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A simple method of production of interspecies embryos between sheep and goat

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Interspecific chimaerism offers new approaches to the study of reproductive incompatibilities between species. Embryonic chimeras of the mouse are well-established tools for studying cell lineage and cell potential. They are also a key part of the analysis of complex phenotypes of mutant mice. By combining embryonic stem cell technology, molecularly tagged mutations and sensitive cell lineage markers, chimeras can provide invaluable insights into the tissue-specific requirement and the mode of action of many mouse genes (Tam and Rossant 2003). Interspecies embryos are generally produced by combining 2 early stage embryos of different species or by injecting embryonic stem cells of 1 species into the blastocyst of another species. The present study was undertaken to produce interspecies embryos between sheep oocytes and goat spermatozoa through simple method of *in vitro* fertilization.

Collection and processing of sheep oocytes: Sheep ovaries were collected from local abattoir and transported to the laboratory in thermo flask containing 0.9% sterile normal saline fortified with antibiotics (50µg/ml gentamicin sulphate) in 32–35°C temperature within 2 h. The ovaries were trimmed properly with the help of a sterile scissors and washed 4 times in 0.9% normal saline supplemented with antibiotics. Oocytes were aspirated by puncturing method in aspiration medium consisting of TCM–199 (HEPES modified) and bovine serum albumin (BSA) (0.3% w/v). Only A and B grade cumulous oocyte complexes (COCs) with more than 3 layers of cumulus cells and with homogenous ooplasm were taken for *in vitro* maturation. The oocytes were washed 5–6 times in maturation medium consisting of TCM 199 (HEPES modified), 10 µg/ml LH, 5 µg/ml FSH, 1 µg/ml estradiol-17ß, 0.05 mg/ml sodium pyruvate, 5.5 mg/ml glucose, 0.0035 mg/ml L-glutamine, 0.05 mg/ml gentamicin, 3 mg/ml BSA and 10% EGS (heat inactivated goat serum). The COCs (15–20 oocytes) were placed in 100µl droplet of maturation medium, covered with paraffin oil in a 35 mm petri dish and incubated in CO_2 incubator (5% CO_2 in air) with maximum humidity at 38.5°C temperature for 24 h.

Collection and processing of goat sperm: Fresh semen was collected from a proven buck by artificial vagina method. Neat semen (50 µl) was placed in 2 ml sp-TALP medium (Parrish *et al*. 1986) in a tube and allowed the spermatozoa to swim-up during incubation for 15 min at 38.5°C temperature under 5% CO_2 in air.

After incubation, the top 1.5 ml was taken and washed 2 times in sp-TALP medium at 296 g for 7 min each to remove the seminal plasma. Finally sperm pellet was suspended in 2 ml of fert-TALP medium containing 50 µg/ml heparin (Katska *et al*. 2004) and incubated at 38.5°C under 5% CO_2 in air in 38.5°C temperature for 1.5 h for capacitation.

In vitro fertilization of sheep oocytes and goat spermatozoa: In vitro matured sheep oocytes after 24 h of maturation with expanded cumulous cells were taken out from maturation medium drops and expanded cumulus cells were removed by repeated gentle pipetting in fert-TALP medium using sterilized Pasteur pipette. The oocytes were washed 5–6 times in fert-TALP medium. About 10–12 denuded oocytes were placed in 50 µl droplet of fert-TALP and covered with mineral oil and incubated at 38.5°C under 5% CO_2 in air for the equilibration of medium prior to *in vitro* fertilization. Capacitated sperm suspension of 50 µl (2×10^6 to 4×10^6 sperm/ml) was added to each 50 µl drop fert-TALP medium containing denuded oocytes and co-incubated for 15 h at 38.5°C and 5% CO_2 in air with maximum humidity.

Culture of presumptive interspecific embryos: At the end of 10 h of co-incubation, oocytes were taken out from fertilization drops and washed 5–6 times carefully in the embryo development medium (EDM) containing TCM 199 (HEPES modification), 30 µg/ml sodium pyruvate, 100 µg/ml L-glutamine, 50 µg/ml gentamicin sulphate, 10 µl/ml essential amino acids, 5 µl/ml non-essential amino acids, 10 mg/ml BSA (fraction-V) and 10% FCS to remove attached spermatozoa without damaging the oocytes by gentle pipetting. The washed oocytes were put in 35 mm petri-dish

