

Interaction between season and culture with insulin-like growth factor-1 on survival of in vitro produced embryos following transfer to lactating dairy cows

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Abstract

Culture of bovine embryos in the presence of insulin-like growth factor-1 (IGF-1) can increase pregnancy rates following transfer to heat-stressed, lactating dairy cows. The objective of the present experiment was to determine whether the effect of IGF-1 on post-transfer embryo survival was a general effect or one specific to heat stress. Lactating recipients ($n = 311$) were synchronized for timed-embryo transfer at four locations. Embryos were produced in vitro and cultured with or without 100 ng/mL IGF-1. At Day 7 after anticipated ovulation (Day 0), a single embryo was randomly transferred to each recipient. Pregnancy was diagnosed at Day 21 by elevated plasma progesterone concentrations, at Days 27–32 by ultrasonography, and at Days 41–49 by transrectal palpation. Transfers were categorized into two seasons, hot or cool (based on the month of transfer). There was a tendency ($P < 0.09$) for an interaction between embryo treatment and season for pregnancy rate at Day 21; this interaction was significant at Days 30 and 45 ($P < 0.02$). Recipients receiving IGF-1 treated embryos had higher pregnancy rates in the hot season but not in the cool season. There was a similar interaction between embryo treatment and season for overall calving rate ($P < 0.05$). There was also an interaction between season and treatment affecting pregnancy loss between Days 21 and 30; recipients that received IGF-1 treated embryos had less pregnancy loss during this time period in the hot season but not in the cool season. The overall proportion of male calves born was 77.5%. In conclusion, treatment of embryos with IGF-1 improved pregnancy and calving rates following the transfer of in vitro produced embryos into lactating recipients, but only under heat-stress conditions.

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1. Introduction

Exposure to heat stress reduces fertility in lactating dairy cows [1,2]. Whereas early embryonic development is very sensitive to the deleterious effects of heat stress, embryos become more resistant as development

progresses [3,4]. Thus, embryo transfer can be used to bypass the period during which the embryo is most sensitive to heat stress and improve fertility as compared to artificial insemination [5–8]. There does remain, however, some detrimental effects of heat stress on pregnancy rates in embryo transfer recipients [9,10].

One strategy to increase pregnancy success for transfer of in vitro produced embryos is to alter embryo culture conditions to improve post-transfer viability of embryos. Addition of insulin-like growth factor-1 (IGF-1) to culture medium can increase development of bovine embryos to the blastocyst stage [11–16],

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increase blastocyst cell number [13,14,17] and reduce the proportion of blastomeres that are apoptotic [13,18]. Treatment of bovine preimplantation embryos with IGF-1 also improved resistance to heat shock by reducing effects of elevated temperature on blastomere apoptosis and development to the blastocyst stage [19,20].

Recently, it was demonstrated that lactating recipient dairy cows exposed to heat stress had higher pregnancy rates when receiving an embryo treated with IGF-1 during culture as compared to control embryos [15]. It is not clear whether this beneficial effect of IGF-I on post-transfer survival was due to actions of IGF-1 on embryonic development in general or, alternatively, was related to the thermoprotective actions of IGF-1 on bovine embryo development [19,20]. Therefore, the objective of the present study was to determine whether the effect of culturing embryos in the presence of IGF-1 on post-transfer survival would be apparent regardless of season, or under heat-stress conditions only.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma (St. Louis, MO, USA) or Fisher Scientific (Fairlawn, NJ, USA) unless specified otherwise. Sperm-Tyrode's Lactate, IVF-Tyrode's Lactate, and HEPES-Tyrode's Lactate were purchased from Caisson Laboratories Inc. (Logan, UT, USA). These media were used to prepare Sperm-Tyrode's Albumin Lactate Pyruvate (TALP), IVF-TALP, and HEPES-TALP as described previously [21]. Oocyte collection medium (OCM) was Tissue Culture Medium-199 (TCM-199) with Hank's salts without phenol red (Atlanta Biologicals, Norcross, GA, USA) and supplemented with 2% (v/v) bovine steer serum (Pel-Freez, Rogers, AR, USA), 2 U/mL heparin, 100 U/mL penicillin-G, 0.1 mg/mL streptomycin, and 1 mM glutamine. Oocyte maturation medium (OMM) was TCM-199 (Invitrogen, Carlsbad, CA, USA) with Earle's salts supplemented with 10% (v/v) bovine steer serum, 2 µg/mL estradiol 17-β, 20 µg/mL bovine FSH (Folltropin-V; Bioniche, Belleville, ON, Canada), 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin sulfate, and 1 mM glutamine. Percoll was from Amersham Pharmacia Biotech (Uppsala, Sweden). Potassium simplex optimized medium (KSOM) that contained 1 mg/mL BSA was from Caisson. On the day of use, KSOM was modified to produce KSOM-BE2 as described previously [22]. Recombinant human IGF-1 was obtained from Upstate Biotech (Lake Placid, NY,

USA). Prostaglandin $F_{2\alpha}$ (PGF) was Lutalyse[®] from Pfizer (New York, NY, USA) and gonadotrophin-releasing hormone (GnRH) was Cystorelin[®] from Merial (Duluth, GA, USA). Controlled internal drug releasing (CIDR) devices were purchased from Pfizer and lidocaine was from Pro Labs (St. Joseph, MO, USA).

