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Original Research Article

Intracytoplasmic sperm injection affects embryo developmental potential and gene expression in cattle

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ABSTRACT

Some reports have linked intracytoplasmic sperm injection (ICSI) with chromosomal abnormalities, low developmental potential and altered gene expression in embryos. ICSI has also been linked with obesity, early aging and increased incidence of tumors in offspring. Other reports have demonstrated that some of these complications disappeared within a few weeks of life or even showed a lack of such associations. The aim of this study was to evaluate and compare embryo development, quality and gene expression in bovine embryos generated by ICSI and by conventional *in vitro* fertilization (IVF) insemination. The results showed differences in cleavage (88.5% in IVF and 64.1% in ICSI) and blastocyst formation rates (36.1% in IVF and 22.3% in ICSI). The proportion of ICM cells to total cell count was higher in ICSI (39.2%) than in IVF embryos (29.5%). However, no differences were observed in the total embryonic cell numbers (159.3 ± 28.5 and 161.2 ± 56.2 for IVF and ICSI, respectively) or in the proportion of apoptotic nuclei to the total embryonic cell numbers (2.12 and 2.64% for IVF and ICSI, respectively). Gene expression analysis showed a down-regulation of insulin-like growth factor 2 (*IGF2*) and overexpression of bcl-2-like protein 4 (*BAX*), octamer-binding transcription factor four (*OCT4*), interferon-tau (*IFNt*), Mn-superoxide dismutase in the mitochondria (*SOD2*), and catalase (*CAT*) in embryos generated by ICSI. In conclusion, our study demonstrated differences in the morphological development of bovine embryos as well as in the expression of genes involved in early development between ICSI and IVF embryos. The results may indicate lower developmental potential of ICSI embryos compared with that of IVF.

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1. Introduction

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technique employed in humans as a method of choice in cases of male infertility [1]. In livestock animals, ICSI has been used to study the interaction between oocytes and spermatozoa during fertilization [2] as an important tool in the conservation of endangered species [3], for the generation of high genetic-value animals with the desired sex [4] and, more recently, as an alternative tool for the generation of transgenic animals [5].

ICSI helps to avoid many of the steps normally necessary for the interaction between gametes during fertilization, such as acrosome reaction, sperm binding to the zona pellucida and the penetration and fusion of the sperm with the oocyte's plasma membrane [6]. In addition, it was observed in some species that sperm accessory structures such as the perinuclear theca, the acrosome and cellular membranes interfere with sperm chromatin decondensation [7]. The acrosome's content was found to be potentially harmful to the injected oocyte [8]. Furthermore, the microinjection of oocytes during ICSI may result in alterations of intracellular ionic concentrations, introducing foreign material and preventing natural selection of sperm [9]. It was reported that ICSI produces normal offspring in some animal species including humans [10]. In contrast, injection of fresh and thawed spermatozoa was found to cause obesity, aberrant growth, abnormal behavior, early aging and increased incidence of tumors in the offspring of mice [11].

Although *in vitro* fertilization (IVF) and ICSI are considered safe in humans, some studies show that they increase the likelihood of complications in the offspring [12,13]. Several reports indicated that embryos of different species produced by ICSI had an increased incidence of chromosomal abnormalities, low developmental potential, abnormal patterns of calcium oscillations and reduced cell numbers in some species [14,15]. It appears that the fertilization method has also a fundamental role in determining the transcriptome of the preimplantation embryo [14] because of differences in the overall pattern of gene expression found between ICSI and IVF mouse embryos [1,14,16]. Although a few studies have compared the *in vitro* developmental potential of bovine embryos generated by these techniques [17–19], there is limited information on gene expression in the embryos themselves [20]. Therefore, the aim of the present study was to assess the effects of ICSI and IVF on preimplantation embryonic development, embryo quality and the expression of 15 genes important for bovine embryo development.

2. Materials and methods

2.1. *Experimental design*

The study included three experiments performed on bovine cumulus–oocyte complexes (COCs). In the first experiment, oocytes from the same batch were matured *in vitro* and were randomly distributed to different treatments (ICSI, IVF and SHAM) to assess *in vitro* embryonic development. A total of 592 oocytes were used for IVF ($n = 244$), ICSI ($n = 256$) and SHAM ($n = 92$) in nine replicates. In addition, pronuclei formation of ICSI embryos was assessed 18 h after insemination ($n = 45$ oocytes). In the second experiment, TUNEL staining was carried out to examine embryo quality of the IVF and ICSI embryos ($n = 10$ good-quality expanded blastocysts per treatment). In the third experiment, expression of selected genes was studied in the IVF and ICSI embryos ($n = 15$ good-quality expanded blastocysts per treatment). All procedures were approved by our Institutional ethics committee and followed the guidelines of the funding source ''Biosafety standards manual, second edition 2008, FONDECYT, CONICYT''. Unless stated otherwise, all chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. *Collection of ovaries, selection of oocytes and* in vitro *maturation (IVM)*

Ovaries were collected from a local slaughterhouse (Frigorifico Temuco, Temuco, Chile). COCs were aspirated from 2 to 7 mm follicles, using an 18-gauge needle. Good quality oocytes surrounded by more than six compact layers of cumulus cells and a uniformly granulated cytoplasm, were selected and matured in TCM-199 medium, supplemented with 10% inactivated fetal bovine serum (FBS; Hyclone Laboratories, Inc., Logan, UT, USA) and 6 μg/mL LH (Sioux Biochemical, Inc., Sioux City, IA, USA), 6 μg/mL FSH (Bioniche Life Science Inc., Belleville, Ontario, Canada) and 1 μg/mL estradiol, and then incubated for 18–24 h (38.5 °C, 5% CO_2, saturation humidity). *In vitro*-matured oocytes were randomly assigned to ICSI IVF or SHAM groups.

2.3. In vitro *fertilization*

In vitro-matured oocytes were co-incubated with 1×10^6 Percoll-separated frozen–thawed sperm per mL (Alta Genetics Inc., Alberta, Canada) for 18–20 h (38.5 °C, 5% CO_2, humidified atmosphere) in IVF-TL medium supplemented with 0.2 mM sodium pyruvate, 6 mg fatty acid-free BSA, 0.025 mg gentamicin sulphate per mL and PHE (80 μM penicillamine, 40 μM hypotaurine, 10 μM epinephrine) and 2 μg heparin. Presumptive zygotes were stripped of cumulus cells *via* vortex and cultured as described below.

2.4. *Intracytoplasmic sperm injection*

Commercial semen was used for IVF and ICSI. Immediately before ICSI, 2 μL of sperm suspension was mixed with 8 μL of PBS containing 10% polyvinylpyrrolidone (PVP; Irvine Scientific, Santa Ana, CA, USA). After 18 h co-culture, oocytes were denuded of granulosa cells by vortexing in the presence of 1 mg/mL hyaluronidase. The mature (MII) oocytes were selected based on the presence of the first polar body. ICSI was performed on an inverted microscope equipped with Hoffman optics (Eclipse TS100F, Nikon Instruments Inc., New York, NY, USA) and hydraulic micromanipulators (Narishige International Inc., New York, NY, USA). Motile sperm with normal morphology were immobilized by tail scoring with the tip of an injection pipette before being aspirated into the injection pipette (9 μm outer diameter). An oocyte was positioned on the holding pipette with the polar body at 12 o'clock. After breaking the oolemma, the spermatozoon was