Timing and dependence upon mitogen-activated protein kinase signaling for pro-developmental actions of insulin-like growth factor 1 on the preimplantation bovine embryo

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ABSTRACT

Insulin like growth factor-1 (IGF1) increases the proportion of embryos that develop to the blastocyst stage. The objective of the present study was to determine whether the pro-developmental actions of IGF1 are exerted before or after Day 4 of development (i.e., on events occurring through the period of genomic activation versus events coincident with compaction and blastocoel formation) and whether mitogen-activated protein kinase (MAPK) signaling pathways mediate effects of IGF1. Treatment with IGF1 increased the proportion becoming blastocysts at concentrations of 10, 100 and 200 ng/mL, with 100 ng/mL being more effective than 10 or 200 ng/mL. At Day 8, the percent of oocytes that became blastocysts was 30, 34, 43, and 36%, respectively (SEM=2.6). As compared to controls (30.4%), IGF1 increased the percent of oocytes that were blastocysts at Day 8 when added from Days 4 to 8 (42%) or Days 0 to 8 post-insemination (40%) but there was no significant effect when IGF1 was added from Days 0 to 4 (37%; SEM = 2.2). Actions of IGF1 to increase blastocyst development were reduced when embryos were co-treated with the MAPK inhibitor PD98059. The percentage of oocytes becoming a blastocyst at Day 8 was 21 versus 37% for 0 and 100 ng/mL in the absence of inhibitor and 24 versus 29% in the presence of inhibitor (IGF1×inhibitor interaction, P \( < 0.05 \); pooled SEM = 1.3). In conclusion, IGF1 promotes development to the blastocyst stage by regulating MAPK-dependent events at Day 4 or later of development.

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1. Introduction

Proper development of the embryo is dependent upon maternal signals. While embryos can grow in simple defined media, the pattern of development can be disrupted. In the cow, for example, in vitro produced embryos suffer from a variety of morphological and molecular abnormalities and competence of the resultant embryo to survive freezing or transfer into recipients is reduced compared to embryos produced in vivo [1,2]. An absence of maternal signals is responsible for some of the problems inherent in embryos produced in vitro because these embryos can be made more similar to embryos produced in vivo if embryos are returned to the oviduct after in vitro fertilization [2].

Growth factors and cytokines that can affect embryonic development have been identified in a variety of species. In the cow, these include vascular endothelial growth factor [3], epidermal growth factor [4], colony stimulating factor 2 [5–7], leukemia inhibitory factor [7] and interleukin-1β [8]. The mechanisms by which embryonic development is improved by these factors are not known. Effects on the proportion of embryos that develop to the blastocyst stage could be caused by stimulation of cell proliferation, inhibition of apoptosis and embryo arrest, or promotion of key events such as maternal RNA degradation, embryonic genome activation, compaction and blastocoel formation.

Here we evaluated how one growth factor capable of regulating embryonic development, insulin-like growth factor-1 (IGF1), increases the proportion of embryos that develop to the blastocyst stage. Insulin-like growth factor-1 is mainly produced in the liver upon stimulation by growth hormone [9] although some local synthesis in the oviduct, endometrium and embryo has been reported [10–14]. Signaling is transmitted through several intracellular pathways with many of the proliferative actions of IGF1 being mediated through activation of the mitogen-activated protein kinase (MAPK) pathway [15]. Treatment with IGF1 can increase the proportion of embryos becoming blastocysts in several species including the bovine [11,16,17]. Insulin-like growth factor-1 also improves resistance of bovine preimplantation embryos to heat shock [18–20] and oxidative...
stress [21], alters expression of several genes at the blastocyst stage [22] and improves embryo survival after transfer into heat-stressed recipients [6,23].

Specific objectives of the current study were to determine whether the pro-developmental actions of IGF1 in culture of in vitro produced embryos are exerted before or after Day 4 of development (i.e., on events occurring through the period of genomic activation versus events coincident with compaction and blastocoel formation) and whether MAPK signaling pathways mediate pro-developmental effects of IGF1.

