Efficacy of Timed Embryo Transfer with Fresh and Frozen In Vitro Produced Embryos to Increase Pregnancy Rates in Heat-Stressed Dairy Cattle

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ABSTRACT

Our objective was to determine whether pregnancy rates in heat-stressed dairy cattle could be enhanced by timed embryo transfer of fresh (nonfrozen) or frozen-thawed in vitro-derived embryos compared to timed insemination. Ovulation in Holstein cows was synchronized by a GnRH injection followed 7 d later by PGF$_2$α and a second treatment with GnRH 48 h later. Control cows (n = 129) were inseminated 16 h (d 0) after the second GnRH injection. On d 7, a fresh (n = 133) or frozen-thawed (n = 142) in vitro-derived embryo was transferred to cows assigned for timed embryo transfer after categorizing the corpus luteum by palpation per rectum as 3 (excellent), 2 (good or fair), 1 (poor), and 0 (nonpalpable). Response to the synchronization treatment, determined by plasma progesterone concentration (ng/ml) $\leq$ 1.5 on d 0 and $\geq$ 2.0 on d 7, was 76.2%. Mean plasma progesterone concentration on d 7 increased as the quality of corpus luteum improved from category 0 to 3. Concentrations of progesterone in plasma were elevated ($\geq$ 2.0 ng/ml) at 21 d in 64.7 (fresh embryo), 40.3 (frozen embryo), and 41.4 $\pm$ 0.1% (timed insemination) of cows, respectively. Cows that received a fresh embryo had a greater pregnancy rate at 45 to 52 d than did cows that received a frozen-thawed embryo or timed insemination (14.3 $>$ 4.8, 4.9 $\pm$ 2.3%). Body condition (d 0) of cows influenced the pregnancy rate and plasma progesterone concentrations. In summary, timed embryo transfer with fresh in vitro-produced embryos in heat-stressed dairy cattle improved pregnancy rate relative to timed insemination.

(Key words: heat stress, embryo transfer, timed insemination, pregnancy rate)

Abbreviation key: BCS = body condition score, CL = corpus luteum, ET = embryo transfer, IVF = in vitro fertilization, TET = timed embryo transfer, TI = timed insemination.

INTRODUCTION

Pregnancy rates to artificial insemination in lactating dairy cattle typically decline during hot seasons (14). In Florida, under hot and humid summer conditions, pregnancy rates can drop to less than 10% (5). Embryonic loss associated with maternal heat stress is one of the major causes for this decreased fertility. Bovine embryos are highly susceptible to heat stress during early developmental stages, specifically from d 0 to 3 after estrus (9, 29). However, later stage embryos (i.e., beyond d 3) are less susceptible to heat-stress-induced losses (9, 11). Putney et al. (30) found no seasonal variation in pregnancy rate to embryo transfer in a large study conducted in the southern United States. Therefore, transferring embryos to recipients at a stage when they are less susceptible to heat stress (i.e., on d 7) may enhance pregnancy rates during periods of heat stress. This hypothesis has been tested by comparing artificial insemination to embryo transfer (ET) with embryos collected from superovulated cows (8, 28) and in vitro-derived frozen-thawed embryos (8). It was demonstrated that pregnancy rates in summer could be increased significantly by transfer of in vivo-derived, frozen-thawed embryos. However, the pregnancy rate from in vitro-derived, frozen-thawed embryos was not different from that following artificial insemination.

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In vivo embryo production is expensive and labor intensive, and the results are often unpredictable because superovulatory responses vary tremendously among donor cows. On the other hand, production of embryos following in vitro fertilization (IVF) is less expensive and less laborious and allows for the production of a large number of embryos within a relatively short time with minimal semen costs. Therefore, transfer of IVF-derived embryos has practical advantages, particularly in large commercial dairy operations such as those prevalent in Florida.

