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The *in vitro* and *in vivo* development of goat embryos produced by intracytoplasmic sperm injection using tail-cut spermatozoa

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Summary

The objective of this study was to assess the efficacy of a novel intracytoplasmic sperm injection (ICSI) procedure, as well as the *in vitro* and *in vivo* developmental competence of goat embryos produced by ICSI. Oocyte–cumulus complexes recovered by LOPU from donors stimulated with gonadotrophins were matured in vitro. Fresh goat semen was used for ICSI following Percoll gradient washing. Tail-cut spermatozoa were microinjected into the ooplasm of goat oocytes using a piezo micropipette-driving system (PiezoDrill). In order to assess developmental competence, the ICSI-derived zygotes were cultured in one of two media systems (mTALP-mKSOM vs G1.3-G2.3) for *in vitro* development or were transferred into recipients for full-term development. The results suggest that cutting sperm tails using the oocyte-holding pipette coupled with the PiezoDrill is an efficient approach for goat ICSI in terms of oocyte survival, pronuclear development and initial cleavage. The mTALP-mKSOM culture system was more suitable for *in vitro* development of ICSI-derived goat embryos than G1.3-G2.3. This first report of full-term development of an ICSI-derived goat embryo suggests that ICSI can be applied to assisted reproduction in goats.

Keywords: Embryo culture, Embryo transfer, Goat, Intracytoplasmic sperm injection, In vitro matured oocytes

Introduction

In mammals, intracytoplasmic sperm injection (ICSI) was first studied by injecting human or hamster spermatozoa into hamster oocytes (Uehara & Yanagimachi, 1976). ICSI can bypass the process of sperm penetration of cumulus cells, corolla oophorus, zona pellucida and oolemma during fertilisation by directly depositing the spermatozoon into the ooplasm. It has been well documented that normal fertilisation can occur and live births have been obtained following ICSI in rabbits (Hosoi *et al*., 1988), cattle (Goto *et al*., 1990), humans (Palermo *et al.*, 1992), mice (Kimura & Yanagimachi, 1995), sheep (Catt *et al*., 1996), horses (Cochran *et al*., 1998), cats (Pope *et al*., 1998), monkeys (Hewitson *et al.*, 1999) and pigs (Martin, 2000).

The efficiency of ICSI depends on the techniques involved in micromanipulation, the treatments for permeabilisation of the sperm membrane and oocyte activation. Due to the elasticity of the oolemma, it is usually difficult to inject a spermatozoon into the ooplasm without damaging the oocyte membrane and cytoplasmic structure. A piezo-driven procedure was developed to overcome the difficulty of penetrating the oolemma (Kimura & Yanagimachi, 1995). As a result, ICSI efficiencies were dramatically improved in mice (Kimura & Yanagimachi, 1995), rats (Hirabayash *et al*., 2002), horses (Choi *et al*., 2002) and cattle (Katayose *et al*., 1999). Decondensation of the sperm nucleus and oocyte activation are initiated by mutual interactions of the factors within the ooplasm and sperm submembrane components (Perry *et al*., 1999). The prerequisite of this process is the permeabilisation of the sperm membrane following fertilisation (Kasai *et al*., 1999). ICSI eliminates sperm membrane breakdown, which occurs during natural fertilisation as sperm penetrate the oolemma. Therefore, a number of treatments have been used for destabilisation of the sperm membrane prior to ICSI, such as freezing and thawing (Catt & Rhodes, 1995; Perreault *et al*., 1988), crushing the sperm

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tail with a micropipette (Keskintepe *et al*., 1997) or laser shot (Montag *et al*., 2000), and removal of the acrosome and tail by sonication (Goto, 1993; Keefer, 1989). As a result, fertilisation rates using ICSI have been considerably improved in various mammalian species. In contrast, the treatments for sperm demembranation led to limited success rates of oocyte fertilisation and activation in ruminant species (Heuwieser *et al*., 1992). It has been shown that bovine spermatozoa must be capacitated to complete nuclear decondensation and pronuclear development following ICSI (Keefer *et al*., 1990). It has also been suggested that species-specific differences in the stability of sperm nuclei exist, probably due to the different disulfide bonding in the sperm nuclei (Perreault *et al*., 1988). Bull spermatozoa pretreated with dithiothreitol (DTT), a reagent used to reduce disulfide bonds, showed improved fertilisation rates after ICSI (Rho *et al*., 1998). Furthermore, it has been demonstrated that the fertilisation and developmental rates of oocytes following ICSI could be enhanced by exposure to the stimuli of calcium ionophore (Keskintepe *et al*., 1997), 6-dimethylaminopurine (Rho *et al*., 1998) and electrical pulses (Yanagida *et al*., 1999).