2. Materials and methods

2.1. Materials

Unless otherwise mentioned, reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA). HEPES-Tyrodes Lactate (TL) and IVF-TL solutions were purchased from Caisson (Sugar City, ID, USA) and used to prepare HEPES-Tyrosides albumin lactate pyruvate (HEPES-TALP), and IVF-TALP as previously described [24]. Oocyte collection medium (OCM) was tissue culture medium–199 (TCM–199) with Hanks salts without phenol red (HyClone, Logan, Utah, USA) supplemented with 2% (v/v) bovine serum albumin, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 1 mM glutamine. Oocyte maturation medium (OMM) was TCM–199 (Gibco, Invitrogen, Grand Island, NY, USA) with Earle’s salts supplemented with 10% (v/v) bovine serum, 2 μg/mL estradiol 17-β, 20 μg/mL bovine follicle stimulating hormone (Folltropin-V; Bioniche, London, Ontario, Canada), 22 μg/mL sodium pyruvate, 50 μg/mL gentamicin sulfate, and 1 mM glutamine. Percoll was from GE Healthcare (Uppsala, Sweden). Frozen semen from various bulls was donated by South-eastern Semen Services (Wellborn, FL, USA). The embryo culture medium was Synthetic Oviduct Fluid-Bovine Embryo 1 (SOF-BE1) [25]. The MAPK kinase inhibitor, PD 98059, was from Sigma-Aldrich. Recombinant human IGF1, which is active in cattle [18–20,22,23], was purchased from Sigma-Aldrich. A vial containing 50 μg of lyophilized IGF1 was rehydrated with 200 μl of water, and this stock solution was then stored at –20 °C in 5 μl aliquots until dilution to the requisite concentration with SOF-BE1 on the day of use.

2.2. In vitro production of embryos

Ovaries were obtained from a mix of beef and dairy cows (>75% beef) from a commercial abattoir (Central Beef Packing Co., Center Hill, FL, USA), and transported in 0.9% (w/v) NaCl solution at room temperature. Cumulus–oocyte complexes (COCs) were obtained by slicing to 2 mm follicles on the surface of ovaries. Those COCs containing at least one layer of compact cumulus cells and even granulation were washed in OCM. COCs were matured for 20–22 h in groups of 10 in 50 μl drops of OMM overlaid with mineral oil at 38.5 °C in an atmosphere of 5% (v/v) CO2 in humidified air. Matured COCs were then washed in HEPES-TALP and transferred in groups of 200 to a 35 mm petri dish containing 1700 μl of IVF-TALP supplemented with 80 μl PHE (0.5 mM penicillamine, 0.25 mM hypotaurine, and 25 μM epinephrine in 0.9% [w/v] NaCl), and fertilized with 120 μl Percoll-purified spermatozoa (~1×10^6 sperm cells). Sperm were prepared from a pool of frozen-thawed semen from three different bulls; a different set of bulls was generally used for each replicate. The day of fertilization was designated Day 0 (0 h). After 6 to 10 h in an atmosphere of 5% CO2 in humidified air, putative zygotes were removed from fertilization wells, denuded of cumulus cells by vortexing for 4 min in HEPES-TALP and hyaluronidase (10,000 U/mL in 600 μl HEPES-TALP medium) and washed in HEPES-TALP. Embryos were then placed in groups of 30 in 50 μl drops of SOF-BE1 overlaid with mineral oil. Embryos were cultured at 38.5 °C in an atmosphere of 5% CO2 in humidified air.

2.3. Concentration-dependent actions of IGF1 to increase blastocyst development

Following fertilization, embryos were washed and cultured in 50 μl of SOF-BE1 (control), or SOF-BE1 containing 10, 100, or 200 ng/ml IGF1. Concentrations were chosen so that the second concentration was within the range of values for IGF1 in blood of lactating cows [26,27]. The percentage of oocytes that cleaved (≥2 cell) was observed at Day 3 after insemination (~72 h post-insemination) and the percentage of embryos that became blastocyst was observed at Day 7 (168 h) and Day 8 (192 h) post-insemination. The experiment was replicated 4 times using 231 to 284 oocytes per group.

