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Intracytoplasmic sperm injection improves *in vitro* embryo production from poor quality bovine oocytes

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ABSTRACT

The objective was to use subzonal sperm injection (SUZI) to understand sperm penetration patterns and to use intracytoplasmic sperm injection (ICSI) to improve production of bovine embryos using poor quality gametes. In experiment 1, poor versus good quality oocytes were fertilized with sperm from two bulls, A and B, with poor and good sperm vigor, respectively. The blastocyst rate was higher for good versus poor quality oocytes (23.3% vs. 11.1%, $P < 0.05$), regardless of the bull used. There was no significant difference in blastocyst rate for bull A (low vigor) regardless of oocyte quality, and for bull B (high vigor), blastocyst rate was better for good versus poor quality oocytes (25.7% vs. 9.2%, $P < 0.05$). In experiment 2, poor quality oocytes were subjected to SUZI. The oocyte penetration rate was lower for bull A than for bull B (29.6% vs. 53.8%, $P < 0.05$), when SUZI was performed within 1 hour after sperm processing. However, when SUZI was performed 2 to 3 hours after sperm processing, penetrating capacity was similar between bulls, but for bull B, penetrating capacity significantly decreased after 3 hours of sperm processing. In an attempt to overcome sperm penetrating disorders, poor and good quality oocytes were subjected to ICSI (experiment 3). Irrespective of the bull or of the oocyte quality grade, there were no differences in cleavage or blastocyst rates. Both bulls had distinct IVF embryo production rates, which we inferred were because of particular individual sperm characteristics. In conclusion, ICSI was an effective means to achieve *in vitro* production of bovine embryos with gametes of variable quality.

1. Introduction

Oocyte and sperm quality might influence the success of *in vitro* production (IVP) of bovine embryos [1,2]. However, under field conditions, genetically valuable cows are frequently subjected to ovum pick-up without considering oocyte quality [3]. Consequently, poor quality oocytes are often retrieved and used for IVP, usually reducing embryo yield. Furthermore, the outcome of IVP might be influenced by bull fertility, semen processing, and storage conditions, or perhaps unknown factors [4,5]. Bull semen that achieves good pregnancy rates in AI programs (using estrus detection or fixed time breeding) does not necessarily achieve good fertility for IVP embryos [6,7]. Furthermore, there are differences among bulls in their contribution to embryonic development [8]; some have a low percentage of embryos produced when used with oocytes from specific donors (because of apparent male–female interactions) [7]. To overcome such obstacles, especially in research, it is imperative to pretest semen intended to be used for IVF. However, in commercial IVP programs, results can be disappointing, especially because a particular sire is typically chosen for reasons other than fertility. Recently, progress has been made to improve *in vitro* embryo production for low quality oocytes and/or semen [9].

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Subzonal sperm injection (SUZI) has been used for *in vitro* evaluation of bull sperm [10], because it assesses sperm penetration competence. Another technique currently employed to circumvent fertilization problems is intracytoplasmic sperm injection (ICSI), widely employed in human reproduction, to enable the use of poor quality gametes. However, ICSI has not been widely used for cattle, because the technique is still under development [11,12]. The general objective of this study was to evaluate SUZI as a strategy to assess sperm penetration capacity, and to evaluate ICSI as a strategy to improve the IVP efficiency of low quality bovine gametes. Specifically, low and high vigor semen was used to fertilize poor or good quality oocytes.

2. Materials and methods

2.1. Experimental design

2.1.1. Experiment 1: Characteristics of sperm/oocyte interaction on embryo development after IVF

Oocytes (N = 1566) classified as either poor or good quality were separately submitted to conventional IVF using semen of two bulls (designated bulls A and B). The aim of this experiment was to evaluate the interaction of distinct seminal characteristics and oocyte quality grades on embryo developmental rates. Bulls A and B were selected based on previous observations of distinct seminal patterns. A sample of zygotes was pooled from each treatment (approximately 44% of total IVF zygotes) for determination of nuclear status, based on morphologic characteristics of the zygotes. Embryo development was compared with nuclear status of zygotes, among all experimental groups (five replications).