Apart from its application in the therapy of male infertility in humans, ICSI technology could also be useful in the generation of transgenic animals using spermatozoa as the DNA carrier (Perry *et al*., 2001) or in gender pre-selection (Hamano *et al*., 1999). Dairy goats are a useful system for producing human therapeutics in their milk by genetic modification. ICSI could be an alternative approach to generate transgenic goats or for propagation of useful genetics. There has been no published report of offspring born using ICSI in goats. In the present study, we used a novel sperm tail-cutting procedure to facilitate the micromanipulation of ICSI in goats. We further characterised the *in vitro* development of goat embryos derived from ICSI in different culture systems. Finally, the full-term developmental competence of ICSI-derived embryos was evaluated.

Materials and methods

Animals

Adult Nigerian Dwarf goats were housed indoors and provided with good-quality hay and water (Keefer *et al*., 2001).

The hormonal treatments for donors and recipients were conducted as previously described (Keefer *et al*., 2001). Briefly, intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Veramix; Upjohn, Orangeville, ON, Canada) were inserted into the vagina of donor goats and left in place for 10 days followed by a luteolytic injection of 125 μg cloprostenol (Estrumate®; Schering Canada, Pointe-Claire, PQ, Canada) 48 h prior to sponge removal. Stimulation of follicular development was induced by gonadotrophin treatment consisting of a total dose equivalent to 70 mg NIH-FSH-P1 (Follitropin-V; Vetrepharm, London, ON, Canada) and 300 IU of eCG (Equinex®; Ayerst Laboratories, Canada) administered intramuscularly 36 h prior to the LOPU procedure. Recipients were synchronised using intravaginal sponges containing 60 mg of medroxyprogesterone acetate for 10 days. The injections of 125 μg of cloprostenol and 300 IU of eCG were given 24 h before sponge removal, which occurred approximately 15 h prior to LOPU.

The animal use and protocols were approved by the Animal Care Committee of Nexia Biotechnologies Inc.

Chemicals and reagents

Unless otherwise stated, all chemicals and reagents were purchased from Sigma (St Louis, MO).

Oocyte recovery and *in vitro* maturation

Nigerian Dwarf goats were fasted 24 h prior to laparoscopy. Anaesthesia was induced with intravenous administration of diazepam (0.35 mg/kg body weight) and ketamine (5 mg/kg body weight) and was maintained with isofluorane via endotracheal intubation. Cumulus–oocyte complexes were recovered by aspiration of follicular contents under laparoscopic observation (Baldassarre *et al*., 1996).

The oocytes were graded according to the following criteria: grade I, oocytes with three or more layers of cumulus cells and homogeneous cytoplasm; grade II, oocytes with one or two layers of cumulus cells and homogeneous cytoplasm; grade III, oocytes with heterogeneous cytoplasm or denuded oocytes. Ten to twenty-five cumulus–oocyte complexes were cultured per 100 μl droplet of maturation medium covered with 4 ml mineral oil in a 35 mm Petri dish (Falcon 1008, Becton Dickinson, NJ) and incubated at 38.5–39 °C in 5% CO_2 for 25–27 h prior to ICSI. The maturation medium was TCM199 with Earle's salts and 25 mM Hepes supplemented with bovine LH (0.02 U/ml; Sioux Biochemicals, Sioux Center, IA), bovine FSH (0.01 U/ml; Sioux Biochemicals), estradiol-17b (1 μg/ml), 0.2 mM sodium pyruvate, kanamycin (50 μg/ml) and 10% heat-inactivated oestrous goat serum (EGS, produced from goats in our production facility).

Semen treatment and oocyte preparation

Fresh semen was obtained using an artificial vagina from a Nigerian Dwarf buck of proven field fertility. Semen samples with >70% sperm motility were used in these experiments. Semen samples were stored at

room temperature in the dark without extender for 2–3 h before being used in ICSI.