2.2. Animals

The experiment was conducted between March 2005 and September 2006 at four locations: Farm 1 (Live Oak, Florida, USA; 30.29434 N, 82.98607 W), Farm 2 (Hague, Florida, USA; 29.77904 N, 82.48001 W), Farm 3 (Bell, Florida, USA; 29.75578 N, 82.86188 W), and Farm 4 (Okeechobee, Florida, USA; 27.24126 N, 80.82988 W). The maximum daily temperatures and average relative humidities for March 15, 2005 through February 9, 2006 (from 10 days before transfers were initiated until completion of all pregnancy diagnoses) are shown in Fig. 1 for data from nearby weather

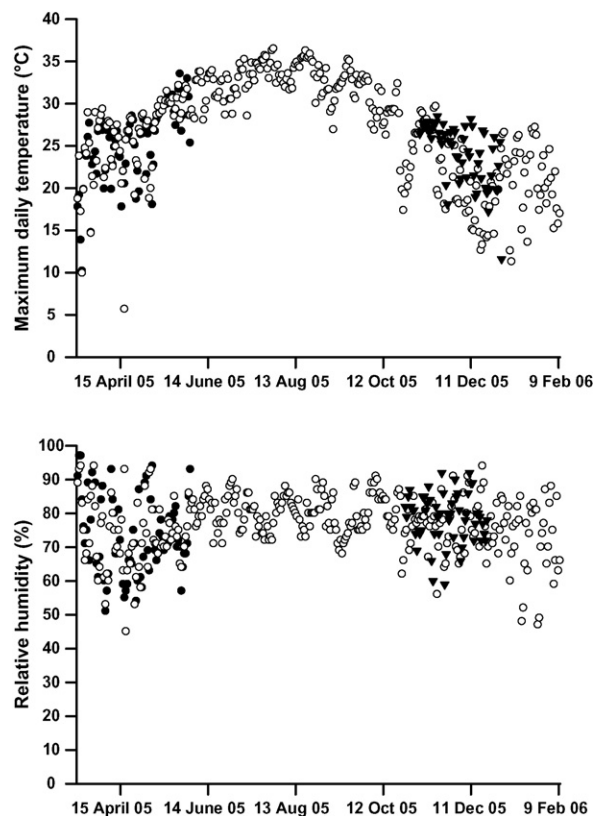


Fig. 1. Scatter plot of daily maximal dry bulb temperatures (top panel) and daily relative humidity (bottom panel) for Farm 1 (solid circles), Farms 2 and 3 (open circles), and Farm 4 (solid triangles) from March 15, 2005 to February 9, 2006.

stations at Live Oak, Florida (Farm 1), Alachua, Florida (Farms 2 and 3), and Ft. Pierce, Florida (Farm 4) as recorded by the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu/>).

At Farm 1, 53 primiparous and multiparous lactating Holstein \times Jersey cows between 63 and 807 days in milk (DIM) (mean = 184.3) were used as recipients from March through April 2005. Cows were housed outdoors on a dirt lot with access to shade cloth structures and sprinklers. All recipients were fed a total mixed ration (TMR) and milked three times per day. Overall, five replicates were completed with 7–15 recipients per replicate. At Farm 2, a total of 99 primiparous and multiparous lactating Holstein cows between 87 and 1014 DIM (mean = 317.1) were used as recipients from March through September 2005. All recipients were housed in a free stall barn equipped with fans and sprinklers, fed a TMR and milked three times per day. A total of 96 recipients received bovine somatotropin (bST; Monsanto, Chesterfield, MO, USA) as per manufacturer's instructions. Overall, six replicates were completed with 11–28 recipients per replicate. At Farm 3, a total of 114 primiparous and multiparous lactating Holstein cows between 36 and 789 DIM (mean = 222.9) were used as recipients from July 2005 through January 2006. All recipients were housed in a free stall barn equipped with fans and sprinklers, fed a TMR and milked three times per day. A total of 82 recipients received bST as per manufacturer's instructions. Overall, seven replicates were completed with 10–20 recipients per replicate. At Farm 4, a total of 44 primiparous and multiparous lactating Holstein cows between 68 and 84 DIM (mean = 78.5) were used as recipients during November 2005. All recipients were housed in a free stall barn equipped with fans and sprinklers, fed a TMR and milked three times per day. A total of two recipients received bST as per manufacturer's instructions. Overall, four replicates were completed with eight to 13 recipients per replicate.

Cows at all four farms were synchronized for timed-embryo transfer. Regardless of the protocol used, Day 0 was defined as the day of anticipated ovulation. Cows at Farm 1 were synchronized using a modified OvSynch protocol [23]. Cows received 100 μ g of GnRH (i.m.) and a CIDR (intravaginal insertion) on Day 10. On Day 3, cows received 25 mg PGF and the CIDR was removed. A second injection of GnRH was administered on Day 1. Cows at Farms 2 and 3 were synchronized as described for Farm 1 without the inclusion of a CIDR [24]. For Farm 4, cows were synchronized using two injections of PGF 14 days apart (Day 18 and 4).

To detect a CL, cows at all locations were examined at Day 7 after anticipated ovulation using an Aloka 500 ultrasound equipped with a 5 MHz linear array transducer. All cows with a CL received epidural anesthesia (5 mL of 2% lidocaine) and a single embryo was transferred to the uterine horn ipsilateral to the ovary with a CL.

