

The effect of in vitro treatment of bovine embryos with IGF-1 on subsequent development in utero to Day 14 of gestation

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Abstract

Culture of bovine embryos with insulin-like growth factor-1 (IGF-1) can improve development to the blastocyst stage and embryo survival following transfer to heat-stressed, lactating dairy cows. Two experiments were conducted to determine whether IGF-1 could improve embryo survival and development at Day 14 after ovulation. In Experiment 1, non-lactating Holstein cows ($n = 58$) were selected as recipients following synchronization for timed-embryo transfer. Embryos were produced in vitro and cultured with or without 100 ng/mL IGF-1. At Day 7 after expected ovulation (Day 0), groups of 7–12 embryos were randomly transferred to each recipient. Embryos were recovered at Day 14. Embryo length and the presence or absence of an embryonic disc was recorded. Recovered embryos were cultured individually for 24 h to determine interferon- τ (IFN- τ) secretion. There was no effect of IGF-1 on embryo recovery rate, embryo length or IFN- τ secretion. In Experiment 2, non-lactating ($n = 56$) and lactating ($n = 35$) Holstein cows were selected as recipients following synchronization for timed-embryo transfer. Embryos were produced as described in Experiment 1. At Day 7 after expected ovulation (Day 0), a single embryo was randomly transferred to each recipient. Embryos were recovered at Day 14. Embryo length and IFN- τ secretion were determined as in Experiment 1. Recovery rate at Day 14 tended ($P = 0.1$) to be higher for recipients that received IGF-1 treated embryos compared to control embryos (43.2% versus 26.1%, respectively). There was no effect of IGF-1 on embryo length or IFN- τ secretion. In conclusion, results suggest that exposure to IGF-1 through Days 7–8 of development does not enhance capacity of embryos to prevent luteolysis. Results of the single embryo-transfer experiment suggested that IGF-1 treatment might affect embryo survival post-transfer as early as Day 14 after ovulation. Further experimentation is warranted to verify this finding.

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1. Introduction

Early embryonic development is coordinately regulated by several molecules secreted by the

maternal reproductive tract and embryo. Among such molecules, growth factors play an important role because they can regulate mitogenesis, differentiation, metabolism and apoptosis [1–3]. Despite their actions on early embryonic development, growth factors are not routinely included in embryo culture medium. The lack of exposure to the proper growth factor milieu may be one reason why embryos produced in vitro differ from their counterparts derived in vivo in terms of metabolism [4], gene expression [5–7], and survival and development after transfer [8–12].

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One growth factor that modifies embryonic physiology is insulin-like growth factor-1 (IGF-1). Addition of IGF-1 to culture medium can reduce the proportion of blastomeres that are apoptotic [13,14], alter the abundance of some developmentally important genes [15], and increase cellular resistance to heat shock [16,17]. Also, IGF-1 can increase development of bovine embryos to the blastocyst stage [13,18–24] and increase blastocyst cell number [13,20,22], although these effects are not always observed [15,19]. Treatment of embryos with IGF-1 in culture can also improve pregnancy rates following transfer of embryos to heat-stressed, lactating dairy cows [21,24].

At present, the reasons for enhanced survival of IGF-1 treated embryos post-transfer are not clear. One possibility is that actions of IGF-1 on embryo development *in vitro* may allow for improved conceptus development or hormone secretion around the time of maternal recognition of pregnancy when the embryo undergoes elongation and secretes interferon- τ (IFN- τ). Treatment of lactating cows with bovine somatotropin tended to increase the proportion of inseminated cows that had a recoverable conceptus at Day 17 of pregnancy as well as conceptus size. In addition, the total amount of IFN- τ in uterine flushings was increased by somatotropin treatment [25]. Effects of somatotropin could be mediated by IGF-1, because concentrations in blood are elevated by somatotropin treatment. Alternatively, these somatotropin-mediated actions are independent of IGF-1. The objective of the present study was to determine whether treatment of embryos with IGF-1 during culture would improve embryo survival to Day 14 after ovulation. Moreover, it was hypothesized that embryos treated with IGF-1 would have increased length and interferon- τ secretion at Day 14 compared to controls.