2.4. Determination of the stage of development at which IGF1 acts to increase blastocyst development

This experiment tested whether IGF1 improves developmental competence by acting from Days 0 to 4 (6–96 h) post-insemination (i.e., on events occurring through the period of genomic activation at the 8–16 cell stage) or from Day 4 (96 h) to Day 8 (192 h) post-insemination (i.e., coincident with compaction and blastocoel formation). Following fertilization, putative zygotes were washed and assigned to one of four treatments: control, IGF1 from Days 0 to 8 (6–92 h) post-insemination, IGF1 from Days 0 to 4 (6–96 h) post-insemination or IGF1 from Days 4 to 8 post-insemination. Embryos were placed in groups of 30 in 50 μl microdrops of SOF-BE1 containing 0 or 100 ng/ml IGF1 at Day 0 (0 h, time of insemination). The concentration of IGF1 used was based on the fact that it produced optimal effects on development in the first experiment. For all treatments, embryos were washed at Day 4 (96 h) and transferred to fresh medium containing SOF-BE1 ± 100 ng/ml IGF1. The percent of oocytes that cleaved was assessed at Day 4 (96 h) post-insemination and the percent that became blastocysts were determined at Day 7 (168 h) and Day 8 (192 h) post-insemination. The experiment was replicated 5 times using 332 to 356 embryos per group.

2.5. Role of MAPK signaling pathway in IGF1 actions

The design was a 2×2 factorial with main effects of IGF1 (0 or 100 ng/ml) and PD 98059 (0 or 100 μM). Embryos were produced as described above and cultured in SOF-BE1 from Days 0 to 4 (0–96 h) post-insemination. At Day 4 (96 h), embryos were placed in groups of 30 in 50 μl microdrops of SOF-BE1 containing 0.1% DMSO (vehicle), SOF-BE1 containing 0.1% DMSO and 100 ng/ml IGF1, SOF-BE1 containing 0.1% DMSO and 100 μM PD 98059 (MAPK inhibitor) or SOF-BE1 containing 0.1% DMSO (vehicle), 100 ng/ml IGF1 and 100 μM PD 98059. Embryo development was assessed at Day 7 and 8 (168 h and 192 h) post-insemination. The experiment was replicated 5 times using 308 to 378 oocytes per group.

2.6. Statistical analysis

Data on the percent of oocytes that cleaved and became a blastocyst were analyzed by least-squares analysis of variance using the Proc GLM procedure of the Statistical Analysis System (SAS for Windows, Version 9.2 Cary, NC). Percent data were transformed by arcsin transformation before analysis. The mathematical model included main effects of replicate, treatment or treatments and all interactions. Replicate was considered random, other main effects were considered fixed and tests of significance were calculated after determination of expected means squares. Probability values were based on analysis of arcsin-transformed data while least-squares means were from analysis of untransformed data. The following
orthogonal contrasts were used to determine differences between individual concentrations of IGF: 0 versus others, 100 versus 10 and 200 and 10 versus 200. For experiment on effects of time of addition of IGF1, identification of means that differed significantly was determined using the pdiff procedure of SAS when the main effect of treatment was significant.

3. Results

3.1. Concentration-dependent actions of IGF1 to increase blastocyst development

Treatment with IGF1 beginning at 6 h post-insemination did not affect the percentage of oocytes that cleaved by Day 3 post-insemination (Fig. 1A) but increased the percentage of embryos that became a blastocyst at Day 7 (P < 0.05; Fig. 1B) and 8 (P = 0.05; Fig. 1C). At Day 7, there was no difference between 10, 100 and 200 ng/mL. At Day 8, the percentage of oocytes that became a blastocyst was higher (P < 0.05) for 100 ng/mL than for 10 or 200 ng/mL.

3.2. Determination of the stage of development at which IGF1 acts to increase blastocyst development

Treatment did not affect cleavage (Fig. 2A) but addition of IGF1 from Days 0 to 8 post-insemination increased (P < 0.05) the percentage of oocytes that became blastocysts at Day 7 (Panel B) and Day 8 (Panel C) post-insemination. Embryos were either cultured without IGF1, IGF1 from Days 0 to 8 post-insemination, Days 0–4 post-insemination or Days 4–8 post-insemination. The main effect of treatment was significant for results at Day 7 (P < 0.05) and 8 (P < 0.01) and differences between individual means (P < 0.05) are indicated by different superscripts above each bar. Data are least-squares means ± SEM of results from 5 replicates involving 332 to 356 oocytes per group.