2.3. Pregnancy diagnosis and calving data

Pregnancy at Day 21 after ovulation was assessed by measurement of peripheral blood progesterone concentrations. Blood samples were taken on Day 21 after anticipated ovulation by coccygeal venipuncture into evacuated heparinized tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Following collection, blood samples were placed in an ice chest until further processing at the laboratory (approximately 3–8 h). Blood samples were centrifuged at $3000 \times g$ for 15 min at 4 °C. Plasma was separated and stored at –20 °C until assayed for progesterone. Plasma progesterone concentrations were determined using the Coat-a-Count[®] progesterone RIA kit (Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 0.1 ng/mL and the intra-assay CV was 5.6%. Cows were classified as pregnant if the progesterone concentration was ≥ 2.0 ng/mL. Pregnancy was also diagnosed at \sim Day 30 of gestation (range = Days 27–32) using ultrasonography and again at \sim Day 45 of gestation (range = Days 41–49) by transrectal palpation. Calving data was recorded for Farms 1, 2, and 4. Data included calf sex and gestation length (Farms 1, 2, and 4) and calf birth weight (Farm 2). In addition, the calf birth weights and calf sexes of cows ($n = 54$) that were bred by AI during the week prior to each embryo transfer replicate at Farm 2 were also recorded.

2.4. Embryo production

For Farms 1, 2, and 4, Holstein cumulus-oocyte complexes (COC) were purchased from BOMED, Inc (Madison, WI, USA; $n = 3$ replicates), Trans Ova Genetics (Sioux Center, IA, USA; $n = 3$ replicates), or Evergen Biotechnologies (Storrs, CT, USA; $n = 9$ replicates). Following collection, COCs were placed into 2 mL cryovials (approximately 50–115 COCs/cryovial) containing maturation medium and shipped overnight in a portable incubator set at 38.5 °C to the laboratory in Gainesville, FL, USA. For Farm 3 ($n = 7$ replicates), COCs were collected as described previously [22] from ovaries (predominately beef cattle) obtained from Central Packing Co. (Center Hill, FL,

USA). Regardless of farm, all COC's were allowed to mature for 21–24 h.

In vitro fertilization and embryo culture were conducted as described elsewhere [23] and all procedures were similar for each farm unless noted otherwise. Following maturation, COCs were washed once in HEPES-TALP and then fertilized with frozen-thawed semen. For Farms 1 and 2, a single Holstein bull was used for each farm. For Farm 3, semen from three randomly selected bulls was used and three different bulls were used for each replicate. For Farm 4, two Holstein bulls were used and alternated for each replicate. Following 20–24 h of co-incubation, presumptive zygotes were then cultured in KSOM-BE2 with or without 100 ng/mL IGF-1 as described elsewhere [15]. For the first 4 replicates (Farms 1 and 2), presumptive zygotes were cultured in a humidified atmosphere of 5% CO₂ and for the remaining 18 replicates (Farms 1–4) presumptive zygotes were cultured in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N₂. Cleavage rate was recorded on Day 3 and the proportion of oocytes developing to the blastocyst stage was recorded on Day 8 (first 7 replicates; Farms 1 and 2) and 7 ($n = 15$ replicates; Farms 2–4).

Grade 1 morula, blastocyst, and expanded blastocyst stage embryos [25] were harvested on Day 7 ($n = 15$ replicates) or Day 8 ($n = 7$ replicates) after insemination and transported to the farm using one of two different methods. For the first six replicates, harvested embryos were loaded into 0.25 mL French straws in holding medium (HEPES-TALP containing 10% fetal bovine serum and 100 μ M β -mercaptoethanol). Straws containing selected embryos were then placed horizontally into a portable incubator (Minitube, Verona, WI, USA) at 39 °C and transported to the respective farm. For the remaining 16 replicates, harvested embryos were placed into 2 mL microcentrifuge tubes containing holding medium, placed into a portable incubator at 39 °C and transported to the respective farm. Once at the farm, embryos were then loaded into 0.25 mL French straws in holding medium. Regardless of transportation method, straws containing embryos were loaded into a 21-in. transfer pipette (IMV Technologies, L'Aigle, France) and randomly transferred to recipients. Of the harvested embryos, 79 were blastocysts and 232 were expanded blastocysts.

2.5. Statistical analysis

The proportion of oocytes that cleaved, that developed to the blastocyst stage on the day of

blastocyst harvest (i.e. Days 7 or 8 after insemination), and the proportion that developed to advanced blastocyst stages (expanded, hatching or hatched) on Days 7 or 8 were calculated for each replicate. Treatment effects were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS (SAS for Windows, Version 9.0, SAS Institute Inc., Cary, NC, USA). The model included the main effects of replicate and treatment. Data were analyzed two ways; first as the entire data set and then separately for replicates in which blastocysts were collected at Days 7 or 8 after insemination. All values reported are least-squares mean \pm S.E.M.