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 [26]. 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 μ g/mL estradiol 17- β , 20 μ g/mL bovine FSH (Folltropin-V; Bioniche, Bellevue, Ontario, Canada), 22 μ g/mL sodium pyruvate, 50 μ g/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-bovine embryo 2 (KSOM-BE2) as described previously [27]. Recombinant human IGF-1 was obtained from Upstate Biotech (Lake Placid, NY, USA) and recombinant human IFN- α (3.84×10^8 IU/mg) was from EMD Biosciences (San Diego, CA, USA). Prostaglandin F $_{2\alpha}$ (PGF) was Lutalyse[®] from Pharmacia & UpJohn (New York, NY, USA) and GnRH was Cystorelin[®] from Merial (Duluth, GA, USA). Controlled internal drug releasing devices (CIDR) were purchased from Pfizer (New York, NY, USA) and lidocaine was from Pro Labs (St. Joseph, MO, USA).

2.2. *In vitro* embryo production

Embryos were produced *in vitro* as described previously [27]. Briefly, cumulus–oocyte complexes (COCs) were obtained by slicing 2–10 mm follicles on the surface of ovaries (predominantly beef cattle) obtained from Central Beef Packing Co. (Center Hill, FL, USA). Those COCs with multiple layers of compact cumulus cells were washed two times in OCM and used for subsequent steps. Groups of 10 COCs were placed in 50- μ L drops of OMM overlaid with mineral oil and matured for 21–24 h at 38.5 °C in an atmosphere of 5% (v/v) CO $_2$ in humidified air. Matured COCs were then washed once in Hepes-TALP and transferred in groups of 30 to four-well plates containing 600 μ L of IVF-TALP and 25 μ L of PHE (0.5 mM penicillamine, 0.25 mM hypotaurine, and 25 μ M epinephrine in 0.9% (w/v) NaCl) per well and fertilized with $\sim 1 \times 10^6$ Percoll-purified spermatozoa from a pool of frozen-thawed semen from three bulls. Spermatozoa were allowed to co-incubate with COCs for 20–24 h at 38.5 °C in an atmosphere of 5% (v/v) CO $_2$ in humidified air. After fertilization, putative zygotes were removed from fertilization wells, denuded of cumulus cells by vortex mixing in Hepes-TALP containing 1000 U/mL hyaluronidase, and randomly placed in groups of 25 in

50- μ L drops of either KSOM-BE2 or KSOM-BE2 containing 100 ng/mL IGF-1 as described previously [21]. All drops of embryos were overlaid with mineral oil and cultured at 38.5 °C in an atmosphere of 5% CO₂ (Experiment 1) or 5% CO₂, 5% O₂ and 90% N₂ (Experiment 2). The proportion of cleaved oocytes was recorded on Day 3 after insemination and the proportion of blastocysts and advanced blastocysts (expanded and hatched) was recorded on Day 7 (Experiment 2) or Day 8 (Experiment 1).

2.3. Experiment 1: group embryo transfer

2.3.1. Animals

Non-lactating Holstein cows at the University of Florida Dairy Research Unit (Hague, FL, USA; 29.77904N, 82.48001W) were used as embryo transfer recipients. Cows were kept on pasture and supplemented with corn silage, grass hay and free-choice mineral. Animals were synchronized for timed-embryo transfer using a modified Ovsynch protocol with the inclusion of a CIDR [28]. Cows received 100 μ g of GnRH (i.m.) and a CIDR (intravaginal deposition) 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 0 (day of expected ovulation). Also on Day 0, the ovaries of all cows were examined using an Aloka 500 ultrasound (Aloka America, Wallingford, CT, USA) equipped with a 5 MHz linear array transducer to determine the presence or absence of a corpus luteum.

2.3.2. Embryo transfer

On Day 8 after fertilization, Grade 1 blastocyst and expanded blastocyst stage embryos [29] were harvested from culture. Selected embryos were placed into holding medium [Hepes-TALP containing 10% (v/v) fetal bovine serum and 100 μ M β -mercaptoethanol] and loaded into 0.25 mL French straws in groups of 7–12 (depending on the replicate). Embryos were loaded so that similar numbers of blastocyst and expanded blastocyst stage embryos were placed into each straw for both treatment groups. The mean number of embryos transferred/recipient was 9.8 for controls (5.1 blastocysts and 4.6 expanded blastocysts) and 10.0 for the IGF-1 group (5.4 blastocysts and 4.5 expanded blastocysts). Once embryos were loaded, the straws were then placed into a portable incubator set at 39 °C and transported to the farm for transfer to recipients.

