concentrations ≥0.5 ng/ml and <1.0 ng/ml on Day 7 were diagnosed pregnant on Days 25–32. Cows that did not respond to GnRH either had follicles <10 mm on Day 0 ($n = 31$), or did not ovulate the follicle ≥10 mm in response to the GnRH2 injection ($n = 50$). Five of the cows with follicles <10 mm on Day 0, also had circulating progesterone concentrations ≥0.5 ng/ml on Day 0. Twenty cows that had been assigned for ET, did not receive an embryo because they did not have a CL on Day 7.

Synchronization rate and CR of cows that were selected on Day 0 based on ultrasonography was not different between AI and ET treatments (Table 1). Moreover, cows with synchronized ovarian status that received AI or ET had similar CR on Days 25–32, 60–66, 100–120 and similar calving rate (Table 1).

Pregnancy loss was similar ($P > 0.10$) for AI and ET cows between Days 25–32 and 60–66 (18.6% [13/70] and 26.2% [22/84] for AI and ET cows, respectively), Days 60–66 to

Table 1
Synchronized ovulation rate and conception rate in lactating dairy cows that received AI or ET

	AI	ET	*P*-value
Synchronized ovulation rate of cows that were selected on Day 0 (%; no./no.)	79.4 (193/243)	81.7 (206/252)	0.49
Conception rate on Days 25–32 of cows that were selected on Day 0 (%; no./no.)	31.1 (74/238)	34.4 (86/250)	0.52
Conception rate on Days 25–32 of cows with synchronized ovulation (%; no./no.)	38.1 (72/189)	42.0 (86/205)	0.45
Conception rate on Days 60–66 of cows with synchronized ovulation (%; no./no.)	31.2 (58/186)	30.7 (62/202)	0.98
Conception rate on Days 100–120 of cows with synchronized ovulation (%; no./no.)	30.9 (58/187)	29.9 (61/204)	0.83
Cows with synchronized ovulation that had live calves (%; no./no.)	25.5 (47/184)	27.6 (56/203)	0.76

Experimental units may not match between lines due to missing cows or missing samples at the time of evaluations.

Table 2
Comparison between cows with single vs. multiple ovulations for serum concentrations of progesterone on Day 7, conception rate at pregnancy diagnosis on Days 25–32 or 60–66 and pregnancy loss for cows with synchronized ovulation

	Single	Multiple	*P*-value
Serum progesterone concentration on Day 7 for cows with synchronized ovulation (ng/ml)	1.88 ± 0.04 (*n* = 331)	2.67 ± 0.17 (*n* = 56)	<0.001
Conception rate on Days 25–32 (%)			
All cows	38.1 (128/336)	50.9 (29/57)	0.10
AI cows	35.6 (57/160)	50.0 (14/28)	0.18
ET cows	40.3 (71/176)	51.7 (15/29)	0.22
Conception rate on Days 60–66 (%)			
All cows	30.5 (101/331)	33.9 (19/56)	0.75
AI cows	29.3 (46/157)	42.9 (12/28)	0.16
ET cows	31.6 (55/174)	25.0 (7/28)	0.43
Pregnancy loss from Days 25–32 to Days 60–66 (%; no./no.)			
All cows	17.9 (22/123)	32.1 (9/28)	0.02
AI cows	14.8 (8/54)	14.3 (2/14)	0.91
ET cows	20.3 (14/69)	50.0 (7/14)	0.01

Experimental units may not match between lines due to missing cows or missing samples at the time of evaluations.

100–120 (0.0% [0/57] and 1.6% [1/62]) and Days 100–120 to calving (13.0% [7/54] and 6.7% [4/60]). Regardless of treatments, pregnancy loss was greater ($P < 0.05$) from Days 25–32 to 60–66, than from Days 60–66 to 100–120.