Logistic regression was performed using the LOGISTIC procedure of SAS to analyze data for the proportion of recipients that were pregnant at Day 21 after ovulation (based on having a plasma progesterone concentration >2.0 ng/mL), Day 30 after ovulation (based on ultrasonography) and Day 45 after ovulation (based on transrectal palpation). Calving rate and pregnancy loss were also analyzed by logistic regression. Calving rate was analyzed two ways: (1) as the proportion of recipients that gave birth to a calf, live or dead (defined as overall calving rate) and (2) as the proportion of recipients that gave birth to a calf that survived at least 24 h (defined as live calving rate). Pregnancy loss was analyzed between three time points as follows: Day 21–30, Day 30–45 and Day 45 to term (except Farm 3). The models for the variables described above included the main effects of season of transfer (hot season: July, August, and September and cool season: January, March, April, and November), embryo treatment, farm-season, days in milk and all two-way interactions. Additional analyses for pregnancy rate, calving rate and pregnancy loss were also conducted. One analysis included a subset of recipients at Farms 2 and 3 only. These were two locations at which transfers were completed in both the cool season and the hot season. Another analysis included a subset of recipients that received embryos cultured in 5% O₂ and were harvested on Day 7. In addition, analyses were also performed separately for transfers in the cool season and hot season, respectively, with farm and embryo treatment as effects. Finally, analyses were conducted separately for control and IGF-1 treated embryos to determine effects of season. All data on pregnancies and calvings are reported as the actual percentage.

Calf birth weight and gestation length were subjected to analysis of variance using the GLM procedure of SAS. Data were analyzed for the data set of all calves and the data set of live calves. The models included embryo treatment, sex of calf and farm-season. All

values are reported as least-squares mean \pm S.E.M. The proportion of calves that were male was analyzed among all calves and all live calves using the LOGISTIC procedure of SAS. The model included season of transfer, embryo treatment, farm-season and all two-way interactions. The effect of breeding type (i.e. artificial insemination or embryo transfer) on calf birth weight and calf sex for a subset of cows at Farm 2 was also analyzed. In addition, Chi-square analysis was used to determine if the sex ratio of all calves and all live calves deviated from the expected 50:50 ratio.

3. Results

3.1. Embryo development

Overall, there was no effect of IGF-1 on cleavage rate at Day 3 after insemination (control – 77.3 \pm 0.8% versus IGF-1 – 78.9 \pm 0.8%), the proportion of oocytes that became blastocysts (control – 16.2 \pm 1.3% versus IGF-1 – 17.2 \pm 1.3%), or the proportion of oocytes that became advanced blastocysts (expanded, hatching or hatched) (7.6 \pm 0.7% versus IGF-1 – 8.4 \pm 0.7%). When only those replicates in which blastocyst development was recorded on Day 8 after insemination ($n = 7$ replicates) were analyzed separately, there was also no effect of IGF-1 on the proportion of oocytes becoming blastocysts (control – 21.9 \pm 1.6% versus IGF-1 – 20.2 \pm 1.6%) or advanced blastocysts (control – 8.9 \pm 0.4% versus IGF-1 – 8.8 \pm 0.4%). However, among replicates in which blastocyst development was recorded on Day 7 after insemination ($n = 15$ replicates), IGF-1 increased the proportion of oocytes

becoming blastocysts ($P < 0.001$; control – 13.9 \pm 0.4% versus IGF-1 – 16.0 \pm 0.4%) and tended to increase the proportion that became advanced blastocysts ($P < 0.07$; control – 7.1 \pm 0.4% versus IGF-1 – 8.2 \pm 0.4%).

3.2. Pregnancy rate

Using plasma progesterone concentrations >2.0 ng/mL as a diagnosis of pregnancy, the proportion of cows pregnant at Day 21 after ovulation was not different between recipients that received control versus IGF-1 treated embryos (Table 1). However, there was a tendency for an increased proportion of recipients with plasma progesterone >2.0 ng/mL in the hot season ($P < 0.06$) compared to the cool season (Table 1). There was also a trend for an interaction between season and treatment ($P < 0.09$), with a higher pregnancy rate for recipients receiving IGF-1 treated embryos than recipients receiving control embryos during the hot season but not the cool season (Table 1).

As shown in Table 1, there was a season \times embryo treatment interaction that affected pregnancy rate at Days 30 and 45 of gestation ($P < 0.01$). In the hot season, recipients that received IGF-1 treated embryos had higher pregnancy rates at both Days 30 and 45 than recipients receiving control embryos. In the cool season, in contrast, there was no difference between recipients receiving IGF-1 treated embryos or control embryos.

Farms 2 and 3 were the two locations where transfers were performed in both seasons. When data from these two farms only were analyzed, there was an interaction between season and IGF-1 ($P < 0.01$) for pregnancy

Table 1

Effect of season and IGF-1 on pregnancy rate at Day 21 (based on elevated plasma progesterone concentrations), Day 30 (based on ultrasonography) and Day 45 of gestation (based on transrectal palpation) and calving rate for all recipient cattle

	Pregnancy rate, number pregnant/total (%) ^a			Calving rate, number calving/total (%) ^b	
	Day 21 ^{c,d}	Day 30 ^{e,f}	Day 45 ^{g,f}	All calves ^h	Live calves
Cool season					
Control	51/76 = 67.1%	27/79 = 34.2%	24/87 = 27.6%	14/62 = 22.6%	12/60 = 20.0%
IGF-1	46/74 = 62.2%	21/77 = 27.3%	19/83 = 22.9%	11/62 = 17.7%	11/62 = 17.7%
Hot season					
Control	41/59 = 69.5%	15/71 = 21.1%	13/71 = 18.3%	5/38 = 13.2%	5/38 = 13.2%
IGF-1	51/63 = 81.0%	34/69 = 49.3%	28/67 = 41.8%	10/30 = 33.3%	9/29 = 31.0%

^a Differences in the number of recipients at each time point is due to some recipients not being diagnosed for pregnancy at all time points.

^b Data exclude Farm 3.

^c Season $P < 0.06$.