At Day 7 after expected ovulation, the ovaries of all cows were scanned again using ultrasonography to

determine the presence or absence of a corpus luteum. Only cows which had a visible corpus luteum on Day 7 and no visible corpus luteum on Day 0 were selected for transfer. A total of 58 cows were selected as embryo transfer recipients based on these criteria. Selected recipients received epidural anesthesia (5 mL of 2% lidocaine) and groups of embryos were then randomly transferred to the uterine horn ipsilateral to the ovary with a corpus luteum.

2.3.3. Embryo recovery, evaluation and culture

On Day 14 after expected ovulation, the ovaries of all cows were scanned again using ultrasonography to determine the presence or absence of a corpus luteum. All 58 recipients selected for transfer on Day 7 had a visible corpus luteum on Day 14. For each recipient, the ipsilateral and contralateral uterine horns were flushed with Dulbecco's Phosphate Buffered Saline (DPBS) to recover embryos. For three replicates, recipients were slaughtered, the reproductive tracts were collected and the uterine horns were flushed with 100 mL of DPS. For seven replicates, embryos were recovered using non-surgical embryo recovery techniques. The uterine horns of each recipient were flushed separately using 18–20 French Foley catheters and the flushing procedure continued until 500 mL of DPBS had been recovered from each uterine horn.

Following embryo recovery, embryo length, embryo stage, and the presence or absence of an embryonic disc was assessed by light microscopy using a stereomicroscope. The stage of each embryo was classified into one of four groups based on embryo shape: (1) spherical, (2) ovoid, (3) tubular, and (4) filamentous. After all measurements were recorded, embryos were then placed into 5 mL of TCM-199 containing 200 U/mL penicillin-G and 0.2 mg/mL streptomycin and cultured at 38.5 °C in 5% CO₂. After approximately 24 h of culture, the medium was harvested and stored at –80 °C until further processing.

2.4. Experiment 2: single embryo transfer

2.4.1. Animals

For seven replicates, non-lactating Holstein cows at the University of Florida Dairy Research Unit (Hague, FL, USA; 29.77904N, 82.48001W) were used for embryo transfer recipients as described for Experiment 1. For four replicates, lactating Holstein cows at a commercial dairy in Florida (Bell, FL; 29.75578N, 82.86188W) were used as embryo transfer recipients. Lactating cows between 64 and 615 days in milk (mean = 193) were housed in a free-stall barn, fed a

total-mixed ration and milked thrice daily. Regardless of location, animals were synchronized for timed-embryo transfer using the Ovsynch protocol [30]. Cows received 100 µg of GnRH (i.m.) on Day –10, followed 7 days later (Day –3) by 25 mg PGF. On Day –1, a second injection of GnRH was administered and the ovaries of all cows were examined ultrasonographically as described in Experiment 1 to determine the presence or absence of a corpus luteum. Day 0 was defined as the day of expected ovulation.

2.4.2. Embryo transfer

On Day 7 after fertilization, Grade 1 embryos [29] at the blastocyst and expanded blastocyst stages were harvested from culture. Embryos were placed into 1.5 mL of holding medium in 2 mL microcentrifuge tubes, placed into a portable incubator set at 39 °C and transported to the farm for transfer to recipients. Upon arrival at the farm, Grade 1 embryos were loaded individually into 0.25 mL French straws in holding medium.

At Day 7 after expected ovulation, the ovaries of all cows were scanned again using ultrasonography to determine the presence or absence of a corpus luteum. Only cows which had a visible corpus luteum on Day 7 and no visible corpus luteum on Day –1 were selected for transfer. A total of 56 non-lactating and 35 lactating cows were selected as embryo transfer recipients. Selected recipients received epidural anesthesia (5 mL of 2% lidocaine) and a single embryo was then randomly transferred to the uterine horn ipsilateral to the ovary with a corpus luteum.