Cows that ovulated one follicle were compared to cows ovulating two or more follicles (Table 2). Multiple ovulating cows had higher circulating progesterone concentrations on Day 7, and a tendency for a greater CR on Days 25–32 than single ovulators (Table 2). However, CR was not different between single and multiple ovulating cows on Days 60–66. Furthermore, there was no difference in CR between AI and ET cows with single or multiple ovulations on Days 25–32 and 60–66 (Table 2). Multiple ovulators had a greater chance of losing the pregnancy between Days 25–32 and 60–66 after ovulation, due to increased losses in multiple-ovulating ET cows (Table 2).

Cows that ovulated a single follicle were analyzed for the effect of follicular size on probability of conception (Fig. 1). Cows that received AI had an increasing probability of conception as follicle size increased from 12 mm (19%) to 19 mm (33%), with no subsequent change in probability of conception for cows ovulating follicles from 19 mm to 22 mm in diameter (Fig. 1). In contrast, cows that received ET had little change in probability of conception between 12 mm (33%) and 18 mm (32%) but subsequently had an increasing probability of conception as follicle diameter increased (up to 22 mm; 40%). We did not have sufficient numbers of cows ovulating follicles <12 mm ($n = 4$) or >22 mm ($n = 8$) to provide meaningful analyses. When data were analyzed by follicle size classes (Table 3), there was a lower CR for AI than ET in cows that ovulated a smaller follicle (≤15 mm) but not average (16–19 mm) or larger (≥20 mm) follicles using data from the

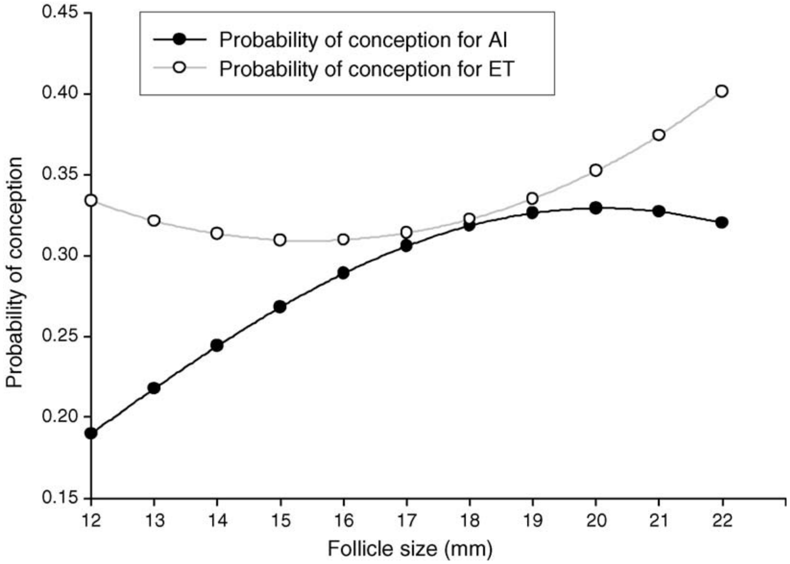

Fig. 1. Effect of size of the ovulatory follicle on estimated probability of conception (using data from pregnancy diagnosis on Days 60–66 in macro GLIMMIX of SAS) in lactating dairy cows ovulating a single follicle that received AI or ET.

pregnancy diagnosis on either Days 25–32 or 60–66 (Table 3). In addition, CR tended ($P < 0.10$) to be lower for cows ovulating smaller follicles versus average or larger ovulatory-sized follicles in AI but not ET cows on Days 60–66 (Table 3). Serum progesterone concentration of synchronized cows was lower in cows ovulating smaller but not average or larger diameter follicles on Day 7 in both AI and ET cows (Table 4).

There was no difference among seasons for synchronization rate or CR on Days 25–32 or 60–66 in all cows or synchronized cows that received AI or ET (Table 5).