Establishment of pregnancy following embryo transfer, among other factors, depends on the availability of well-synchronized recipients. Since efficiency of estrus detection is poor during summer months (1, 40, 43), selection of recipients becomes a difficult task, making embryo transfer less practical during summer. Synchronization of recipients using the protocol (GnRH, d 0 – PGF2α, d 7 – GnRH, d 9) developed for timed insemination (26, 38) eliminates the need for estrus detection. Efficiency of the timed insemination protocol has been demonstrated under Florida conditions (3, 4, 7, 18, 38) and may therefore be a dependable system for the efficient management of recipients for embryo transfer. In the present study, we used this approach to examine systems of reproductive management of dairy cattle potentially relevant to hot climates or seasons. Primary components of the systems were in vitro production of embryos, both fresh and frozen; their shipment to herd sites remote from the laboratory of production; synchronization of recipients without estrus detection; and the application of timed embryo transfer. As pointed out by Rutledge and Seidel (34), individual technologies may have little value used in isolation, but synergism among such technologies may lead to useful systems of production.

The primary objective was to determine whether pregnancy rates in heat-stressed dairy cattle could be enhanced by timed embryo transfer of fresh or frozen-thawed IVF-derived embryos compared to timed insemination.

MATERIALS AND METHODS

Cows

Four hundred and four lactating Holstein cows, 50 to 140 DIM, (parity range: 1 to 4) were used. Cows were housed in two barns of a 10,000-cow commercial dairy farm located in Okeechobee County, Florida (27°12′N, 80°46′W). Barns were open-sided with concrete flooring and self-locking stanchions. Cows had free access to an adjacent sod-based area. Barns were equipped with fans and misters for evaporative cooling. Fans were operational 24 h daily for forced ventilation, and misters operated in 15-min (3 min on, 12 min off) cycles between 0800 and 2000 h daily. Cows were milked three times daily and allowed to eat a TMR ad libitum. Feed was replenished immediately following each milking. The study was conducted during the hot, humid months of August to October, 1996, in six replicates (three in each barn). The average temperature (24 h mean) for the season was 25.9°C; peak day-time temperatures exceeded 32.2°C for 25 d during the experimental period (6) and a mean relative humidity of 79%. Body condition scores (BCS) were obtained around d 0 (the day after the second GnRH treatment) on 299 cows [scoring scale 1 to 5 (10)] in 0.25 increments. The mean cumulative conception rates in cows not included in the study but maintained under identical management conditions and inseminated at detected estrus during the corresponding period, were 9.4% (1441 services) and 9.6% (1653 services) for the two barns.

Production and Transport of Embryos

Embryos for transfer without freezing were produced at the University of Wisconsin-Madison, we used Holstein oocytes obtained from an abattoir (Peck Packing Co., Milwaukee, WI) during August to October. Transport of ovaries, recovery, and maturation of oocytes were as described in Saeki et al. (35, 36). After 20 to 22 h of maturation, frozen-thawed sperm after postthaw separation on a Percoll gradient (21) were used to fertilize the oocytes as described by Parrish et al. (23). Sperm from four different bulls were used and assigned randomly for IVF to each batch of oocytes. Sperm and eggs were coincubated for 18 to 20 h. The cumulus cells were then removed and the zygotes placed in CR1aa medium (33) for culture. Heat-treated fetal calf serum (10%) was added to culture drops on day 5 (d 0 = fertilization).

Embryos that were removed from in vitro culture conditions and transferred to recipient utero without freezing will be referred to as “fresh” throughout this paper. For fresh transfers, morula or early blastocyst-stage embryos were removed from culture on d 6, placed in TL-Hepes (22) without glucose supplemented with 0.22 mM sodium pyruvate, 25 µg of gentamicin/ml, and 10% heat-treated fetal calf serum, in 5 ml of sterile, polystyrene Falcon tubes (Beckton and Dickinson, East Rutherford, NJ). Tubes were sealed with Parafilm, placed in a battery-powered portable incubator (Mini-tub, Inc., Verona, WI) set at 39°C, and shipped to Florida by counter-to-counter air courier (2 to 3 h transit). From the air terminal, embryos were transported by car to the farm site (1-h transit) and remained in the portable incubator overnight.