^d Season \times treatment $P < 0.09$.

^e Treatment $P < 0.06$.

^f Season \times treatment $P < 0.01$.

^g Treatment $P < 0.07$.

^h Season \times treatment $P < 0.05$.

Table 2

Effect of season and IGF-1 on pregnancy rate at Day 21 (based on elevated plasma progesterone concentrations), Day 30 (based on ultrasonography) and Day 45 of gestation (based on transrectal palpation) and calving rate among recipient cattle that received embryos that were cultured in 5% O₂ and harvested on Day 7

	Pregnancy rate, number pregnant/total (%) ^a			Calving rate, number calving/total (%) ^b	
	Day 21	Day 30 ^{c,d}	Day 45 ^{c,f}	All calves	Live calves
Cool season					
Control	27/42 = 64.3%	13/40 = 32.5%	11/45 = 24.4%	5/22 = 22.7%	4/21 = 19.1%
IGF-1	27/39 = 69.2%	13/37 = 35.1%	11/42 = 26.2%	5/22 = 22.7%	5/22 = 22.7%
Hot season					
Control	41/59 = 69.5%	15/71 = 21.1%	13/71 = 18.3%	5/38 = 13.2%	5/38 = 13.2%
IGF-1	51/63 = 81.0%	34/69 = 49.3%	28/67 = 41.8%	10/30 = 33.3%	9/29 = 31.0%

^a Differences in the number of recipients at each time point is due to some recipients not being diagnosed for pregnancy at all time points.

^b Data exclude Farm 3.

^c Treatment $P < 0.03$.

^d Season \times treatment $P < 0.06$.

^e Treatment $P < 0.05$.

^f Season \times treatment $P < 0.09$.

rate at Day 21 (cool season: control – 27/35 = 77.1% and IGF-1 – 21/34 = 61.8%; hot season: control – 41/59 = 69.5% and IGF-1 – 51/63 = 81.0%), Day 30 of gestation (cool season: control – 12/35 = 34.3% and IGF-1 – 5/34 = 14.7%; hot season: control – 15/71 = 21.1% and IGF-1 – 34/69 = 49.3%) and Day 45 of gestation (cool season: control – 10/35 = 28.6% versus IGF-1 – 6/37 = 16.2%; hot season: control – 13/71 = 18.3% versus IGF-1 – 28/67 = 41.8%).

A third analysis considered only those embryos that were cultured in 5% oxygen and harvested at Day 7 ($n = 15$ replicates; Table 2). There was an interaction between IGF-1 and season for pregnancy rate at Day 30 ($P < 0.06$) and a tendency ($P < 0.09$) for an interaction at Day 45.

A fourth analysis was performed in which data were analyzed for each season separately. In this analysis, there was no effect of IGF-1 on pregnancy rate in the cool season at Day 21, 30, or 45, but in the hot season IGF-1 treatment increased pregnancy rates at Days 30 and 45 ($P < 0.01$).

A fifth analysis was performed to determine the effect of season for each treatment separately. There was a tendency ($P < 0.08$) for control embryo recipients to have lower pregnancy rates at Day 30 in the hot season than the cool season. There were no effects of season for pregnancy rate at Day 21 or 45. Among IGF-1 recipients, pregnancy rate at Days 21, 30, and 45 were higher ($P < 0.02$) in the hot season compared to the cool season. Similar seasonal effects were apparent for the subset of cows receiving embryos collected at Day 7 after insemination but differences were not significant (Table 2).

3.3. Calving rate

These data were available for a subset comprising Farms 1, 2, and 4. There was an interaction ($P < 0.05$) between season and embryo treatment affecting overall calving rate (Table 1). At Farm 2 where transfers were done in both the cool and hot seasons, there was an interaction ($P < 0.03$) between season and embryo treatment on overall calving rate (cool season: control – 4/13 = 30.8% versus IGF-1 – 1/17 = 5.9%; hot season: control – 5/38 = 13.2% versus IGF-1 – 10/30 = 33.3%) and live calving rate (cool season: control – 4/13 = 30.8% versus IGF-1 – 1/17 = 5.9%; hot season: control – 5/38 = 13.2% versus IGF-1 – 9/29 = 31.0%).

When data were analyzed from the cool season only, there was no effect of IGF-1 on overall calving rate or live calving rate (Table 1). However, when data from the hot season were analyzed separately, IGF-1 tended to increase both overall calving rate ($P < 0.06$) and live calving rate ($P < 0.09$). When data were analyzed separately for each treatment group, recipients that received IGF-1 treated embryos tended ($P < 0.10$) to have a higher overall calving rate in the hot season compared to the cool season, but there was no difference in live calving rate. For control embryo recipients, there was no significant effect of season on either overall or live calving rate although, numerically, calving rates were greater for the cool season.

3.4. Pregnancy loss

Pregnancy loss was 52.8% (96/182) between Days 21 and 30 of gestation. A total of 10.8% (10/93) and

Table 3
Effect of season and IGF-1 on pregnancy loss among all recipient cattle

	Pregnancy loss, number of losses/total pregnancies (%) ^a		
	Days 21–30 ^{b,c}	Days 30–45	Day 45 to term ^d
Cool season			
Control	24/48 = 50.0%	3/26 = 11.5%	2/15 = 13.3%
IGF-1	24/42 = 57.1%	3/22 = 13.6%	2/13 = 15.4%
Hot season			
Control	29/41 = 70.7%	1/14 = 7.1%	4/9 = 44.4%
IGF-1	19/51 = 37.3%	3/31 = 9.7%	2/12 = 16.7%

^a Differences in the number of pregnancies at each time point is due to some recipients not being diagnosed for pregnancy at all time points as well as not having calving data for Farm 3.

