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Research and in Reproduction for Commercialization in Malaysia

Abdullah, R. B.* and W. E. Wan Khadijah

Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Email: ramli@um.edu.my

Malaysia gives a high priority in agriculture including livestock animals as the third engine of economic growth. In goats, the country imports more than 95% of goat meat requirement to meet the market demand with per capita consumption of 0.7 kg per year. This trend is increasing annually as the public realizes the goat meat is healthy to consume and less lipid contents compared with other meats. Goat population in Malaysia is very low and approximately 400,000 heads. Therefore, there is an urgent need to increase the goat population in the country to keep up the domestic requirement as well as possible export. Through conventional breeding technique, it is impossible to meet the goal and hence using reproductive technologies such as artificial insemination, embryo transfer and cloning would be logical choices to overcome these constraints.

In Malaysia, some efforts are made to conduct research and development in reproductive biotechnology in goat to enhance the goat production for the industry. Most of the research in goat reproduction has been conducted at the University of Malaya(UM). Other institutions involved are the Malaysian Agriculture Research and Development Institute (MARDI), the University Putra Malaysia (UPM) and Department of Veterinary Services.

Through application of artificial insemination using frozen semen, a synthetic goat breed (Jermasia) was developed by the University of Malaya through crossbreeding between imported German Fawn goat and local Katjang goat. This genotype is a dual-purpose breed for milk and meat production. This breed is adaptable to Malaysian climate and relatively resistant to parasites. At present, the demand for this breed by the local entrepreneurs is very high; however, the demand cannot be met due to low number of Jermasia breed. Therefore, serious efforts have been made to multiply this breed commercially as well as to further improve the performance through assisted reproductive techniques (ARTs). These techniques include artificial insemination (AI), cryopreservation of sperm, embryo as well as oocytes, oestrus synchronization and superovulation, *in vitro* maturation, fertilization and culture (IVMFC), intracytoplasmic sperm injection (ICSI), embryo transfer (ET) and nuclear transfer (NT) are being applied as

options for the generation of Jermasia kids.

Artificial insemination in goat is still limited to research in the university, government agencies and private sector. At the University of Malaya, AI technique has been successfully established using frozen semen with kidding rate of more than 70% (Abdullah *et al.*, 2002). However, when AI in goat is applied under field condition, the pregnancy and kidding rate are variable. This was thought to be due to the poor detection of oestrus, not optimized oestrus synchronization, sub-standard management especially feeding and lack of AI appreciation by the farmers involved. CIDR has been used routinely in goat research for artificial insemination programme (Siti Aina, 2005) and LOPU experiment (Mohd Noor Hisham, 2007) as well as embryo transfer project (Shariffah, 2009, personal communication). Cryopreservation of goat semen is technically challenging due to the presence of lipases in seminal plasma which interact with egg yolk to create substances that are toxic to sperm. Currently, the use of Tris egg yolk cryopreservation diluents has been recommended for goat sperm. Abdullah *et al.* (1997, 2002) reported that goat sperm were successfully frozen using TCAYE extender.

In modern goat farming as proposed by the government, the female goats are programmed to produce kids in large number at specified kidding times. To overcome the difficulties faced by goat producers in accurate oestrus detection and to determine proper time of insemination in a flock, attention was focused on developing technology to control the time of oestrus and ovulation. For better understanding of the physiology of oestrous cycle, time of ovulation and proper time of insemination, hormonal profiles for progesterone and oestradiol in different breeds of mature female goats were investigated (Massita *et al.*, 1999). This would help in better application of reproductive technologies such as AI and embryo transfer. Further in-depth research on normal and abnormal oestrous cycles, superovulation and oestrus synchronization is suggested before this technology is applied extensively. The most widely used method in goat is the treatment of progesterone for 10 ~ 17 days followed by luteolytic dose of prostaglandin administered in the period 36 hours prior to removal of intravaginal sponge or CIDR. CIDR is preferable because sponges frequently cause discomfort and may adhere to the vaginal wall causing problems upon removal. Ovulation in goat can be synchronized more precisely by administering GnRH around the time of oestrus which improves the success of fixed-time AI and collection of oocytes/embryos at a controlled stage of development for specific applications such as oocytes for IVP, ICSI, SCNT and zygotes for pronuclear microinjection.

Superovulation associated with embryo recovery and transfer to synchronized recipient females is considered as an effective means of increasing the contribution of superior females to the gene pool of the population. The successful application of multiple ovu-

lation and embryo transfer (MOET) technology largely depends on superovulation for which the essential factor is the treatment with gonadotrophins. In Malaysia, superovulation in goats was initiated in 1989using FSH or PMSG (Rosnina, 1989). The effect of FSH on superovulatory response of local goats was investigated; and it was found that superovulatory response based on number of corpora lutea was higher in FSH group (13.4±8.4) than in PMSG treated group (6.4±5.1), however, the difference was non-significant (Rosnina *et al.*, 1992). They reported that does responded throughout the year with more than 50% of does responding during rainy months compared with less than 35% responding during dry months. In our laboratory, PMSG alone (Shamsul, 1997) or in combination with hCG (Mohd Noor Hisham, 2007) was used to superovulate does during LOPU and embryo transfer programme. Due to high variability as well as lower oocyte recovery (OR) rate and embryo recovery, a combination of recombinant ovine FSH (OvagenTM ICPbio Limited, New Zealand) and hCG (Ovidrel; Laboratories Serono, Swittzerland) is currently preferred which resulted in less variability as well as high oocyte recovery rate and embryo recovery (Phua, 2006; Anna Aryani, 2007 Mohd Noor Hisham, 2007). These have subsequently been improved further (Rahman *et al.*, 2007; Abdullah *et al.*, 2008) and are still routinely in use.