2.1.2. Experiment 2: Determination of sperm penetration patterns

Based on bull B having greater penetrating competence in poor quality oocytes (experiment 1), experiment 2 aimed to further confirm the higher penetrating capacity of bull B. Oocytes (N = 498) classified as poor quality underwent SUZI using semen from bull A and bull B (three replications).

2.1.3. Experiment 3: Comparison of ICSI and IVF on embryo production

To overcome limitations of sperm–oocyte interactions that impair IVF efficiency, the objective of experiment 3 was to compare ICSI and conventional IVF with oocytes of poor or good quality (N = 426), using semen from bulls A and B (three replications).

2.2. Media and reagents

Except where otherwise indicated, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Recovery of oocytes, classification, and IVM

Bovine ovaries were recovered from a local abattoir and transported to the laboratory at 25 °C to 30 °C in 0.9% sodium chloride solution, up to 6 hours after slaughter. Follicles of 2 to 8 mm in diameter were aspirated with the aid of a vacuum pump (approximate pressure 10–15 mL water flow/min). Cumulus-oocyte complexes in follicular fluid and examined under a stereomicroscope were classified based on morphologic criteria [13]. Oocytes were classified as of good quality if they had a homogeneous cytoplasm with no or few dark clusters, and also if they were completely surrounded with at least three compact layers of cumulus cells. Poor quality oocytes had a heterogeneous cytoplasm with several dark clusters, completely or partially surrounded by noncompact and slightly dark cumulus cells. Degenerated oocytes were not used.

Oocytes were matured *in vitro* in 400 μL of modified tissue culture medium (TCM-199; Cultilab, Campinas, São Paulo, Brazil), with 5.95 mg/mL HEPES, 2.62 mg/mL $NaHCO_3$, 0.025 mg/mL sodium pyruvate, 5 μg/mL porcine follicle-stimulating hormone (NIH-FSH-P1; Folltropin-V; Bioniche Animal Health, Belleville, Ontario, Canada), 50 μg/mL porcine pituitary luteinizing hormone (LH-P; Lutropin-V; Bioniche Animal Health), and 10% estrous mare serum [14], under mineral oil, for 18 to 24 hours at 38.5 °C and 5% CO_2 in air, with saturated humidity.

2.4. Semen preparation

Frozen-thawed semen collected from two *Bos taurus taurus* sires (bulls A and B), known to be efficient for IVF, was used. Straws of semen were thawed in a water bath at 37 °C for 20 seconds. After thawing, although each bull had approximately 40% motile sperm, sperm vigor was greater for bull A versus bull B (2 vs. 4, respectively). Sperm vigor was evaluated at magnification ×400 and rated on a scale of 0 to 5, as described in Howard et al. [15]. Briefly, the scales were: 0, sperm with no movement; 1, sperm with slight side-to-side movement, but no forward progression; 2, sperm with side-to side movement and occasional slow forward progression; 3, sperm with side-to-side movement with slow forward progression; 4, sperm with steady forward progression; and 5, sperm with rapid, steady, forward progression. All seminal postthaw evaluations were performed on slides warmed to 37 °C. Thawed semen was selected using mini-Percoll [16] gradients (90% and 45%), and resuspended in Sperm-TALP medium.

2.5. IVF

Selected sperm and oocytes were incubated (for 20 hours) in Fert-TALP medium with 6 mg/mL BSA, 0.022 mg/mL sodium pyruvate, 30 μg/mL heparin, and PHE (penicillamine 30 μg/mL; hypotaurine 15 μmol/L, and epinephrine 1 μmol/L) at 38.5 °C with 5% CO_2 in air and saturated humidity. The final semen concentration used for IVF was 1.5×10^6 motile sperm/mL.

2.6. Evaluation of nuclear status of presumptive zygotes

After IVF, cumulus cells were removed from presumptive zygotes by successive pipetting. Approximately 44% of zygotes were pooled and used for evaluation of nuclear status, with the remainder subjected to conventional IVC (to assess embryo development). For evaluation of nuclear