Table 3
Conception rate of cows with synchronized ovulation of a single follicle that received AI or ET compared to size of the single-ovulatory follicle at the time of GnRH2

	AI	ET	*P*-value
Days 25–32 pregnancy diagnosis			
Smaller-size ovulatory follicles (10–15 mm)	23.7 (9/38)	42.3 (11/26)	0.05
Medium-size ovulatory follicles (16–19 mm)	41.2 (21/51)	37.3 (19/51)	0.81
Larger-size ovulatory follicles (20–30 mm)	34.3 (12/35)	51.0 (25/49)	0.38
Days 60–66 pregnancy diagnosis			
Smaller-size ovulatory follicles (10–15 mm)	18.4^{A} (7/38)	38.5 (10/26)	0.05
Medium-size ovulatory follicles (16–19 mm)	35.3^{B} (18/51)	27.4 (14/51)	0.43
Larger-size ovulatory follicles (20–30 mm)	28.6^{B} (10/35)	40.8 (20/49)	0.75

Different superscript letters (A and B) within each column; $P < 0.10$.

Table 4
Serum progesterone concentration on Day 7 for AI and ET cows with synchronized ovulation of a single follicle compared to size of the ovulatory follicle

	AI cows	ET cows	All cows
Smaller-size ovulatory follicles (10–15 mm)	1.70 ± 0.11^a ($n = 38$)	1.43 ± 0.09^a ($n = 26$)	1.59 ± 0.08^a ($n = 64$)
Medium-size ovulatory follicles (16–19 mm)	2.03 ± 0.11^b ($n = 51$)	1.97 ± 0.11^b ($n = 51$)	2.00 ± 0.08^b ($n = 102$)
Larger-size ovulatory follicles (20–30 mm)	2.12 ± 0.13^b ($n = 35$)	2.14 ± 0.15^b ($n = 49$)	2.13 ± 0.10^b ($n = 84$)

Different superscript letters (a and b) within each column; $P < 0.05$.

Table 5
Comparison among seasons for synchronized ovulation rate and conception rate in lactating dairy cows that received AI or ET

	Winter	Spring	Summer	Fall	*P*-value
Synchronized ovulation rate of all cows (%; no./no.)	75.2 (112/149)	77.9 (120/154)	67.9 (76/112)	67.4 (91/135)	0.25
Conception rate of all cows on Days 25–32 (%; no./no.)	33.3 (49/147)	30.7 (47/153)	24.5 (27/110)	28.6 (38/133)	0.71
Conception rate (%; Days 25–32) of cows with synchronized ovulation					
All cows	42.0 (47/112)	39.5 (47/119)	36.5 (27/74)	41.6 (37/89)	0.83
AI cows	43.1 (22/51)	34.5 (20/58)	35.9 (14/39)	39.0 (16/41)	0.63
ET cows	41.0 (25/61)	44.3 (27/61)	37.1 (13/35)	43.8 (21/48)	0.71
Conception rate of all cows on Days 60–66 (%; no./no.)	28.3 (41/145)	23.8 (36/151)	20.0 (22/110)	17.6 (23/131)	0.37
Conception rate (%; Days 60–66) of cows with synchronized ovulation					
All cows	35.5 (39/110)	30.8 (36/117)	29.7 (22/74)	26.4 (23/87)	0.56
AI cows	38.0 (19/50)	26.3 (15/57)	30.8 (12/39)	30.0 (12/40)	0.38
ET cows	33.3 (20/60)	35.0 (21/60)	28.6 (10/35)	23.4 (11/47)	0.12

Experimental units may not match across lines due to missing cows or missing samples at the time of the evaluations.

4. Discussion

The results of the present study have shown that overall, in comparison to fixed-time AI, ET failed to enhance conception or calving rates in lactating dairy cows, even during the warmer times of the year. These findings contrasted with data from previous studies performed in Florida [8–11]. Those studies have reported improvements between 83% and 153% in CR for heat-stressed cows receiving Day 7 embryos in comparison to heat-stressed cows that received AI during a natural estrus or synchronized ovulation

(Ovsynch). Whereas, CR following AI in Florida ranged from 5% to 20% during the hot summer months [11], CR during summer in our study was approximately 30% for synchronized cows. One contrasting difference between our study in Wisconsin and the Florida studies is that our cows were rarely exposed to ambient temperatures above 32 °C during the critical days of the experiment in the summer of 2002. Environmental temperatures below 30 °C seemed to have little impact on CR in lactating cows [16].