Embryos for freezing were removed from culture at d 7 as expanded or expanding blastocysts and cryopre-
erved for direct transfer in modified Dulbecco’s PBS containing 1.5 M ethylene glycol and 0.4% BSA following a previously described procedure (42) with the addition of 0.2 M sucrose. Most of the frozen embryos were produced in Wisconsin and air-transported to Florida in one shipment and held in liquid nitrogen, until used. One batch of frozen embryos (38% of total) was produced at the University of Florida following the same culture and freezing procedures described.

Synchronization of Ovulation

All animals received GnRH (Cystorelin®, Sanofi Animal Health Inc., Overland Park, KS; 100 μg, i.m.) followed 7 d later by PGF₂α (Lutalyse®, Pharmacia-Upjohn, Kalamazoo, MI; 25 mg, i.m.) and a second GnRH treatment given again at 48 h after PGF₂α. Cows were not observed for estrus but were randomly assigned to timed insemination 16 h after the second injection of GnRH (TI, n = 129) or timed embryo transfer to receive either a fresh (TET-Fresh, n = 133) or frozen-thawed (TET-Frozen, n = 142) embryo. Embryos were transferred approximately 7.5 d after the time of second GnRH injection.

Embryo Transfer

On the morning of d 7 (d 0 = d of TI in control cows), fresh embryos were removed from the portable incubator and evaluated at 40× under a stereo-zoom microscope. Good-quality embryos (expanded and expanding blastocysts) were packaged individually in straws. The straws were laid horizontally (to prevent loss of embryos due to drifting) in a container, protected from direct light, and carried to the barn for transfer.

Prior to embryo transfer, cows were palpated for the presence of a corpus luteum (CL) and for abnormalities of the reproductive tract. Three cows with palpable abnormalities such as pyometra or extensive uterine adhesions were excluded from the study. Based on size and palpable characteristics, CL were categorized as 3 (excellent quality: well-defined large CL with prominent crown, approximate size ≥2.0 cm), 2 (good to fair quality: easily palpable small CL with rounded surface but no prominent crown; ≥1.0 cm and <2.0 cm), 1 (poor quality: ill-defined CL, or corpus hemorrhagicum; ≤1.0 cm), or 0 (nonpalpable: no palpable CL). An embryo was deposited in the uterine horn ipsilateral to the side of the CL after aseptic preparation of the vulva and adjacent areas. Even in cows with no palpable CL (category 0), an embryo was transferred to the uterine horn ipsilateral to the larger ovary. Embryos were transferred intentionally to cows with no palpable CL because all TI cows with normal reproductive tracts were inseminated. Thus, all cows with no palpable abnormalities of the reproductive tract in each of the three groups were either inseminated or received an embryo.

All transfers were performed nonsurgically under epidural anesthesia by two veterinarians (JDA and MD) experienced in embryo transfer. Frozen embryos stored under liquid nitrogen were carried to the barn and thawed after the recipient animal had been prepared for transfer. Thawing was done by holding the straw in air for 10 s and then immersing in 30°C water for 20 s. The straw was wiped dry and placed in an ET catheter, and the embryo was transferred immediately.

Recipients assigned to receive frozen embryos were prepared after completion of all fresh transfers to avoid prolonged exposure of fresh embryos to high ambient temperature. The transfer of fresh embryos was completed within 3 h after removing embryos from the portable incubator. Frozen embryos were thawed in-straw, one at a time. The interval between thawing and completion of transfer was usually less than 3 min.