^b Treatment $P < 0.07$.

^c Season \times treatment $P < 0.01$.

^d Data exclude Farm 3.

20.4% (10/49) of pregnant recipients lost their pregnancies from Days 30 to 45 and Day 45 to term, respectively.

There was an interaction ($P < 0.01$) between season and embryo treatment affecting pregnancy loss from Days 21 to 30. Pregnancy loss in the cool season was not different between recipients that received control versus IGF-1 embryos but recipients in the hot season that received control embryos had more pregnancy loss than recipients receiving IGF-1 treated embryos (Table 3).

For recipients at Farms 2 and 3 where transfers were done in both seasons, there was also an interaction ($P < 0.02$) between season and embryo treatment affecting pregnancy loss from Days 21 to 30 (cool season: control – 15/27 = 55.6% versus IGF-1 – 14/

Table 4
Effect of season and IGF-1 on pregnancy loss among recipient cattle that received embryos that were cultured in 5% O₂ and harvested on Day 7

	Pregnancy loss, number of losses/total pregnancies (%) ^a		
	Days 21–30 ^{b,c}	Days 30–45	Day 45 to term ^d
Cool season			
Control	13/25 = 52.0%	2/12 = 16.7%	0/5 = 0%
IGF-1	12/24 = 50.0%	3/14 = 21.4%	1/6 = 16.7%
Hot season			
Control	29/41 = 70.7%	1/14 = 7.1%	4/9 = 44.4%
IGF-1	19/51 = 37.3%	3/31 = 9.7%	2/12 = 16.7%

^a Differences in the number of pregnancies at each time point is due to some recipients not being diagnosed for pregnancy at all time points as well as not having calving data for Farm 3.

^b Treatment $P < 0.05$.

^c Season \times treatment $P < 0.07$.

^d Data exclude Farm 3.

19 = 73.7%; hot season: control – 29/41 = 70.7% versus IGF-1 – 19/51 = 37.3%) and Days 21–45 (cool season: control – 16/26 = 61.5% versus IGF-1 – 16/21 = 76.2%; hot season: control – 31/41 = 75.6% versus IGF-1 – 23/49 = 46.9%).

Among the subset of recipients that received embryos that were cultured in 5% O₂ and harvested on Day 7, pregnancy loss between Days 21 and 30 was lower ($P < 0.05$) for recipients that received IGF-1 treated embryos than for controls (Table 4). There was also a tendency for an interaction ($P < 0.07$) between season and IGF-1 on pregnancy loss between Days 21 and 30 (Table 4). When pregnancy loss data were analyzed from the cool season only, there was no effect of IGF-1 on pregnancy loss. However, when data from the hot season were analyzed separately, IGF-1 embryo recipients had lower pregnancy loss ($P < 0.04$) from Days 21 to 30. When data were analyzed separately among treatment groups, recipients that received control embryos had higher ($P < 0.05$) pregnancy loss between Days 21 and 30 in the hot season compared to the cool season. Conversely, recipients that received IGF-1 treated embryos had lower pregnancy loss between Days 21 and 30 in the hot season compared to the cool season ($P < 0.06$).

3.5. Gestation length

There were no effects of embryo treatment on gestation length whether all calves or only live calves were analyzed. Recipients that received embryos in the hot season had shorter ($P < 0.04$) gestation lengths than recipients that received embryos in the cool season (all calves: cool season – 278.8 \pm 1.1 days versus hot season – 274.3 \pm 1.4 days; live calves: cool season – 278.5 \pm 1.1 days versus hot season – 274.4 \pm 1.4 days).

3.6. Calf sex ratio and birth weight

The calf sex ratio was different ($P < 0.002$) than the expected 50:50 ratio. In particular, there was a

Table 5
Effect of IGF-1 on calf birth weight (least-squares means \pm S.E.M.) and sex ratio

	Birth weight (kg)	<i>n</i>	Male calves (%)
All calves			
Control	46.6 \pm 3.0	9	15/19 = 79.0%
IGF-1	48.2 \pm 2.9	11	16/21 = 76.2%
Live calves			
Control	47.4 \pm 2.2	9	13/17 = 76.5%
IGF-1	46.8 \pm 2.1	10	15/20 = 76.0%

preponderance of male calves among all calves born ($31/40 = 77.5\%$) as well as live calves only ($28/37 = 75.7\%$). There were no effects of embryo treatment, season of transfer, farm-season or gestation length on calf sex ratio (Table 5). The proportion of male calves born following artificial insemination at Farm 2 was 50% for all calves ($27/54$) as well as all live calves ($26/52$); this was lower ($P < 0.04$) than the proportion of male calves born following embryo transfer at Farm 2 (all calves: $16/20 = 80\%$ and live calves: $15/19 = 79.0\%$).

Calf birth weight was recorded for 20 calves at Farm 2. There were no effects of embryo treatment, season of transfer, or calf sex on calf birth weight (Table 5). Of the 20 calves, one was born dead. This calf was from the IGF-1 treatment group and weighed 68.2 kg at birth. For the 19 calves born alive, there was also no effect of embryo treatment, season of transfer, or calf sex on calf birth weight (Table 5). Calves born following AI at Farm 2 had lower ($P < 0.001$) birth weights than for calves born following embryo transfer. This was true for all calves (artificial insemination – 41.1 ± 0.8 versus embryo transfer – 48.2 ± 1.3 kg) as well as all live calves (artificial insemination – 41.2 ± 0.8 versus embryo transfer – 47.1 ± 1.3 kg).