Other possible reasons for the lack of improvement in CR in cows that received ET instead of AI may be related to the fact that lactating dairy cows have other reproductive problems that are not overcome by ET, such as clinical or subclinical uterine infections, stress, negative energy balance, infectious diseases not restricted to the uterus, hormonal imbalances and nutritional deficiencies (reviewed by Sartori [17]). Moreover, sire could be a confounding factor in this study, since we did not necessarily use the same sires for the embryo donors and the AI-treated cows. There does appear to be a sire effect on pregnancy loss [18]. It is also important to consider that the transferred embryos in this study were generated in superovulated animals. A combination of the hormonal, chemical and mechanical manipulations that the embryo donors or the embryos receive during embryo production, recovery and transfer may have compromised embryo quality. Some studies have questioned the quality of oocytes/embryos from cows undergoing superovulation [19–21]. For example, treatment of cows with eCG or FSH inhibited the normal vacuolization process of the nucleoli of oocytes [21]. Moreover, studies have suggested that a higher superovulatory response increased the likelihood of production of inferior-quality embryos, although embryos may look normal upon recovery [19]. These problems with embryo quality may be more apparent in lactating dairy cows than when embryos are transferred to other, higher fertility recipients.

The diameter of the ovulatory follicle in single-ovulating cows also appeared to be related to CR. Ovulation of smaller follicles appeared to lower CR in AI but not ET cows, possibly due to reduced circulating progesterone concentrations during early embryonic development (before Day 7). There is a positive correlation between the size of the ovulatory follicle and luteal tissue volume, as well as between size of the ovulatory follicle and serum progesterone concentration on Days 6 or 7 [14]. In some studies, pregnant cows had higher circulating progesterone concentrations by Days 4–7 than inseminated, non-pregnant cows [22–24]. Similar to our observation of reduced CR in AI cows ovulating smaller follicles, intentional ovulation of smaller follicles (by strategic use of follicular aspiration during Ovsynch [25]) reduced subsequent CL volume, circulating progesterone and CR following timed AI. In beef cattle, ovulation of smaller follicles (<12 mm) during Ovsynch and timed AI also reduced CR [26]. In addition to differences in progesterone concentrations, cows ovulating smaller follicles would also be expected to have reduced circulating estradiol concentrations that could compromise sperm and/or oocyte transport, fertilization and uterine environment for early embryonic development. Other investigators have previously indicated that lower concentrations of estradiol before ovulation may contribute to poor fertilization and early embryonic development [27,28]. It is well known that estradiol treatment increases uterine contractions, ciliary beats and fluid movement [29] as well as sperm transport in ewes [30]. Moreover, alterations in serum estradiol concentrations may alter expression of estrogen-dependent secretory proteins that are present in the oviduct at the time of fertilization and embryo development [28,31]. In addition to possible effects of hormonal differences, decreased CR could be due to reduced fertility of oocytes originating from smaller follicles (reviewed by Merton et al. [32]).

In addition, there was a tendency for greater CR on Days 25–32 in multiple versus single ovulating cows receiving either AI or ET. Cows ovulating multiple follicles had higher circulating progesterone concentrations on Day 7 and would have been expected to have higher circulating estradiol concentrations during Ovsynch. As mentioned above, these hormonal changes could have positive effects on fertilization, early embryonic development, or even later conceptus development [33,34]. In addition, cows receiving AI would have the possibility for fertilization of two oocytes, thereby increasing the probability of pregnancy. The tendency for increased pregnancies at Days 25–32 in ET cows also suggests other potential positive effects of multiple ovulation on early embryo development; although, this effect seemed to be lost after Days 25–32 (discussed below).