Blood Collection and Progesterone Assay

Blood samples were collected from cows of all three groups on d 0 (16 h after the second GnRH), d 7 (day of embryo transfer) and d 21 by coccygeal venipuncture into 7-ml Vacutainer tubes (Becton Dickinson, East Rutherford, NJ) containing EDTA as anticoagulant. Blood samples were unavailable from 16 cows on d 21. Tubes were placed in ice immediately upon collection, maintained in ice, and centrifuged (1000 × g, 30 min at 4°C) within 24 h. Plasma samples were stored at –20°C until analysis for progesterone by radioimmunoassay (16). Every fifth plasma sample was assayed in duplicate. The intra- and interassay coefficients of variation for the progesterone assays were 10.9 and 13.2%, respectively.

Assessment of CL status

The accuracy of rectal palpation for the positive and negative diagnosis of CL was determined by using plasma progesterone concentrations as the standard. Positive predictive value was calculated as (a/a + b) × 100, where a is the number of correct positive diagnoses (CL present) and b is the number of incorrect positive diagnoses. Negative predictive value was calculated as (c/c + d) × 100, where c is the number of correct negative diagnoses (no CL present) and d is the number of incorrect negative diagnoses (24, 31). All cows with ≥1.5 ng of progesterone were considered as a correct positive diagnosis and those with <1.5 ng/ml as an incorrect diagnosis for categories 1, 2, and 3 of CL. In category 0, cows with <1.5 ng of progesterone/ml were considered...
as correct negative diagnosis and cows with \( \geq 1.5 \) ng of progesterone/ml as incorrect negative diagnosis.

**Pregnancy Diagnosis**

After TI or TET, animals were observed for signs of estrus by farm personnel twice daily. Cows returning to estrus were inseminated as per routine managerial practice of the farm. Cows that failed to return to estrus were palpated per rectum between 45 and 52 d for pregnancy diagnosis. The presence of fluid in the uterine horn, a palpable amniotic vesicle, and fetal membrane slip were considered positive indicators of pregnancy (12). Pregnancy rate on d 45 was calculated as the percentage of cows receiving TET or TI that were confirmed pregnant on rectal palpation after excluding 14 cows (TI = 7, TET-Fresh = 3, TET-Frozen = 4) that left the farm during the course of the experiment.

**Statistical Analyses**

Results were analyzed by least-squares ANOVA by the general linear models procedure of SAS (37). The mathematical model for the dependent variable pregnancy at d 45 to 52 included the effects of treatment, barn, parity, and DIM. The influence of batch and ET personnel were also considered, but excluded from further analysis because no effects could be detected. Orthogonal contrast procedures compared pregnancy rates to TI versus TET (Fresh and Frozen) and also TET-Fresh versus TET-Frozen. Differences in pregnancy rates among TI, TET-Fresh, and TET-Frozen groups were further tested by CATMOD procedures of SAS. Regression analysis was used with data on BCS. Differences in progesterone concentrations among various CL categories were also tested by orthogonal contrast procedures. The distribution of cows among the various CL categories was determined by the PROC FREQ procedures of SAS and tested by chi-square analysis. The relationship between progesterone and CL quality was examined by ANOVA and PROC CORR procedures of SAS.

**RESULTS**

**Plasma Progesterone on d 7 and CL Quality**

Cows with plasma progesterone concentrations \( \leq 1.5 \) ng/ml on d 0 and \( \geq 2.0 \) ng/ml on d 7 were considered to have responded to the synchronization treatment. Based on this criterion, 76.2% of the cows were estimated to have undergone a synchronized ovulation. Plasma progesterone concentrations of d 7 did not differ \( (P > 0.05) \) among treatments (TI: 6.2 \( \pm 0.3 \), TET-Fresh: 5.6 \( \pm 0.3 \), and TET-Frozen: 6.2 \( \pm 0.3 \) ng/ml) or among cows that were eventually confirmed pregnant or not pregnant \( (6.5 \pm 0.5 \) vs. \( 6.0 \pm 0.2 \) at d 45 to 52 by rectal palpation. However, the mean concentration (ng/ml) of plasma progesterone increased as the palpable quality of CL improved from category 0 \( (2.99 \pm 0.49) \) to 1 \( (4.62 \pm 0.54) \), 2 \( (5.44 \pm 0.38) \) and 3 \( (7.13 \pm 0.25) \). The linear relationship had a positive correlation \( (r = 0.46; P < 0.01) \). Orthogonal contrast procedures revealed that progesterone concentrations of cows in CL-categories 0 and 1 differed \( (P < 0.01) \) from cows in CL-categories 2 and 3. Category 0 differed from category 1 \( (P < 0.03) \) and category 2 differed \( (P < 0.01) \) from category 3. Positive predictive value and negative predictive value of rectal palpation for the detection of a CL were 95 and 48%, respectively.