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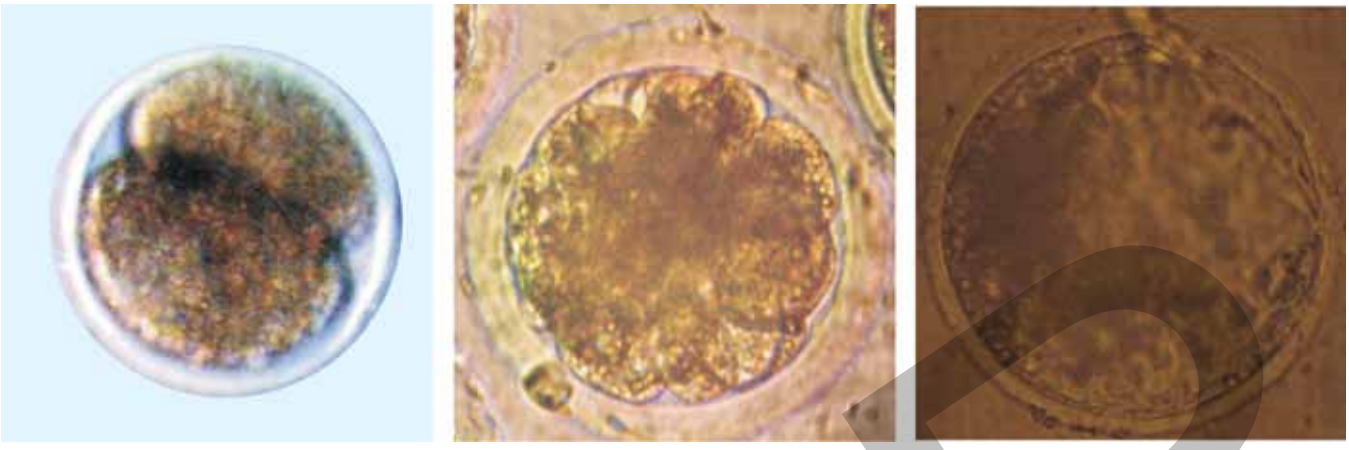

Figs 1–3. **1.** Two cell stage interspecies embryo. **2.** Morula stage interspecies embryo **3.** Blastocyst stage interspecies embryo.

having 100 µl drop of EDM and covered with mineral oil. This petri-dish was incubated in 5% CO_2 in air at 38.5°C and oocytes were examined for cleavage after 35 h. Cleaved oocytes were isolated from the droplet and put into 100 µl EDM supplemented with goat oviductal epithelial cells for further development.

Interspecies embryos are generally produced by combining 2 early stage embryos of different species or by injecting embryonic stem cells of one species into the blastocyst of another species. Interspecies chimeric calves were produced by aggregation of *Bos indicus* and *Bos taurus* demi-embryos (Williams *et al*. 1990). Recently interspecies nuclear transfer (NT) becomes an effective alternative for production of interspecific embryos and can be used very successfully to replicate animals when supply of recipient oocytes is limited or *in vitro* embryo production systems are incomplete. Interspecies nuclear transfer had been used for production of interspecies embryos between yak and dog (Murakami *et al.* 2005). The percentages of fusion and subsequent embryo development to the 8-cell stage of interspecies NT embryos were reported comparable to those of intraspecies NT embryos (cow-cow NT embryos) but the percentage of development to blastocysts was significantly lower ($P < 0.05$) in yak-cow NT embryos than that in cow-cow NT embryos (Murakami *et al.* 2005). The feasibility of interspecies somatic cell NT (iSCNT) was demonstrated by blastocyst formation and the production of offspring in several studies. Tecirlioglu *et al*. (2006) reported the production of interspecies embryos for horse-cow/mouse by interspecies SCNT. Interspecies nuclear transfer was used for production of embryos reconstructed from cat somatic cells and bovine ooplasm (Thongphakdee *et al*. 2008). Most recently in 2010, production of cloned asian elephant embryos using an interspecies somatic cell nuclear transfer (iSCNT) technique was reported (Sathanawongs *et al*. 2010). Lee *et al*. (2007) reported a simple method for mass production of chimeric embryos by coculturing denuded embryos and embryonic stem cells in an eppendorf vial for 3 h. In the present study, a simple method of *in vitro* fertilization was used for production of interspecies embryos between sheep and goat. The present method is very simple and reproducible. The cleavage (Fig. 1) was recorded 36–48 h post insemination and morula (Fig. 2) and blastocyst stage embryos (Fig. 3) were obtained on day 5 and day 7 respectively.

Table 1. *In vitro* production of interspecies embryos

No of ovaries	A+B quality oocytes	Cleavage (%)	Morula (%)	Blastocyst (%)
397	1140	673 (59.03)	291 (43.24)	89 (30.58)

The cleavage percentage was 59.03% . Among the cleaved embryos, 43.24% reached the morula stage and among morula, 30.58**%** reached blastocyst stage (Table 1).

It may be concluded that interspecies embryos between sheep and goat can be produced successfully *in vitro* up to the blastocyst stage.

SUMMARY

The present study was undertaken to produce interspecies embryos between sheep oocytes and goat spermatozoa through simple method of *in vitro* fertilization. Sheep oocytes were collected from slaughter house derived ovaries and were matured *in vitro*. Goat semen was collected by artificial vagina method and was processed for removal of seminal plasma and *in vitro* capacitation. Interspecies embryos were produced by co-incubation of *in vitro* matured sheep oocytes and *in vitro* capacitated goat spermatozoa. Total 89 interspecies blastocysts were produced. The study demonstrates a simple method of production of interspecies embryos between sheep and goat.

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