Defined medium (Brackett & Oliphant, 1975) was modified by addition of 6 mg/ml fatty-acid-free bovine serum albumin (BSA) and 100 µg/ml gentamicin sulphate (mDM). Percoll solution contained 90% Percoll (v/v) plus the following ingredients: 80 mM NaCl, 3.1 mM KCl, 0.29 mM NaH_2PO_4, 1.97 mM $CaCl_2$, 0.39 mM $MgCl_2$, 10 mM HEPES, 26 mM lactic acid, 25 mM $NaHCO_3$. The Percoll gradient was prepared by carefully adding 2 ml 45% Percoll solution (1 ml 90% Percoll solution diluted with 1 ml mDM) onto the top of 2 ml 90% Percoll solution in a 15 ml centrifuge tube (Sarstedt, Newton, NC).

The sperm cells were washed with Percoll gradient. Briefly, 100 µl fresh semen was layered on top of 4 ml Percoll gradient followed by centrifugation at 857 *g* for 30 min. The pellet was suspended in 6 ml of mDM and was centrifuged at the same speed for 10 min. Then, the pellet was resuspended in 1 ml mDM at room temperature.

Oocytes of grade I and II were pooled and briefly treated with 0.2% hyaluronidase solution in EmCare medium (Immuno-Chemical Products, Auckland, NZ). The cumulus cells were carefully stripped off the zona pellucida by pipetting sequentially through two glass pipettes with 250 µm and 200 µm inner diameters, respectively. Denuded oocytes with first polar bodies were selected and cultured in *in vitro* maturation (IVM) medium in 5% CO_2, at 38.5–39 °C until the ICSI procedure.

Micromanipulation procedure for ICSI

ICSI was performed in a 100 mm Petri dish (Sarstedt, Newton, NC) containing 100 µl EmCare plus1% fetal calf serum (FCS) covered with mineral oil (Sage Biopharma, WI). A straight oocyte-holding micropipette was set up with an angle of 45° to the bottom of the dish. The tip of the holding pipette had a 50 µm inner diameter, 175 µm outer diameter, and an unpolished straight cut edge. A bevelled micropipette with 6–7 µm inner diameter and a sharp spike on the tip (Humagen, VA) was used for sperm injection. The micromanipulation system was an Olympus inverted microscope with Normarski optics and a Narishige hydraulic micromanipulator attached to a PiezoDrill (Burleigh, NY).

A 5 µl aliquot of washed sperm suspension in mDM was mixed with 100 µl 10% polyvinylpyrrolidone (PVP) (wt. 360 000; ICN Biochemicals, OH) in EmCare medium. A 20 µl sperm-containing microdroplet was prepared beside the micromanipulation droplet under mineral oil. A motile spermatozoon was selected and its tail was partially cut with the holding pipette against the bottom of the dish (Fig. 1A). Immediately following tail-cutting, the spermatozoa with about 5 µm tail left were picked up using the injection pipette (Fig. 1B). The injection pipette containing a tail-cut spermatozoon was inserted into the ooplasm and piezo pulses (bandwidth 100 kHz, frequency 20 Hz, duration 75 µs, amplitude 75 V) were applied to break the oolemma. Following the pulses, the spermatozoon was discharged into the ooplasm with a minimum amount of the vehicle medium (Fig. 1C). It typically took approximately 15 s to complete the ICSI procedure for each oocyte. Following ICSI, the oocytes were cultured in B_2 medium (Laboratoire CCD, France) containing 10% FCS in 5% CO_2, at 38.5–39 °C for about 2 h. Oocytes injected only with vehicle medium (sham injection) served as controls.

Embryo culture

Oocytes undergoing ICSI were cultured using two culture regimes. (1) mTALP-mKSOM: During the first 24 h, the embryos were cultured in modified Tyrode's medium (Parrish *et al.*, 1986) supplemented with 20% EGS (mTALP) in 5% CO_2, 7% O_2 and 88% N_2, at 38.5–39 °C. From 24 to 96 h, the embryos were cultured in mKSOM + 0.3% BSA (fatty acid free) in 5% CO_2, 7% O_2 and 88% N_2, at 38.5–39 °C; from 96–192 h, the medium was changed to mKSOM + 1% fatty-acid-free BSA. The mKSOM was KSOM (Erbach *et al.*, 1994) modified by the addition of 2.5 mM Hepes, 0.4 mM taurine supplemented with Eagle's essential amino acids (1x) and non-essential amino acids (1×). (2) G1.3-G2.3 (Vitrolife, Englewood, CO): During the first 72 h, the oocytes were cultured in G1.3 and then they were transferred into G2.3 in 5% CO_2, 7% O_2 and 88% N_2, at 38.5–39 °C from 72 to 192 h.