**Pregnancy Rate**

The pregnancy rate (percentage) at d 45 to 52 was higher \( (P < 0.01) \) in cows allocated to TET-Fresh compared to TET-Frozen or TI group when all experimental animals were included in the analysis (Table 1). Pregnancy rates among the subset of cows considered to have responded to the synchronization treatment were slightly higher but maintained the same pattern. The percentage of cows that had an elevated \( (\geq 2.0 \) ng/ml) concentration of plasma progesterone on d 21 was much higher than the proportion of cows later confirmed pregnant at day 45 to 52 (Table 1). Plasma progesterone concentrations on d 21 differed among treatments when either all experimental cows or only synchronized cows were considered (Table 2). However, no differences in progesterone concentrations among treatments were evident when only the cows that had elevated progesterone \( (\geq 2.0 \) ng/ml) concentrations on d 21 were used in the analysis. Despite the positive correlation between CL category and progesterone concentration, no correlation was detected between CL category at day of transfer and subsequent pregnancy at d 45 to 52.

**Body Condition versus Pregnancy and Plasma Progesterone**

Only 299 of the 404 cows in the experiment were scored for body condition, but they were evenly distributed among all three treatment groups. There was an association between body condition and pregnancy rate \( (\text{pregnancy rate} = -0.914 + 0.373 \times \text{BCS}; P < 0.01) \), with approximately a 37% increase in pregnancy projected for a whole unit increase in BCS. If only the best cows \( (\text{that is cows that responded to synchronization}, \text{received a BCS} \geq 2.75 \) and had \( \geq 5.0 \) ng/ml concentration of progesterone in plasma on d 7) were considered \( (n = 148) \), pregnancy rates increased
TABLE 1. Least square means (±SEM) of pregnancy rate at d 45 to 52 determined by palpation per rectum, and pregnancy estimate at d 21 based on elevated concentrations (≥2 ng/ml) of progesterone in plasma, for all cows in the study and only cows that responded to the synchronization treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cows (%) with elevated progesterone at d 21</th>
<th>P</th>
<th>Cows (%) with elevated progesterone at d 21</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All cows</td>
<td>Synch cows</td>
<td>All cows</td>
<td>Synch cows</td>
</tr>
<tr>
<td>TI (a)</td>
<td>4.9 ± 2.3</td>
<td>6.7 ± 3.2</td>
<td>41.4 ± 0.1</td>
<td>46.7 ± 4.2</td>
</tr>
<tr>
<td>TET-Fresh (b)</td>
<td>14.3 ± 2.3</td>
<td>17.5 ± 3.0</td>
<td>64.7 ± 0.1</td>
<td>64.9 ± 4.2</td>
</tr>
<tr>
<td>TET-Frozen (c)</td>
<td>4.8 ± 2.3</td>
<td>6.1 ± 3.8</td>
<td>40.3 ± 0.1</td>
<td>40.5 ± 4.2</td>
</tr>
<tr>
<td>Orthogonal contrasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) vs (b, c)</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>(b) vs (c)</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

1. All cows included in the study.
2. Cows that responded to synchronization treatment.
3. Excludes 14 cows that left the herd before palpation for pregnancy.
5. Not significant.

substantially to 25.4 ± 0.06 (n = 50), 12.0 ± 0.05 (n = 48) and 10.2 ± 0.05% (n = 50), for TET-Fresh, TET-Frozen, and TI groups, respectively. The difference in pregnancy rate between TET-Fresh and TET-Frozen was significant at P ≤ 0.08. Body condition score also influenced the plasma progesterone concentration of d 7 with an increase of 2.9 ng of progesterone/ml detected for a whole unit increase in BCS (progesterone = –1.808 + 2.918 (BCS); P < 0.01).