2.4.3. Embryo recovery, evaluation and culture

At Day 14 after expected ovulation, the ovaries of all cows were examined again using ultrasonography to determine the presence or absence of a corpus luteum. All 91 recipients selected for transfer had a visible corpus luteum at Day 14. Non-surgical embryo recovery procedures were used at Day 14 after expected ovulation as described in Experiment 1. Embryos were also evaluated and cultured as in Experiment 1 except that the presence or absence of an embryonic disc was not recorded in Experiment 2.

2.5. Analysis of interferon- τ secretion

The quantity of biologically active IFN- τ in embryo culture medium after 24 h culture was determined using an antiviral assay based on the inhibition of vesicular stomatitis virus-induced lysis of Madin-Darby bovine kidney cells [31]. The dilution of sample that prevented

virus-induced lysis by 50% was converted to ng/mL of IFN- τ by comparison to activity of a recombinant bovine IFN- τ standard [32] that was included in the assay. The specific activity of the bovine IFN- τ standard (1.68×10^8 IU/mg) was determined by comparison to a recombinant human IFN- α standard also included in the assay (EMD Biosciences, San Diego, CA, USA; 3.84×10^8 IU/mg).

2.6. Statistical analysis

Percentage data were transformed by arcsin transformation before analysis. Probability values for percentage data are based on analysis of arcsin-transformed data while least-squares means are from analysis of untransformed data.

The proportion of oocytes that cleaved, that developed to the blastocyst stage (i.e. total blastocysts including non-expanded, expanded, etc.) on Day 7 (Experiment 2) or Day 8 (Experiment 1) and that developed to advanced blastocyst stages (expanded, hatching or hatched) on Day 7 (Experiment 2) or Day 8 (Experiment 1) were calculated for each replicate in each experiment. Treatment effects were analyzed using least-squares analysis of variance using the General Linear Models procedure of SAS (SAS for Windows, version 9.0, SAS Inst., Inc., Cary, NC, USA). The model included the main effects of replicate and treatment. All values reported are least-squares means \pm S.E.M.

Recovery rate in Experiment 1, as well as embryo length and IFN- τ secretion in both experiments, was analyzed by analysis of variance using the GLM procedure of SAS. The statistical model in Experiment 1 included treatment, flush type (i.e. slaughter versus live animal), cow (flush type \times treatment) and treatment \times flush type. For Experiment 2, the statistical model included replicate, treatment, lactation and all two-way interactions. For IFN- τ secretion, data were analyzed with and without embryo length as a covariate. All values obtained from the GLM procedure are reported as least-squares means \pm S.E.M. The correlation between embryo length and IFN- τ secretion was analyzed using the CORR procedure of SAS.

Embryo recovery in Experiment 2 and the proportion of embryos that had a visible embryonic disc at Day 14 after ovulation in Experiment 1 were analyzed by logistic regression using the LOGISTIC procedure of SAS. The statistical model for each experiment was the same as described above. The data are reported as the actual percentage.

Embryo stage in both experiments was analyzed using both the LOGISTIC and CATMOD procedures of

SAS. The statistical models for each experiment were the same as described above. The statistical values obtained after analysis with LOGISTIC and CATMOD were similar and only statistical inferences from the LOGISTIC analysis are reported. Data are reported as the actual percentage.

3. Results

3.1. Experiment 1: group embryo transfer

3.1.1. Embryo development in vitro

Addition of IGF-1 to culture increased ($P < 0.05$) cleavage rate on Day 3 (control, $80.9 \pm 0.8\%$ versus IGF-1, $84.0 \pm 0.8\%$). However, there was no effect of IGF-1 on the proportion of oocytes that developed to the blastocyst stage (control, $27.3 \pm 1.6\%$ versus IGF-1, $28.7 \pm 1.6\%$) or advanced blastocyst stages (control, $14.9 \pm 0.6\%$ versus IGF-1, $14.7 \pm 0.6\%$) on Day 8 after insemination.