The cultured embryos were assessed for cleavage under a dissecting microscope or were sampled for evaluation of pronuclear development using Hoechst 33342 staining (Choi *et al.*, 2002) and fluorescence microscopy at 20–22 h following ICSI (Fig. 1D). The cell number of the blastocysts was counted using fluorescence microscopy following Hoechst 33342 staining (Choi *et al.*, 2002).

Embryo transfer

Recipient goats were fasted and anaesthetised in the same manner as the donors. The oocytes cleaved or exhibiting visible pronuclei at 20–22 h culture were surgically transferred into the oviduct ipsilateral to ovulations by means of a TomCat catheter (Sovereign, Canada) threaded into the oviduct through the fimbriae. Pregnancy was detected by ultrasound using a SonoVet 600 scanner (Medison, Korea) with a - transrectal 7.5 MHz linear-array probe at about 30 and 60 days following embryo transfer.

Embryos produced using an *in vitro* fertilisation

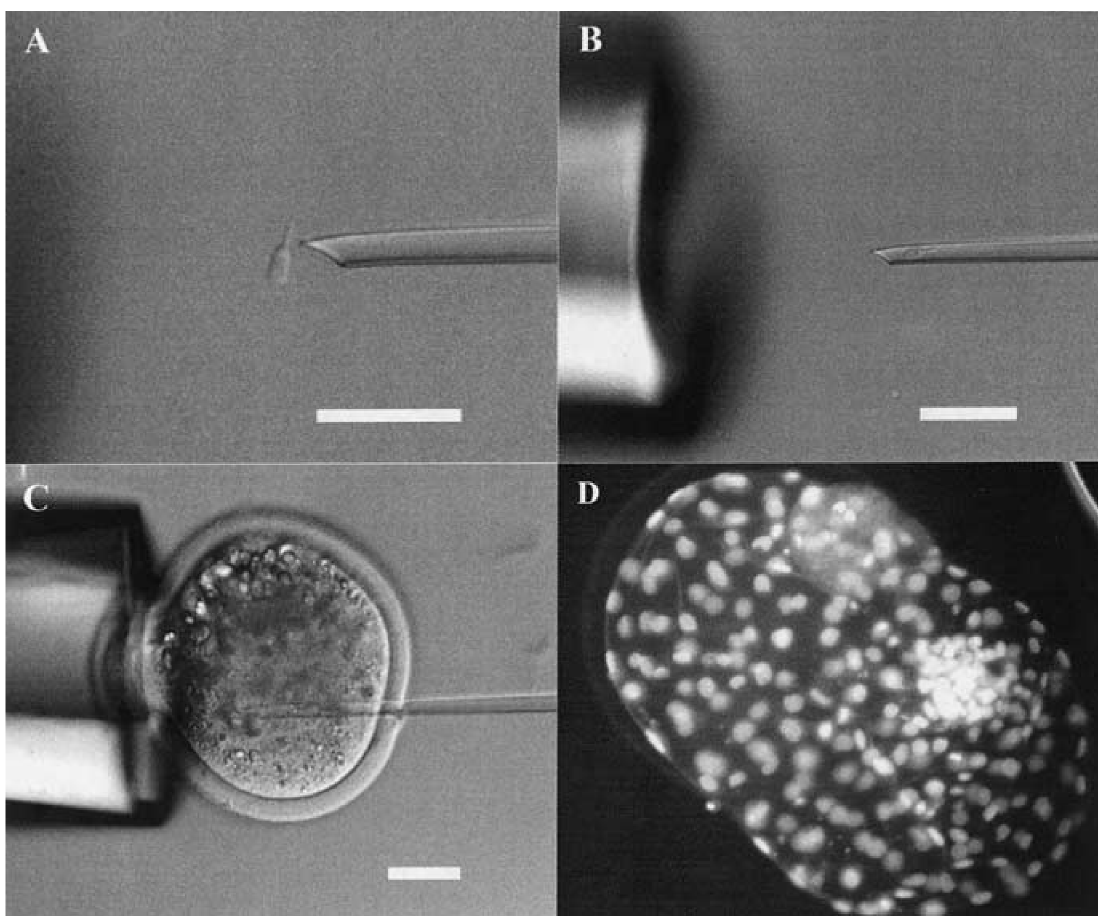

Figure 1 Goat ICSI using a tail-cut spermatozoon (*A*–*C*). A sperm tail was cut by the oocyte-holding pipette against the bottom of the dish, leaving a middle piece approximately 5 μm long (*A*). The tail-cut spermatozoon was aspirated into the injection pipette (*B*) and delivered into the ooplasm with the aid of a PiezoDrill (*C*). Hoechst staining showed cell nuclei in an ICSI-derived expanded blastocyst cultured for 8 days in mKSOM medium (x400) (*D*). Scale bar in (*A*)–(*C*) represents 20 μm.

procedure (Wang *et al.*, 2002) and at the same developmental stage as ICSI-derived embryos were transferred into recipient goats as the control group.

Statistical analysis

The proportional data of oocyte intactness following ICSI and embryo development at various stages were arcsin-transformed and were analysed with one-way or two-way ANOVA assay followed by Bonferroni post-tests. The cell number of blastocysts was analysed with Student's *t*-test. The statistical software was GraphPad Prism® (San Diego, CA). A probability of < 0.05 was considered to be statistically significant.

Results

Efficiencies of ICSI in goats