DISCUSSION

This is the first report of timed embryo transfer in dairy cows using fresh or frozen-thawed in vitro produced embryos. Results of the present field study support the hypothesis that transfer of IVF-derived embryos to heat-stressed dairy cattle can increase pregnancy rate when compared to TI. This beneficial effect of ET, however, was only apparent when fresh IVF-derived embryos were used because transfer of frozen-thawed embryos resulted in low pregnancy rates. The beneficial effects of TET are all the more remarkable because transfer was performed without estrus detection. Although not all cows responded to the synchronization treatment, results show the potential for ovulation control as a tool in embryo transfer. Under severe heat stress conditions, the length of estrous behavior is shortened (1, 43), and its intensity is diminished (13). As a result, during summer months up to 80% of estrous periods may go undetected (41). Considering that pregnancy rate is the product of estrus detection rate and conception rate, the strategy to eliminate estrus detection through timed embryo transfer and timed insemination was convenient to implement. Fixed days of the week could be designated for each operation and involved no estrus detection. Timed insemination has been shown to be effective under heat stress conditions in Florida (3, 7). Although TET with in vitro-produced fresh embryos in the present study increased pregnancy rates compared to TI, pregnancy rates generally remained low in all groups. Further opportunities exist

TABLE 2. Least square means (±SEM) of plasma progesterone concentrations on day 21 for all cows in experiment, cows which had synchronized ovulation and cows that had elevated concentrations (≥2 ng/ml) of plasma progesterone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>All cows</th>
<th>Synchronized cows</th>
<th>Cows with elevated progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 388)</td>
<td>(n = 303)</td>
<td>(n = 196)</td>
</tr>
<tr>
<td>TI (a)</td>
<td>1.6 ± 1.5</td>
<td>3.4 ± 0.5</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>TET-Fresh (b)</td>
<td>3.8 ± 1.5</td>
<td>5.9 ± 0.5</td>
<td>8.4 ± 0.5</td>
</tr>
<tr>
<td>TET-Frozen (c)</td>
<td>1.5 ± 1.5</td>
<td>3.7 ± 0.4</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>Orthogonal contrasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) vs (b, c)</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>NS2</td>
</tr>
<tr>
<td>(b) vs (c)</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

2. Not significant.
for enhancing the effectiveness of TET protocols during summer.

Putney et al. (28) and Drost et al. (8) demonstrated that pregnancy rates in heat-stressed dairy cows could be enhanced by transfer of frozen-thawed embryos collected from superovulated cattle, but not by transfer of frozen-thawed IVF-derived embryos. Drost et al. (8) reported pregnancy rates of 21% following AI versus 35 and 19%, respectively, following transfer of in vivo and in vitro-derived frozen-thawed embryos to recipient cattle at 7 d after a detected estrus. The latter study was conducted in a commercial dairy in North Central Florida, whereas the present study was in South Florida, a region where heat stress conditions are more intense and the summer decline in fertility is greater (2, 5). This may explain the severely depressed pregnancy rates observed in the present study even after transfer of fresh embryos. Considering the morphological differences between embryos produced in vivo and in vitro (15, 25), it is possible that IVF-derived embryos are more susceptible to thermal stress than embryos obtained from superovulated donors.