3.1.2. Embryo recovery and development at Day 14

Supplementation of culture medium with IGF-1 did not affect the proportion of embryos recovered at Day 14 (Table 1). Among embryos recovered, there was also no effect of IGF-1 on embryo length, IFN- τ secretion, or the proportion of embryos with a visible embryonic disc (Table 1). In addition, treatment with IGF-1 did not affect embryo stage at Day 14. The percent of recovered embryos that were classified spherical, ovoid, tubular and filamentous was 13.3, 32.5, 33.7 and 20.5% for control embryos and 12.9, 32.9, 32.9, and 21.4% for embryos treated with IGF-1.

Recovery rate and embryo length were affected by flush type (i.e. slaughter versus live animal). Recovery rate tended to be greater ($P < 0.06$) for embryos that were recovered after slaughter than for embryos collected by non-surgical procedures ($37.8 \pm 6.2\%$ versus $21.8 \pm 4.1\%$, respectively). In addition, embryo

Table 1
Effect of IGF-1 on recovery rate, embryo length, IFN- τ secretion and embryonic disc formation at Day 14 after ovulation in Experiment 1

Variable	Control	No.	IGF-1	No.
Recovery rate (%)	30.4 ± 5.1	294	29.2 ± 5.5	260
Embryo length (mm)	5.4 ± 5.5	83	5.9 ± 5.8	70
IFN- τ (ng/mL)	29.2 ± 7.5	51	22.5 ± 7.5^a	54
	26.8 ± 9.0	51	22.5 ± 8.5^b	54
Embryonic disc (%)	75.8	62	77.9	54

^a Analysis includes embryo length as a covariate.

^b Analysis performed without embryo length as a covariate.

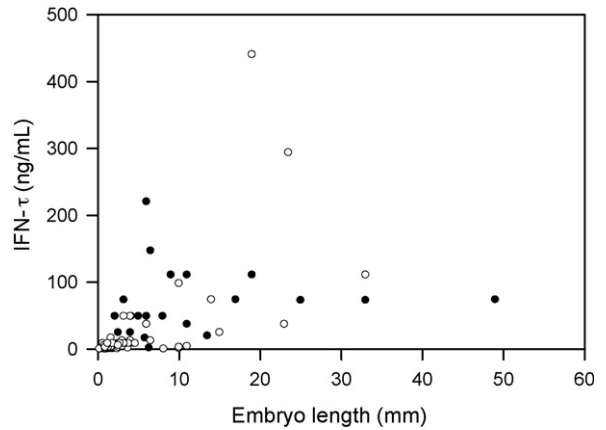


Fig. 1. Relationship between embryo length and IFN- τ secretion for control embryos (black circles) and IGF-1 embryos (open circles) recovered at Day 14 in Experiment 1. The correlation between embryo length and IFN- τ secretion was $r = 0.5$ ($P < 0.001$).

length was greater ($P < 0.01$) for embryos recovered following slaughter than for embryos recovered from live recipients (7.7 ± 0.87 mm versus 3.7 ± 0.85 mm, respectively). There was no effect of flush type on IFN- τ secretion, the proportion of embryos with a visible embryonic disc or embryo stage at Day 14. Moreover, there was no interaction between embryo treatment and flush type on any of the variables analyzed.

Embryo length was a significant covariate for IFN- τ secretion ($P < 0.001$) and there was a positive correlation ($r = 0.5$; $P < 0.001$) between embryo length and IFN- τ secretion (Fig. 1).

3.2. Experiment 2: single embryo transfer

3.2.1. Embryo development in vitro

Addition of IGF-1 to embryo culture did not affect cleavage rate on Day 3 (control, $85.2 \pm 1.2\%$ versus IGF-1, $85.3 \pm 1.2\%$) or the proportion of oocytes that developed to advanced blastocyst stages on Day 7 (control, $11.3 \pm 2.2\%$ versus IGF-1, $14.2 \pm 2.2\%$). However, the proportion of oocytes that developed to the blastocyst stage on Day 7 tended to be increased ($P < 0.08$) for embryos treated with IGF-1 compared to controls ($23.8 \pm 1.8\%$ versus $18.6 \pm 1.8\%$, respectively).