Thirty-two oocytes were sampled from two trials of ICSI at 20–22 h of culture for evaluation of pronuclear development. The result showed 26 of 32 (81%) with two normal pronuclei, 5 of 32 (16%) with condensed sperm heads and 1 of 32 (3%) without sperm (data are not shown in the tables).

Identical survival rates of the oocytes (97%) were observed from both groups of ICSI at 24–25 h versus 26–27 h after oocyte maturation culture (Table 1). Furthermore, the culture media (G1.3 vs mTALP) and oocyte age (24–25 h vs 26–27 h) had no significant effect on oocyte cleavage following ICSI (71% to 90%). As expected, no oocyte cleavage was observed in sham-injected groups (Table 1).

In vitro development of ICSI-derived embryos

A significantly higher proportion of ICSI-derived embryos developed to the blastocyst stage after 192 h culture in mTALP-mKSOM (35%) than those cultured in G1.3-G2.3 (9%), $p < 0.05$ (Table 2). However, the average cell number of blastocysts from both culture groups was similar (61, SEM = 7.3 vs 64,

Table 1 Effect of oocyte age and culture medium on ICSI efficiencies

Groups	Replicates	No. of oocytes injected	No. of intact oocytes (%)	No. of lysed oocytes	Culture media	No. of oocytes cultured	No. of oocytes cleaved (%)
ICSI at 24–25 h	5	113	110 (97)[a]	3	G1.3	63	45 (71)[b]
					mTALP	46	36 (78)[b]
ICSI at 26–27 h	5	114	111 (97)[a]	2	G1.3	61	44 (72)[b]
					mTALP	49	44 (90)[b]
Sham injection	5	47	46 (98)[a]	1	G1.3	27	0 (0)[c]
					mTALP	19	0 (0)[c]

[a,b,c] Data within the same column with different superscripts differ significantly ($p < 0.05$).

Table 2 *In vitro* development of ICSI-derived embryos cultured either in G1.3-G2.3 or mTALP-mKSOM

Groups	Replicates	No. of oocytes cultured	Embryo stage reached after 192 h in culture (%)				Average cell number of blastocysts (*n*)
			2–4 cells	5–16 cells	Morula	Blastocyst	
ICSI G1.3-G2.3	5	55	41 (74)[a]	22 (40)[a]	8 (15)[ab]	5 (9)[a]	64 (5)[a]
ICSI mTALP-mKSOM	4	49	44 (89)[a]	38 (78)[b]	20 (41)[a]	17 (35)[b]	61 (17)[a]
Sham G1.3-G2.3	5	27	0[b]	0[c]	0[b]	0[a]	0[b]
Sham mTALP-mKSOM	4	19	0[b]	0[c]	0[b]	0[a]	0[b]

The oocytes were injected with a spermatozoon at 26–27 h after maturation culture.
[a,b,c] Data within the same column with different superscripts differ significantly ($p < 0.05$).