As reported previously (8), transferring frozen-thawed IVF derived embryos had no advantage. It is known that postthaw survival of IVF-derived embryos is affected by the freeze-thaw process. Cells of the inner cell mass are less viable in frozen-thawed IVF-derived bovine embryos than in unfrozen embryos (39). In addition, the freeze-thaw process may have affected the thermoresistance of IVF-derived embryos rendering them more susceptible to the elevated uterine temperature of heat-stressed cows. The low pregnancy rate in the TI cows compared to the higher rate of pregnancy in TET-Fresh group indirectly confirms the observation of Putney et al. (27) that early cleavage-stage embryos are highly susceptible to heat stress. Further support to this observation comes from the low (9.5%) conception rate among observational controls that were inseminated at detected estrus. This comparison must be evaluated cautiously as the 9.5% conception rate reported for observational controls is a cumulative value (not restricted to first service) obtained from cows that were inseminated at detected estrus. On the other hand, the pregnancy rate reported for TI cows is based on a single fixed time insemination with no estrus detection.

Inaccuracies associated with rectal palpation of CL were evident upon comparing progesterone concentrations. Although palpation accuracy was over 95% for the presence of a CL (categories 3, 2, and 1), the accurate diagnosis of the absence of a CL (category 0) was only 48% efficient. The positive and negative predictive values attained in the present study are in agreement with previous reports (20, 24, 32). One study (20) reported a low negative predictive value of 41%. Together, these observations indicate that palpation per rectum is not a reliable technique to determine the absence of a CL because approximately 50% of the cows in category 0 were diagnosed inaccurately. It is likely that a large percentage of ovaries bear small, embedded CL, making detection by palpation difficult. Three cows determined to have no palpable CL were diagnosed pregnant by palpation per rectum at 45 to 52 d. In a previous study, Niemann et al. (19) reported six of their 10 recipients pregnant despite having poor quality CL on the day of transfer. The same researchers reported a 35% pregnancy rate from 17 recipient cows determined to have plasma progesterone concentrations lower than 2.0 ng/ml on the day of ET.

The major difference in the percentage of cows that had elevated plasma progesterone concentrations on d 21 and that were diagnosed pregnant at 45 to 52 d suggests that substantial embryo losses may have occurred between 21 and 45 d. Other factors such as heat stress-induced extension of cycle length, luteal cysts, and subclinical uterine infections, could have contributed to this difference. Embryo losses at least partially contribute to these differences since the difference in the percentage of cows with progesterone >2.0 ng/ml on d 21 among treatments parallels the difference in pregnancy rates at d 45 to 52 (TET-fresh vs TET frozen and TI control). Recently, Massip et al. (17) reported that the majority of embryonic losses occurred from 21 to 45 d after transfer of in vitro-derived fresh and frozen-thawed bovine embryos. More recent evidence suggests that a majority of embryonic loss occurs before d 42 in heat-stressed cows (41).

Several previous studies have shown that a relationship exists between body condition and pregnancy rates (4, 18). Results of the present study detected a 37% increase in pregnancy rate for a whole unit gain in body condition. Similarly, a relationship was detected between body condition and plasma progesterone concentration. These results reemphasize the importance of returning cows to a positive energy state as soon as possible to avoid major losses due to reproductive wastage.

In conclusion, transfer of fresh IVF-derived embryos to heat-stressed dairy cattle increased pregnancy rates in the present study. However, transferring frozen-thawed IVF-derived embryos was of no advantage. On-farm transfer of fresh IVF-derived embryos has practical difficulties and requires trained personnel and equipment for the evaluation and packaging of embryos at farm sites. If techniques that allow transportation of fresh embryos prepacked in straws (without the risk of losing embryos) could be developed, transfer of fresh IVF-derived embryos may be realistic under some situa-
tions. On the other hand, if pregnancy rates comparable to transfer of fresh IVF-derived embryos could be achieved with frozen-thawed IVF-derived embryos, timed embryo transfer to enhance pregnancy rates during summer months may become a practical alternative. Further research is therefore needed to enhance freezability of IVF-derived embryos. Timed embryo transfer without estrus detection proved convenient and practical under field conditions. Even under extreme heat stress conditions, cows in better body condition tend to have increased rates of pregnancy. Since progesterone concentration increases with CL quality, recipient cattle in good body condition and well-defined CL should be used, where possible, to increase the chances of pregnancy under heat stress conditions.

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