3.2.2. Embryo recovery and development at Day 14

There was a tendency ($P = 0.1$) for recovery rate to be higher for recipients that received IGF-1 treated embryos than for those recipients that received control embryos (Table 2). There was no effect of IGF-1 on embryo length or IFN- τ secretion (Table 2). Additionally, IGF-1 did not affect embryo stage. The percent of

Table 2
Effect of IGF-1 on recovery rate, embryo length and IFN- τ secretion at Day 14 after ovulation in Experiment 2

Variable	Control	No.	IGF-1	No.
Recovery rate (%)	26.1	46	43.2 ^a	37
Embryo length (mm)	24.1 \pm 9.2	12	28.8 \pm 8.6	15
IFN- τ (ng/mL)	284.5 \pm 56.2	10	329.2 \pm 47.5 ^b	14
	264.0 \pm 131.1	10	354.5 \pm 113.6 ^c	14

^a Treatment $P = 0.1$.

^b Analysis includes embryo length as a covariate.

^c Analysis performed without embryo length as a covariate.

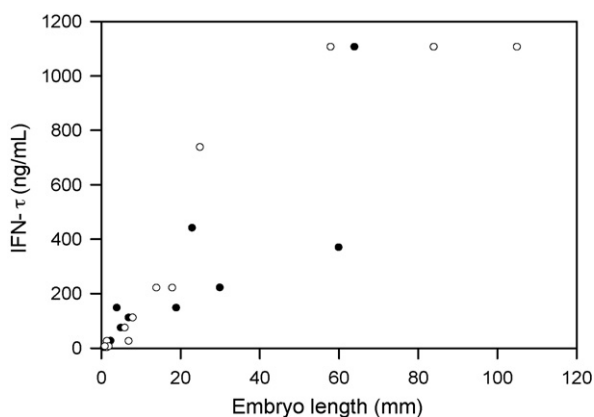


Fig. 2. Relationship between embryo length and IFN- τ secretion for control embryos (black circles) and IGF-1 embryos (open circles) recovered at Day 14 in Experiment 2. The correlation between embryo length and IFN- τ secretion was $r = 0.9$ ($P < 0.001$).

recovered embryos that were classified as spherical, ovoid, tubular and filamentous was 0, 8.3, 33.3, and 58.3% for control embryos and 6.3, 18.8, 37.5, and 37.5% for embryos treated with IGF-1.

There was no effect of lactation or an interaction between embryo treatment and lactation on recovery rate, embryo length, IFN- τ secretion, or embryo stage at Day 14 after ovulation.

As in Experiment 1, embryo length was a significant covariate for IFN- τ secretion ($P < 0.001$) and there was a positive correlation ($r = 0.9$; $P < 0.001$) between embryo length and IFN- τ secretion (Fig. 2).

4. Discussion

The objective of the present set of experiments was to test the hypotheses that supplementation of embryo culture medium with IGF-1 for the first 7–8 days after insemination would increase post-transfer embryo survival at Day 14 after ovulation, improve embryonic development, increase embryo length, and enhance embryonic IFN- τ secretion. Results on embryonic

survival were equivocal because there was a tendency for IGF-1 to increase survival at Day 14 in one experiment but there was no effect in the other. In both experiments, however, results were more consistent with respect to embryo development, length and IFN- τ secretion. In particular, treatment with IGF-1 during culture did not alter stage of development at Day 14, embryo length or the amounts of IFN- τ secreted by recovered embryos when placed in culture.

The fact that there was a tendency ($P = 0.10$) for IGF-1 to increase the proportion of transferred embryos that were recovered at Day 14 in Experiment 2 was consistent with an earlier experiment that treatment of lactating dairy cows with bST at insemination and 11 days later tended to increase the proportion of recipients that had a recoverable conceptus at Day 17 of gestation [25]. One interpretation of the tendency for improved embryonic survival in Experiment 2 is that effects of bST on embryonic survival previously reported [25] involved actions of IGF-1. Recently, it was reported that addition of IGF-1 to embryo culture altered the relative abundance of some developmentally important gene transcripts [15]. Embryos treated with IGF-1 had an increased abundance of Na/K ATPase and desmocollin II transcripts and a decreased abundance of heat shock protein 70 transcripts. Such differences in gene expression may be important for embryo survival to Day 14 of gestation.