SEM = 10.9, respectively; $p > 0.05$). No embryo development at first cleavage or beyond was observed in control groups.

In vivo development of ICSI-derived embryos

One of 4 recipients (25%) that received 6 ICSI-derived 2-cell embryos cultured in mTALP was diagnosed pregnant at 60 days following embryo transfer, and gave birth to one healthy male kid (Fig. 2). No pregnancy was detected in 6 recipients transferred with ICSI-derived embryos cultured in G1.3. Two of 5 recipients (40%) transferred with IVF-derived embryos were pregnant at 60 days, but only one recipient delivered one female kid (Table 3).

Discussion

An efficient ICSI procedure was demonstrated in this study (97% survival rate following ICSI and 71–90% cleavage rates). We hypothesise that the modification of ICSI technology including sperm tail-cutting and utilisation of a PiezoDrill contributes to the efficiency of this goat ICSI procedure. During fertilisation, the sperm plasma membrane over the acrosome cap is lost during the acrosome reaction. The remaining plasma membrane mingles with the oocyte's plasma membrane during sperm–oocyte fusion (Horiuch *et al.*, 2002). Therefore, the spermatozoon delivers only

Figure 2 The male kid produced by ICSI at 1 day after birth.

Table 3 Full-term development of ICSI-derived embryos versus IVF-derived embryos following transfer into recipients

Groups	No. of embryos transferred		No. of recipients used	No. of embryos per recipient	No. of pregnant recipients at 60 days (%)	No. of live births
	PN	2-cell				
ICSI mTALP	0	24	4	6	1 (25)	1
ICSI G1.3	7	29	6	6	0	0
IVF	4	26	5	6	2 (40)*	1

The oocytes were injected with spermatozoa at 24–25 h after maturation culture.
*One recipient lost a pregnancy between 60 days and the due date.

nuclear components into the ooplasm in natural fertilisation. The permeabilisation treatment of the sperm membrane prior to ICSI helps sperm nuclear decondensation. Although the process of decondensation occurs readily in humans (Palermo *et al.*, 1992), mice (Kimura & Yanagimachi, 1995) and rabbits (Deng & Yang, 2001) following ICSI by scoring of the sperm tail to disrupt the plasma membrane, bull and rat sperm heads remained condensed in oocytes following similar treatment (Perreault *et al.*, 1988). Keskintepe *et al.* (1997) reported that a low proportion of goat oocytes (~50%) cleaved after 48 h of culture following ICSI using the sperm tail-scoring procedure. Palermo *et al.* (1996) found that the fertilisation rate of human oocytes following ICSI using testicular spermatozoa increased significantly when sperm tails were scored more harshly. To ensure disruption of the sperm tail membrane and to facilitate the procedure of sperm loading and injection, we cut the sperm tail at the middle piece using an oocyte holding pipette. Wei & Fukui (2002) described a sperm tail-cutting procedure in cattle using a 20 μm sharpened bevelled pipette by micromanipulation, but the tail-cut spermatozoa would have to wait in the medium for more than 30 min prior to injection into the ooplasm while the sperm injection pipette is set up again after sperm tail-cutting (personal communication). The procedure for sperm tail-cutting used in this study did not require change of micropipettes, and hence speeded up ICSI to about 15 seconds after each sperm tail-cutting. This might be critical for minimising the damage to goat sperm chromatin incurred from rapid influx of ionic components into the medium, as has been reported in the case of ICSI in mice (Tateno *et al.*, 2000). In addition, the amount of vehicle medium delivered into the ooplasm was minimised when injecting a spermatozoon with about a 5 μm tail. Removing sperm tails is particularly helpful when trying to inject spermatozoa with long tails (Tateno *et al.*, 2000). Sonication is a simple and effective way to separate sperm tails and heads. However, the sperm centrioles, which are essential for fertilisation and development to term in species other than mice, might be lost during sonication treatment (Hamano *et al.*, 1999). Since centrioles are located in the sperm neck region (Roberts, 1986), our procedure of removing most, but not all, of the sperm tail results in retention of sperm centrioles, which in turn permits normal embryo development.