Although relatively high, pregnancy loss did not differ between cows receiving AI or ET and was greater during the late embryonic/early fetal period than during later periods. That pregnancy losses ranged from 18% to 26% between Days 25–32 and 60–66 for cows receiving AI or ET seemed greater than the data reported in the literature for beef cows and heifers that received AI or ET (reviewed by Sartori [17]). However, recent studies with high-producing lactating cows have shown similar high pregnancy loss after AI [35–37] or ET [38]. The high pregnancy loss in ET cows was not consistent with our second hypothesis that ET could reduce the high pregnancy loss in lactating dairy cows. This unexpected result argues against the role of the oocyte or early embryonic development (before Day 7) and was more consistent with problems of the reproductive tract or circulating hormone concentrations causing the high pregnancy loss in dairy cows. Nevertheless, this high pregnancy loss in ET cows could still have been the result of the use of primarily frozen embryos from superovulated animals.

One interesting finding was the greater pregnancy loss in ET cows experiencing multiple ovulations as compared to cows with single ovulation. This difference was not observed, however, for AI cows. A recent study [39] reported that cows with multiple CL lost pregnancy more often than cows with one CL after AI (27% versus 9%, respectively). The contrasting results between that study and our study for AI cows may be due to no difference in serum progesterone concentrations between single- and multiple-ovulating cows in their study, whereas in our study multiple-ovulators had higher circulating progesterone. Nevertheless, although differences between pregnancy loss for single- versus multiple-ovulating ET cows were significant ($P = 0.01$), it is reasonable to think that the low numbers of pregnant ET cows with multiple ovulations ($n = 14$) make random stochastic variation between groups another possible explanation for these unexpected results.

In conclusion, ET did not improve CR in lactating dairy cows in comparison to fixed-time AI. Possible reasons include: (a) environmental conditions during the year in Wisconsin were not harsh enough to decrease AI success; (b) lactating dairy cows have other reproductive dysfunctions other than possible oocyte and early embryonic development problems and these problems are not overcome by ET; (c) superovulated animals (embryo donors) may not yield optimal quality embryos. Moreover, it may be possible to improve the success of AI or ET by developing methods to optimize the size and number of ovulating follicles during the synchronization procedures.

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References

[1] Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci 2001;84:1277–93 (ADSA Foundation Scholar Award).

[2] Royal M, Mann GE, Flint APF. Strategies for reversing the trend towards subfertility in dairy cattle. Vet J 2000;160:53–60.

[3] Royal MD, Darwash AO, Flint APE. Webb R, Woolliams JA, Lamming GE. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000;70:487–501.

[4] Washburn SP, Silvia WJ, Brown CH, McDaniel BT, McAllister AJ. Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds. J Dairy Sci 2002;85:244–51.

[5] Ryan DP, Prichard JF, Kopel E, Godke RA. Comparing early embryo mortality in dairy-cows during hot and cool seasons of the year. Theriogenology 1993;39:719–37.

[6] Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci 2002;85:2803–12.

[7] Wiebold JL. Embryonic mortality and the uterine environment in 1st-service lactating dairy cows. J Reprod Fertil 1988;84:393–9.

[8] Al-Katanani YM, Drost M, Monson RL, Rutledge JJ, Krininger CE, Block J, et al. Pregnancy rates following timed embryo transfer with fresh or vitrified in vitro produced embryos in lactating dairy cows under heat stress conditions. Theriogenology 2002;58:171–82.

[9] Ambrose JD, Drost M, Monson RL, Rutledge JJ, Leibfried-Rutledge ML, Thatcher MJ, et al. 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 1999;82:2369–76.

[10] Putney DJ, Drost M, Thatcher WW. Influence of summer heat-stress on pregnancy rates of lactating dairy-cattle following embryo transfer or artificial insemination. Theriogenology 1989;31:765–78.

[11] Drost M, Ambrose JD, Thatcher MJ, Cantrell CK, Wolfsdorf KE, Hasler JF, et al. Conception rates after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. Theriogenology 1999;52:1161–7.

[12] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF(2-Alpha) and GnRH. Theriogenology 1995;44:915–23.

[13] DeJarnette JM, Marshall CE. Effects of pre-synchronization using combinations PGF(2 alpha) and (or) GnRH on pregnancy rates of Ovsynch- and Cosynch-treated lactating Holstein cows. Anim Reprod Sci 2003;77:51–60.