4. Discussion

The objective of the present experiment was to determine whether culturing embryos in the presence of IGF-1 would increase pregnancy and calving rates following the transfer of in vitro produced bovine embryos to lactating dairy cows. In this study, pregnancy and calving rates were increased by IGF-1 in the hot season but not the cool season. Although heat stress tended to reduce post-transfer survival of control embryos, treatment of embryos with IGF-1 blocked this effect and, in fact, caused an increase in pregnancy rate greater than the reduction caused by heat stress. The calves born as a result of IGF-1 treatment were similar to those derived from control embryos. Thus, IGF-1 treatment improved the efficacy of in vitro embryo transfer during summer without additional alterations in gestation length or calf birth weight. Results also indicated some limitations to the transfer of in vitro produced embryos, including high fetal loss, increased calf birth weight, and skewed sex ratio.

Treatment of embryos with IGF-1 improved embryo survival following transfer in the hot season while not having any effect on pregnancy and calving rates in the cool season. This interaction between embryo treatment and season of transfer on pregnancy rates was seen for

the entire data set, for the subset of recipients at Farms 2 and 3 where transfers were done in both seasons, and for the subset of recipients that received embryos that were cultured in 5% O₂ and harvested on Day 7. In addition, when data from the cool season were analyzed, there was no effect of IGF-1 on pregnancy and calving rates. In contrast, when data from the hot season were analyzed, IGF-1 embryo treatment increased pregnancy and calving rates. That IGF-1 increased pregnancy rate in the hot season was in agreement with a previous report in which treatment of embryos with IGF-1 increased pregnancy and calving rates in heat-stressed, lactating dairy cows [14].

The mechanism by which IGF-1 improves post-transfer embryo survival during heat stress is not known. However, IGF-1 is a survival factor for the preimplantation embryo and can reduce deleterious effects of heat shock on development to the blastocyst stage and apoptosis [18,20]. Although embryos have acquired substantial resistance to elevated temperature by the blastocyst stage of development [3,4], results from the current study and others [9,10] indicate that there is a reduction in post-transfer survival of embryos during heat stress. Such an effect could represent actions on the embryo or mother (for example, reduced blood progesterone concentrations [26]). One possibility is that the increased survival for IGF-1 treated embryos represents an improved capacity of the embryo to withstand exposure to maternal hyperthermia following transfer.

Perhaps IGF-1 altered developmental processes in a way that resulted in blastocysts with increased capacity for survival when maternal function was compromised (as may be the case during heat stress). An increase in embryo development to the blastocyst stage following addition of IGF-1 to bovine embryo culture medium has been reported many times [11–17]. In the present study, IGF-1 treatment increased blastocyst development on Day 7 after insemination but had no effect on Day 8. Although statistically significant, the increase in blastocyst development on Day 7 was only 2.1%; this was similar to the increase in embryo development for IGF-1 treated embryos observed in a previous report from our laboratory [15], but smaller than previous reports with IGF-1 [13,14,16,17]. Differences in the effect of IGF-1 on embryo development may be partly explained by differences in culture systems because there are reports that effects of IGF-1 on embryonic development depend upon culture conditions [11,27].

The effects of IGF-1 to increase pregnancy rate in the summer involve more than simply reversing the deleterious effects of season on embryonic survival.

In that regard, pregnancy and calving rates for IGF-1 embryo recipients in the hot season were higher than the pregnancy and calving rates of the control embryo recipients in the cool season. It is not clear at the present time why there would be a synergistic effect between IGF-1 and heat stress on embryo survival. Perhaps positive effects of IGF-1 can be offset by other actions of IGF-1 that reduce embryonic survival and the predominating effect (positive, negative, or no effect) depends upon characteristics of the oocyte used to produce embryos or the recipient. Indirect evidence for this idea comes from studies with the IGF-1 secretagogue, bovine somatotropin. Administration of somatotropin can increase the proportion of cows pregnant following timed AI if cows are lactating [28–30]. In contrast, somatotropin administration decreased the proportion of non-lactating cows pregnant following timed AI [31].

One possibility is that IGF-1 treated embryos are able to overcome alterations in uterine function caused by heat stress. For example, the secretion of prostaglandin $F_{2\alpha}$ from the endometrium of pregnant cows is increased by heat shock [32]. Since IGF-1 treated embryos can be more advanced in development [14,15] and have increased cell numbers [13,14,16] they may be able to block this increase in prostaglandin $F_{2\alpha}$ secretion by producing more interferon- τ . Conversely, during cool periods when prostaglandin $F_{2\alpha}$ secretion is less likely to be altered, this effect of IGF-1 may not be beneficial.

Overall pregnancy loss between Day 21 and term in the present study was 70.2% (80/114). A total of 50.2% (96/182) of pregnancies were lost between Days 21 and 30 of gestation; this period was a major source of pregnancy loss. It is likely that the Day 21 pregnancy rate is an overestimate and therefore should be interpreted carefully. Other factors such as recipient asynchrony, extended estrous cycles (>21 days), luteal cysts and subclinical uterine infections could have contributed to elevated plasma progesterone. It is also important to note, however, that similar pregnancy losses between Days 21 and 22 and Days 42–52 have been reported in lactating dairy cows following AI and embryo transfer [6,7,33].