In contrast, there was no effect of IGF-1 on embryonic survival in Experiment 1. Embryos were transferred in groups in this experiment and perhaps cooperation or interactions between embryos obscured differences in embryonic survival caused by IGF-1. There were other differences in the design of Experiments 1 and 2, however. In addition, an earlier study with lactating recipients also indicated no improvement in embryonic survival caused by culture with IGF-1 unless recipients were heat-stressed [24]. Clearly, additional studies are required to clarify the question as to whether IGF-1 enhances embryonic survival at Day 14 of pregnancy.

Among those embryos that did survive to Day 14, there was no effect of IGF-1 on embryo stage of development, length, or IFN- τ secretion. One interpretation of these results is that IGF-1 is not a critical factor in the first 7–8 days of culture for events such as trophoblast elongation and IFN- τ secretion that are required for rescue of the corpus luteum from luteolysis. Treatment of lactating dairy cows with bST increased conceptus length and total IFN- τ in uterine flushings [25]. It is likely that this action of bST was either independent of IGF-1 or that IGF-1 mediated effects on conceptus length and IFN- τ secretion does not occur until after Day 7.

Embryonic capacity for IFN- τ secretion was measured by determining release of IFN- τ into culture medium. Perhaps this technique could obscure the IGF-1 treatment effect if embryonic secretion became dysregulated during culture. However, the method has been shown useful for determining regulation of IFN- τ secretion in several experiments that demonstrated dependence of IFN- τ secretion on embryo age [33] and gender [34] as well as effects of maternal heat stress [35] and administration of progesterone [36] and human chorionic gonadotropin [37]. Suitability of the culture system is supported by the finding that, as expected [38], IFN- τ secretion in vitro increased in magnitude as conceptus length increased.

Embryo recovery rates at Day 14 in Experiments 1 and 2 were 27.6% (153/554) and 33.7% (28/83), respectively. Similar embryo recovery rates have been reported previously for in vitro produced embryos recovered at Day 14 [39,40]. There was however, an effect of flush type on recovery rate and embryo length in Experiment 1. In particular, more embryos were recovered and embryos were longer when recipients were flushed following slaughter compared to non-surgical embryo recovery using live recipients. Whereas the mean embryo length for embryos recovered using non-surgical recovery procedures (3.7 ± 0.85 mm) was similar to previous reports in which embryos were recovered from live recipients at Day 14 following group embryo transfer (1.3–4.9 mm; [39–41]), the mean embryo length for embryos recovered after slaughter was much longer (7.7 ± 0.87 mm). These results suggest the possibility that non-surgical embryo recovery is not the optimal method for recovering intact, elongated embryos following group embryo transfer.

Culture conditions can have a significant effect on the proportion of embryos at Day 14 with a visible embryonic disc [39]. Embryos without a visible disc are not capable of establishment of pregnancy following transfer [39]. There was however, no effect of IGF-1 on the proportion of embryos recovered at Day 14 in Experiment 1 that had a visible embryonic disc. Although detection of the embryonic disc using stereomicroscopy can be imprecise, the proportion of embryos with a visible embryonic disc in experiment 1 ($88/116 = 75.9\%$) is similar to previous reports [39,40].

Addition of IGF-1 to embryo culture improves the proportion of embryos becoming blastocysts in several studies [13,18–24], but not in all [15,19,42]. In the present experiments, IGF-1 treatment tended to increase the proportion of oocytes that developed to the blastocyst stage at Day 7 (Experiment 2), but there

was no effect of IGF-1 on blastocyst development at Day 8 (Experiment 1). Similar results were also observed in a recent study from our laboratory in which IGF-1 increased embryo development to the blastocyst stage on Day 7, but not on Day 8 [24]. Inconsistencies in the effect of IGF-1 on embryo development may be partly explained by differences in culture systems since there are reports that the actions of IGF-1 to stimulate embryonic development depend upon culture conditions [19,42].

In conclusion, we inferred that exposure to IGF-1 through Days 7–8 of development did not enhance capacity of embryos to prevent luteolysis as evaluated by conceptus length and IFN- τ secretion. Results of the single embryo-transfer experiment suggested that IGF-1 treatment might affect embryo survival post-transfer as early as Day 14 after ovulation. Further experimentation is warranted to verify this finding.

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