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Effect of ICSI and embryo biopsy on embryo development and apoptosis according to oocyte diameter in prepubertal goats

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Abstract

ICSI and embryo biopsy are routine methods used for assisted reproduction. However, their impact on embryo quality is still poor studied. Moreover, oocyte size is also a crucial factor for blastocyst production. In this study effect of oocyte size, ICSI and embryo biopsy was assessed in terms of incidence of apoptosis and blastocyst development. IVM-oocytes from prepubertal goats were fertilized by ICSI or IVF. Embryos obtained were divided depending on oocyte size, biopsied at day-4 post-insemination/injection and cultured for additional 4–5 days. Apoptotic cell number was assessed by TUNEL staining in day-4 embryos and blastocysts obtained. In each diameter group, ICSI did not affect embryo development, blastocyst cell number and embryo apoptotic grade in comparison to IVF. Embryo biopsy did not affect blastocyst rate and apoptotic cell number, but decreased blastocyst cell number ($P = 0.0018$). Moreover, there was a negative relationship between blastocyst cell number and apoptotic grade ($P < 0.05$). In conclusion, ICSI and embryo biopsy do not have negative effect on embryo quality and development. However, oocyte size has a positive relationship on blastocyst yield and quality.

Keywords: Apoptosis; Embryo biopsy; Goat; ICSI

1. Introduction

Oocytes derived from slaughterhoused ovaries are highly heterogeneous. This heterogeneity comes from the different grades of growth and atresia of the oocytes obtained. Previous studies in our laboratory with prepubertal goats have shown that oocyte size has an important impact on oocyte quality and it is a good indicator of blastocyst development rate [1].

Low fertilization rates and high polyspermy are observed in in vitro fertilized slaughterhouse prepubertal goat oocytes [2,3]. Intracytoplasmic sperm injection (ICSI) is an alternative to IVF to male infertility (in human) [4] or when high fertilization abnormalities occur. A major application of this technique includes the use of genetically important male gametes to procreate domestic animals, as well as to inject sperm for which spermatozoa motility is not required: freeze-dried sperm [5,6], or killed sperm [7]. Moreover, this technique could be used in the production of transgenic animals using sperm as a vector of foreign DNA [8]. ICSI could also be useful in the evaluation of oocyte quality because it reduces the

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variation due to sperm penetration and allows potential fertilization and embryo development of each one of the MII-oocytes injected [1].

Since Uehara and Yanagimachi [9] obtained the first animal born by ICSI, a hamster, other live births have been obtained with this fertilization technique (human: [4]; mice: [10]; cattle: [7]; sheep: [11]; horse: [12]; pig: [13]; and goats: [14]). In goats, the first study using ICSI as a fertilization method with development until blastocyst stage was reported by Keskintepe et al. [15]. The first kid born by this method was reported by Wang et al. [14] using a piezo-drill ICSI; Jimenez-Macedo et al. [1] studied different protocols of ICSI to fertilize prepubertal goat oocytes obtaining embryos from these oocytes. Later, these authors [1] obtained ICSI-derived blastocyst from oocytes larger than 125 μm of prepubertal goats.

However, micromanipulation techniques such as ICSI and embryo biopsy could be considered as external aggressions. The grade of embryo development after ICSI and biopsy could be indicative of the robustness of the embryo, and indirectly, of the competence of the oocyte which it derives from. Embryo biopsy in human assisted reproduction is a useful technique for preimplantation genetic diagnosis (PGD) [16]. It is also used to obtain stem cells without the destruction of the embryo [17,18]. In domestic mammals, embryo biopsy is important because it allows embryo sex identification. Sexing preimplantational embryos from livestock species is of a great importance in transgenesis because it provides a way to choose the desired sex. Embryo sexing has been reported in mice [19], pig [20] and cattle [21].

Previously, we have measured oocyte size of prepubertal goats to study their developmental competence after ICSI [1] and IVF [22]. The objective of this work is to study embryo development and blastomere apoptosis of biopsied and non biopsied-derived embryos produced by ICSI and IVF of prepubertal goat oocytes with different diameters.

2. Materials and methods

2.1. Recovery of oocytes and in vitro maturation

Ovaries from prepubertal goats (1–2 months old) were obtained from a local abattoir and transported to the laboratory at 38.5 °C in PBS solution Dulbecco phosphate-buffered saline (PBS, P-4417, Sigma Chemical Co., St. Louis, MO, USA) containing 50 μg/mL of gentamycin sulphate. The ovaries were rinsed with the same solution. Cumulus-oocyte complexes (COCs) were recovered after slicing the ovaries submerged in slicing medium: TCM199 (M-2520, Sigma), supplemented with 2.2 mg/mL $NaHCO_3$, 2% (v/v) steer serum (Donor Bovine Serum, CanSera, Ontario, Canada) and 50 μg/mL gentamycin at 38.5 °C. Only COCs with at least four intact layers of compact cumulus cells and homogeneous cytoplasm were selected.

Selected COCs were washed in IVM medium. Groups of 20–25 COCs were matured in 100 μL drops of IVM medium: TCM199 (M-7528, Sigma) supplemented with 275 μg/mL sodium pyruvate (P-3662, Sigma), 146 μg/mL L-glutamine (G-5763, Sigma), 10% (v/v) steer serum, 10 μg/mL *o*-LH (L-5269, Sigma), 10 μg/mL *o*-FSH (F-8174, Sigma), 1 μg/mL 17 (estradiol (E-2257, Sigma), 100 μM cysteamine (M-9768, Sigma), and 50 μg/mL gentamycin. Oocytes were incubated for 27 h at 38.5 °C in a humidified atmosphere of 5% CO_2 in air under mineral oil (M-3516, Sigma).

2.2. In vitro fertilization

Fresh semen was collected by artificial vagina from Murciano-Granadino bucks of proven fertility. The sperm motility was evaluated under a microscope. The motile sperm fraction was selected by swim-up: 70 μL of semen were placed in conical tubes under 2 mL of defined medium [23] modified by Younis et al. [24], referred here as mDM, and incubated for 1 h in a humidified atmosphere of 5% CO_2 in air at 38.5 °C. After incubation, 600 μL from the supernatant were removed and centrifuged at 200 × *g* for 10 min. The sperm pellet was resuspended 1:1 (v/v) with mDM medium containing heparin and ionomycin (I-0634, Sigma) (final concentration: 10 μg/mL and 200 nM, respectively) and incubated for 15 min in a humidified atmosphere of 5% CO_2 in air at 38.5 °C.

After maturation, groups of 20–25 oocytes (IVF group) were placed into 100 μL fertilization microdrops of modified Tyrode medium (TALP; [25]), and supplemented with 1 μg/mL hypotaurine (H-1384, Sigma) under mineral oil. The treated spermatozoa were co-incubated with the COCs for 24 h with a final concentration of 4×10^6 spermatozoa/ml in a humidified atmosphere of 5% CO_2 in air at 38.5 °C.

2.3. Oocyte size measurement and intracytoplasmic sperm injection

One matured oocyte (with the first polar body visible) per drop was placed into a microdrop of 10 μL of TALP medium covered with mineral oil. Before