Interestingly, Days 21–30 of gestation was also the time during which IGF-1 had a major effect on embryo survival. The beneficial effect of IGF-1 on embryo survival during this time period was only evident during the hot season. Although there was no difference in pregnancy loss between IGF-1 and control embryos from Days 21–30 in the cool season, there was significantly less pregnancy loss from Days 21–30

for IGF-1 embryos compared to controls during the hot season. Perhaps IGF-1 treatment from Days 1 to 7 after insemination affects events after the time of maternal recognition of pregnancy and during the peri-attachment period of gestation. These events could include overall growth of the embryo or the program of gene expression. One possibility is that IGF-1 treatment increases conceptus size but this effect is only beneficial for embryo survival under stressful conditions, e.g. hyperthermia. Such a dichotomy has been observed for the effect of somatotropin on conceptus length and pregnancy rates in dairy cattle. Although somatotropin treatment increased conceptus length at Day 17 in both lactating and non-lactating dairy cows, only lactating dairy cows have improved pregnancy rates following somatotropin treatment [31,34]. Another possible explanation involves the formation of the embryonic disc. Whereas only 35–72.6% of in vitro produced embryos recovered at Days 14–16 have a detectable embryonic disc [35,36], the addition of IGF-1 to embryo culture increased the number of cells in the inner cell mass [16]. Thus IGF-1 treatment may result in a more viable embryonic disc which is more capable of withstanding heat stress.

Embryonic loss between Days 30 and 45 was 10.8%, within the range reported for lactating dairy cows following AI [9,30,33,37] or embryo transfer with superovulated embryos [9,37] during similar time periods. Fetal loss (from Day 45 to calving) in the present study was 20.4%. In a previous report from our laboratory in which in vitro produced embryos were transferred to lactating dairy cows, pregnancy loss from Day 53 of gestation to calving was 24.0% [14]. These values seemed high compared to values ranging from 7.6 to 13.1% for fetal loss between Days 50 and 60 of gestation and calving for pregnancies established with in vitro produced embryos [38,39] and values of 10.0% for fetal loss rate between Days 40 and 50 of gestation and term for lactating cows in Florida bred by AI [40]. It is also possible that the oocyte or culture system used to produce embryos resulted in a large proportion of conceptuses incapable of completing fetal development. Another possible contributing factor is lactational status because lactating dairy cows were used as recipients here compared with the heifer recipients used elsewhere [38,39]. Fetal losses in females impregnated by AI are higher in lactating cows than heifers [40]. In another study from our laboratory, pregnancy losses between Day 67 of gestation and term were 6.7% when single in vitro produced embryos were transferred into heifers or crossbred dairy cows producing low amounts of milk [41].

The sex ratio of calves born in the present study was significantly different from the sex ratio of calves born following AI, as well as the expected 50:50 ratio with 31/40 (77.5%) calves being male. Several previous studies have reported a skewed sex ratio in favor of males following the transfer of in vitro produced embryos with a range of 55.4–82.0% [39,42,43]. The sex ratio of 77.5% in this study was higher than that reported in a previous study from our laboratory in which the sex ratio was 64.3% males [15]. The increase in the proportion of male calves in this study was likely due to the fact that most of the embryos in the present study were selected on Day 7 following insemination compared to Day 8 in the previous report. Male embryos develop to the blastocyst stage in vitro faster than female embryos [44,45]. In addition, the high proportion of male calves was most likely due in large part to a skewed sex ratio at the time of embryo selection. The sex ratio of in vitro produced embryos at or beyond the morula stage in our laboratory was 69.5% males [15]. Although an increase in the maximum air temperature around the time of conception has been associated with an increase in the proportion of male calves [46], there was no effect of season on calf sex ratio in the present study.

The mean birth weight of embryo transfer calves was 6–7 kg higher than calves born following AI, consistent with previous reports in which birth weights of calves produced following in vitro embryo production were higher than for calves produced following AI [43,47]. Caution must be used in interpreting the observed difference because sires differed between embryo transfer calves and artificial insemination calves. Although addition of IGF-1 to bovine embryo culture increased blastocyst cell number [13,14,16], it had no effect on calf birth weight. This result agrees with a previous report from our laboratory in which IGF-1 treatment improved pregnancy rates but did not alter calf birth weight [15].

There are important practical implications of the present findings. Embryo transfer has been proposed as a tool for increasing pregnancy rate in the summer because the embryo becomes more resistant to elevated temperature as it advances in development [3,4]. Indeed, use of embryo transfer has been shown to improve pregnancy rates during heat stress in Florida [5–8] and Brazil [48]. While embryos can be produced following superovulation, in vitro embryo production can be a more practical alternative for the large-scale production of embryos [49]. The improvement in pregnancy rates caused by culture with IGF-1 resulted in a pregnancy rate at Day 45 for embryo recipients in

the hot season of 41.8% (28/67). This pregnancy rate is much higher than the pregnancy rates (14–19%) in two previous studies evaluating the effectiveness of in vitro embryo transfer in the summer [6,8]. We inferred that addition of IGF-1 to embryo culture improved the effectiveness of in vitro embryo transfer in the summer when compared to AI, and resulted in pregnancy rates comparable to those achieved using AI in cool weather.

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