3.3. Effect of inhibition of MAPK on actions of IGF1 to promote development

Development at both Days 7 and 8 was affected by an inhibitor by IGF1 interaction (Fig. 3; P < 0.05). These interactions reflected the fact that IGF1 increased development in the absence of the inhibitor but not in the presence of PD 98059.
formation. BlastocoeI formation requires actions of ATP1A1 to increase blastocoeI fluid and facilitate tight junction formation [37,38] and IGF1 tended to increase expression of ATP1A1 in Day 7 blastocysts [22].

The effects of IGF1 before Day 4 are less clear. While there was no significant effect of IGF1 from Days 0 to 4 on the percentage of oocytes that developed to the blastocyst stage, values were intermediate between untreated controls and embryos treated with IGF1 from Days 4 to 8 or 0 to 8. More research is needed to define effects of IGF1 early in development. Receptors for IGF1 are present as early as the two-cell stage [35]. Nonetheless, IGF1 was without effect on embryo thermotolerance at the two-cell stage [20]. Perhaps, embryonic genome activation is required for IGF1 to affect embryonic function.

The concentrations at which IGF1 increased competence for development to the blastocyst stage in the present study is within the range of those found in the blood of lactating and non-lactating cows [26,27]. Surprisingly, IGF1 was more effective at increasing development at 100 ng/mL than at 200 ng/mL. The reasons for this effect are not known. Perhaps, at higher concentrations, IGF1 activated receptors for other ligands that activate signaling pathways inhibitory to development.

4. Discussion

Results presented here indicate that the pro-developmental effects of IGF1 involve actions mediated by the MAPK pathway and events occurring during the period from Days 4 to 8 after insemination. Moreover, IGF1 exerts its pro-developmental effects at concentrations that are within the range of those found in the blood of lactating and non-lactating cows.

That the pro-developmental effects of IGF1 involve actions mediated by the MAPK pathway is indicated by the observation that inhibition of this pathway with PD98059 blocked actions of IGF1 on development to the blastocyst stage. The MAPK pathway is one of the main signaling pathways (the PI3K pathway being the other) through which IGF1 alters cellular function [15]. Proliferative actions of IGF1 involve activation of the MAPK pathway [28,29]. It is possible, therefore, that the main mechanism by which IGF1 increases blastocyst development is through an increase in proliferation. In this way, more embryos could reach a critical cell number necessary for differentiation into the blastocyst. Other molecules that stimulate proliferation also can increase the proportion of embryos that develop to the blastocyst stage [3,4]. That IGF1 may increase blastocyst formation by increasing the proportion of embryos that reach a cell number critical for blastocyst formation is supported by the finding that cell number of bovine blastocysts did not differ between control and IGF1-treated embryos [22].

An alternative explanation for the effect of IGF1 on blastocyst formation, that IGF1 increases cell number by blocking apoptosis, is less likely. Although IGF1 can block induction of apoptosis in preimplantation bovine embryos [19,21,30] and cause alterations in gene expression that would contribute to an anti-apoptotic state [20], the anti-apoptotic actions of IGF1 involve the PI3K pathway [19,30]. Moreover, in the absence of a pro-apoptotic signal, there was no effect of IGF1 on the proportion of blastocyst lacerotomes that are apoptotic [22].

The fact that IGF1 was as effective at increasing embryonic competence to form a blastocyst when added between Days 4 and 8 as when added between Days 0 and 8 means that actions of IGF1 to increase blastocyst formation involve regulation of events associated with events after embryonic genome activation [31] and when the embryo is undergoing compaction [31,33]. DNA methylation [34], proliferation and blastocoeI formation [32,35,36]. There is evidence that IGF1 increases development to the blastocyst stage, at least in part, by regulating expression of genes involved in blastocoeI formation.

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### References