[14] Sartori R, Rosa GJM. Wiltbank MC. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci 2002;85:2813–22.

[15] Littell RC, Miliken GA, Stroup WW, Wolfinger RD. SAS system for mixed models. Cary, NC: SAS Institute Inc., 1996.

[16] Thatcher WW, Collier RJ. Effects of climate on bovine reproduction. In: Morrow DA., editor. Current therapy in theriogenology. Philadelphia: WB Saunders Co.; 1986. p. 301–9.

[17] Sartori R. Fertilization and embryonic mortality in cattle. Acta Scientiae Veterinariae 2004;32(Suppl.): 35–50.

[18] Lopez-Gatius F, Santolaria P, Yaniz J, Rutllant J, Lopez-Bejar M. Factors affecting pregnancy loss from gestation Day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 2002;57:1251–61.

[19] Armstrong DT. Recent advances in superovulation of cattle. Theriogenology 1993;39:7–24.

[20] Foote RH, Ellington JE. Is a superovulated oocyte normal? Theriogenology 1988;29:111–23.

[21] Greve T, Callesen H, Hyttel P, Hoier R, Assey R. The effects of exogenous gonadotropins on oocyte and embryo quality in cattle. Theriogenology 1995;43:41–50.

[22] Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, et al. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. J Anim Sci 1996;74:1943–52.

[23] Gumen A, Guenther JN, Wiltbank MC. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J Dairy Sci 2003;86:3184–94.

[24] Henricks DM, Lamond DR, Hill JR, Dickey JF. Plasma progesterone concentrations before mating and in early pregnancy in beef heifer. J Anim Sci 1971;33:450–4.

[25] Vasconcelos JLM. Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 2001;56:307–14.

[26] Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2 alpha for ovulation control in postpartum suckled beef cows. J Anim Sci 2001;79:2253–9.

[27] King RS, Anderson SH, Killian GJ. Effect of bovine oviductal estrus-associated protein on the ability of sperm to capacitate and fertilize oocytes. J Androl 1994;15:468–78.

[28] DeSouza MM, Murray MK. An estrogen-dependent secretory protein, which shares identity with chitinases, is expressed in a temporally and regionally specific manner in the sheep oviduct at the time of fertilization and embryo development. Endocrinology 1995;136:2485–96.

[29] Hawk HW. Sperm survival and transport in the female reproductive tract. J Dairy Sci 1983;66:2645–60.

[30] Hawk HW, Cooper BS. Improvement of sperm transport by administration of estradiol to estrous ewes. J Anim Sci 1975;41:1400–6.

[31] Binelli M, Hampton J, Buhi WC, Thatcher WW. Persistent dominant follicle alters pattern of oviductal secretory proteins from cows at estrus. Biol Reprod 1999;61:127–34.

[32] Merton J, de Roos APW. Mullaart E, de Ruigh L, Kaal L, PLAM Vos, et al. Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology 2003;59:651–74.

[33] Mann GE, Lamming GE, Robinson RS, Wathes DC. The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. J Reprod Fertil 1999;(Suppl. 54):317–28.

[34] Kerbler TL, Buhr MM, Jordan LT, Leslie KE, Walton JS. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. Theriogenology 1997;47:703–14.

[35] Chebel RC, Santos JEP. Cerri RLA. Galvao KN, Juchem SO, Thatcher WW. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology 2003;60:1389–99.

[36] Fricke PM, Caraviello DZ, Weigel KA, Welle ML. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J Dairy Sci 2003;86:3941–50.

[37] Pursley JR, Silcox RW, Wiltbank MC. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J Dairy Sci 1998;81:2139–44.

[38] Chagas e Silva J, da Costa LL, Silva JR. Plasma progesterone profiles and factors affecting embryo-fetal mortality following embryo transfer in dairy cattle. Theriogenology 2002;58:51–9.

[39] Starbuck MJ, Dailey RA, Inskeep EK. Factors affecting retention of early pregnancy in dairy cattle. Anim Reprod Sci 2004;84:27–39.