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PhD Thesis title: Goat embryo production from *in vitro* matured heterogeneous oocytes fertilised by intracytoplasmic sperm injection (ICSI) technique

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Abstract

This study was conducted to produce goat embryos from heterogeneous oocytes retrieved by laparoscopic ovum pick-up (LOPU), ovariectomy and slicing of abattoir ovaries fertilised by intracytoplasmic sperm injection (ICSI) technique. For LOPU technique, selected does were oestrus synchronised and then superstimulated. Recovered oocytes were then cultured in microdrops of *in vitro* maturation (IVM) medium in presence of CO_2 (5%) at 38.5°C for 27 hours. Frozen buck semen was thawed and motile sperm were selected by swim-up. ICSI was performed on the lid of a culture dish (35 mm) under an inverted microscope fitted with micromanipulators. After catching and immobilising a motile sperm, it was then aspirated into the injection needle. Each mature oocyte was positioned in the ICSI microdrop with its first polar body at 6 or 12 o'clock on the holding pipette; and sperm was injected, head-first, into the ooplasm. Injected oocytes were then cultured for 7 to 8 days in mSOF medium in the CO_2 incubator. A sample of embryos from each stage and uncleaved oocytes were stained with Hoechst 33342 to examine the number of nuclei of the embryos or pronuclear status. The data were presented as mean±SEM and were analysed using one-way ANOVA. The significant differences among treatments were further analysed by DMRT and $P<0.05$ was considered significant.

In Experiment 1, two protocols, *i.e.*, P-1 and P-2 were compared. While 54.1% fertilisation and 29.8% cleavage rates were obtained with P-2, only 28.6% fertilisation rate without any cleavage was achieved with P-1. Some embryos with P-2 progressed to morula (4.0%); and this is a first report of goat embryo development by ICSI in Malaysia.

Experiment 2 evaluated the effect of time intervals (36, 60 and 72 hours) between FSH+hCG treatment and LOPU on oocyte recovery (OR) as well as IVM and ICSI outcomes. Significantly higher OR ($P<0.001$), IVM ($P<0.001$), fertilisation ($P<0.05$), cleavage ($P<0.05$) and morula development ($P<0.05$) rates were obtained with 60 and 72 hours interval groups than 36 hours. No pregnancy was achieved after embryo transfer (ET) at morula stage to recipient does.

In Experiment 3, the effect of calcium ionophore (Ca^{2+} ionophore) on oocyte activation and embryo development by ICSI was evaluated. A significantly ($P<0.001$) higher fertilisation (88.3%), cleavage (81.3%) and morula (32.5%) development rates were obtained with pre- & post-ICSI activation regimen than pre-ICSI, post-ICSI or ICSI control and all four sham (without sperm) injection groups. Staining of uncleaved oocytes and embryos

revealed significantly (P<0.001) higher rates of 2 PN (47.5%) and oocyte activation (82.5%) with pre- & post-ICSI regimen than other groups.

Experiment 4 examined the effect of oocyte grading on IVM and embryo development by ICSI. Significantly higher (P<0.001) maturation (95.2%) and morula development (73.3%), and higher (P<0.05) fertilisation (74.2%) and cleavage (32.7%) rates were obtained from Grade A oocytes than Grades B, C and D. Similar results were obtained significantly when different grades of oocytes were treated with Ca^{2+} ionophore (pre- & post-ICSI regimen). However, no embryos progressed to blastocyst; and no pregnancy was achieved after ET at morula stage to recipient does.

In Experiment 5, developmental competence of oocytes of IVM and embryo of IVC from dysmorphic and normal groups were compared. Significantly (P<0.05) higher maturation rates were obtained from oocytes having normal ooplasm (73.7%) than paler (54.6%) and big fat globules (56.3%); while no significant difference with darker ooplasm (69.0%). After ICSI, no significant difference was found between dysmorphic and normal oocytes in fertilisation and cleavage rates. However, dysmorphic oocytes were developmentally less competent than normal ones as most of these oocytes were either arrested or degenerated at around 5 to 8-cell stage; whereas, morula (12.6%) were obtained from normal oocytes. This is the first report of goat embryo development by ICSI from dysmorphic oocytes.

Experiment 6 evaluated the effect of oocyte source on OR, IVM and embryo developmental competence. LOPU technique provided better quality oocytes as evidenced by the recovery of higher proportion of Grade A oocytes (32.6%) than abattoir (19.0%) source. IVM rate was significantly (P<0.01) higher in LOPU (73.8%) than abattoir (54.0%) source. Following ICSI, higher (P<0.05) fertilisation rate, cleavage and morula were produced from LOPU than abattoir source.

It can be concluded from the present study that goat embryos could be produced through ICSI technique in Malaysia. Prolonging time interval between FSH+hCG treatment and LOPU from 36 to 60 and 72 hours could improve OR rate, oocyte quality, IVM and embryo development rates. Pre- & post-ICSI Ca^{2+} ionophore treatment regimen improves oocyte activation, fertilisation and embryo development rates. Grade A oocytes are more developmentally competent than other grades. Dysmorphic oocytes could also be matured and developed to embryos but are less competent than normal oocytes. LOPU can provide higher quality oocytes, better maturation and embryo development rates than abattoir source. These findings would be able to fill up some gaps of scientific information in goat ICSI. Therefore, considering the current goat population of Malaysia, ICSI coupled with LOPU technique would be an appropriate assisted reproductive technology (ART) to generate *in vitro* goat embryos which will, in turn, improve country's goat population.