Since the oolemma of goat metaphase II oocytes is characterised by extreme elasticity (unpublished observation), like that of bovine oocytes (Gagne *et al.*, 1995), it could not be broken with micropipette insertion. In cattle, previous investigators often encountered pseudo-ICSI owing to failure to break the oocyte membrane. In fact, spermatozoa could have been expelled from the ooplasmic furrow created by the injection pipette. Extra manoeuvring of the pipette might result in oolemma penetration, but oocyte degeneration following ICSI was unavoidable (Keskintepe & Brackett, 2000). In this study, we employed the PiezoDrill, a device that generates mechanical pulses through a piezo-actuator to break the oolemma during ICSI. We demonstrated that recoverable local rupture of goat oolemma can occur when a certain level of pulse energy is exerted. The high survival rate of goat oocytes after ICSI confirmed other reports of the application of the piezo-actuator for ICSI in mice (Kimura & Yanagimachi, 1995), horses (Choi *et al.*, 2002), rats (Hirabayash *et al.*, 2002) and cattle (Hamano *et al.*, 1999; Horiuch *et al.*, 2002).

A number of reports have suggested that oocyte activation could be improved following ICSI with stimulation by calcium ionophore in goats (Keskintepe *et al.*, 1997), 6-dimethylaminopurine in cattle (Rho *et al.*, 1998), and electrical pulses in humans (Yanagida *et al.*, 1999) and pigs (Lai *et al.*, 2001). We found that it was not necessary to artificially activate goat oocytes following ICSI with our protocol since 90% of oocytes

cultured in mTALP medium cleaved (Table 1), which is consistent with findings in cattle (Katayose *et al.*, 1999).

The high proportions of polyspermic *in vitro* fertilisation has long been problematic in assisted reproduction in some mammalian species including goats (Palomo *et al.*, 1999; Bhatia *et al.*, 2002) and pigs (Nagai *et al.*, 1988; Funahashi & Day, 1997). Although many efforts were made to improve the *in vitro* fertilisation system by modification of oocyte maturation media (Wang *et al.*, 1997; Abeydeera *et al.*, 1998), capacitation (Bhatia *et al.*, 2002) and adjustment of sperm concentration (Abeydeera & Day, 1997), it remains difficult to obtain consistent improved normal fertilisation rate in goats or pigs comparable to those of bovine IVF system (Yang *et al.*, 1993). ICSI is an alternative approach to produce a high proportion of normal mammalian zygotes *in vitro*. In this study, we demonstrated that 81% of the goat oocytes fertilised with ICSI developed two normal pronuclei, and excluded the possibility of polyspermic fertilisation. Similar efficiencies have been reported in mice (Kuretake *et al.*, 1996), rabbits (Deng & Yang, 2001) and cattle (Horiuch *et al.*, 2002).

Previous studies have shown that mKSOM can efficiently support *in vitro* embryo development of bovine embryos from the 1-cell to blastocyst stage (Liu & Foote, 1995). In this study, a significantly higher proportion of blastocyst development was observed in mTALP-mKSOM (35%) compared with G1.3-G2.3 sequential culture media (9%). G1.3-G2.3 is an improved version of the previous G1.2-G2.2 sequential culture media (Gardner & Lane, 1997). A similar blastocyst developmental rate (~ 10%) of goat IVF-derived embryos cultured in G1.2-G2.2 sequential media was reported by Ongeri and co-workers (2001).

To further determine the developmental competence of the embryos generated from ICSI, embryos were transferred. A recipient that received embryos cultured in mTALP delivered a healthy male kid. This is the first report of goat offspring developed from ICSI-derived embryos. No pregnancy was established in recipients transferred with embryos cultured in G1.3. Although a small number of recipients were implanted with embryos cultured in G1.3, the result was consistent with *in vitro* development of embryos in G1.3-G2.3 medium.

In conclusion, cutting the sperm tail using a holding pipette coupled with PiezoDrill-assisted injection was an efficient approach for goat ICSI in terms of oocyte survival, pronuclear development and initial cleavage. The mTALP-mKSOM culture system was found to be more suitable for *in vitro* development of ICSI-derived goat embryos than G1.3-G2.3. This first case of full-term development of an ICSI-derived goat embryo suggests that ICSI can be applied to assisted reproduction in goats.

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