Laporascopic ovum pick-up (LOPU) is a quick and less invasive technique and the protocol can be generally completed in less than 30 minutes by an experienced surgeon; it is cost effective and can be repeated several times on the same animal without the complications that accompany laparotomy or surgical oocyte collection. It is, therefore, generally more suitable for oocyte recovery from live animals and allows for repeated production of oocytes/ embryos from a single donor. LOPU is also an advantages technique for use in prepubertal or aged goats which would be unable to reproduce using AI or MOET. LOPU is performed under general anesthesia after standard surgical preparation andovarian follicles are aspirated under laparoscopic observation. While other LOPU-IVP research groups (Baldassarre *et al.*, 2007) obtained optimum OP rates (13.4 ~ 15.7 oocyte per doe) after performing LOPU at 36 hours of FSH+hCG treatment, OR rates in our laboratory (Phua, 2006; Anna Aryani, 2007; Mohd Noor Hisham, 2007) was always less than 7 oocytes per goat. Therefore, we increased the time interval between FSH and hCG treatment and the onset of LOPU from 36 hours to 60 and 72 hours and obtained significantly higher OR rates (8.6 and 16.1 per doe, respectively, at 60 and 72 hours intervals) (Abdullah *et al.*, 2008). With slight modification of superstimulation protocol (decreasing hCG dose rate from 500 IU to 250 IU per doe), 14.9 to 17.6 oocytes per doe were retrieved when LOPU performed 60 hours post-FSH-hCG treatment (Rahman *et al.*, 2008) which is similar to the results of Baldassarre *et al.*(2007) who

used slightly different protocol consisting of a single dose of FSH combined with a moderate dose of PMSG (e.g., 80 mg of FSH and 300 IU eCG). Using 60 mg FSH and 300 IU PMSG in their superovulation protocol, Gibbons *et al.*(2007) reported lower OR rate (5.6-8.0 oocytes per doe) than our study. Therefore, LOPU at 60 or 72 hours post FSH+hCG treatment could be the preferred protocol to optimize yields of good quality oocytes for IVM and ICSI embryos in goat and provide a flexibility of time interval.

Research on IVMFC in goat was carried out to optimize conditions for maturation, fertilization and culture *in vitro* (Phua, 2006 Anna Aryani, 2007; Chan, 2008). The main problems faced by the researchers include the poor quality of oocytes obtained from abattoir and difficulty to obtain goat embryo cleavage especially the blastocyst stage. Currently the media used for IVM, IVF and IVC were buffered TCM 199 supplemented with pyruvate, heat-inactivated goat serum and hormones (Rahman, 2008), BO medium with 25 μg/ml of heparin (Anna Aryani, 2009) as well as mSOF medium with heat-inactivated goat serum (Rahman, 2008). IVMFC are favoured techniques in circumstances such as the production of offspring from subfertile males and females, increasing the number of progeny from selected mature and juvenile females and salvage of oocytes or sperm from valuable dead or dying animals. Currently, our laboratory is engaged in goat IVMFC using oocytes that have mainly been obtained from LOPU. So far, now morula stage embryos have been successfully produced (Phua, 2006; Anna Aryani, 2007 Chan, 2008).

Cryopreservation of caprine oocytes and embryos has been attempted using established protocols in our laboratory, such as conventional freezing, quick freezing, direct plunging and vitrification using the mouse model. Currently, we are actively involved in goat embryo freezing particularly using vitrification methods such as cryoleaf and the results are encouraging (Chan, 2008; Azieatul Ashikin, 2009, personal communication; Tan, 2009, personal communication).

Intracytoplasmic sperm injection is a process whereby a single sperm is injected directly into the ooplasm of a matured, metaphase II oocyte. It is a valuable tool for production of embryos which may otherwise not be possible due to male factor infertility. In our laboratory, embryos have been produced both from normal and dysmorphic oocytes using this techniques (Rahman *et al.*, 2008). Currently, we are actively involved in goat ICSI studies using oocytes retrieved from both LOPU and abattoir sources (Kong, 2009, personal communication Farizah, 2009, personal communication).

Preimplantation embryos obtained from *in vitro* production or cryopreservations are transferred into the foster mother (recipient) to undergo a pregnancy perioduntil birth. In goats, the use of embryo transfer techniques in breeding programme is limited compared

with cattle. This is probably due to its excessive cost when compared to thevalue of the animal. Assisted reproductive technologies such as AI and MOET have been introduced to overcome reproductive inefficiencies in goats, and accelerate genetic gain. However, due to its high cost, MOET cannot replace AI as a routine reproductive technology. However, MOETcan be used as a means of increasing the rate of genetic gain when superior does are bred to high genetic-merit AI sires. Our laboratory is currently actively engaged in goat ET by minor surgery, using embryos derived from IVMFC, ICSI, AI or natural mating. Currently, the does are superovulated and mated to fertile bucks (or AI) and subsequently flushed either at 2-cell stage or blastocyst stage before embryo transfer being performed by oviduct and uterine embryo transfer, respectively. The extra embryos are cryopreserved using vitrification method. Confirmation of pregnancy was made through ultrasound scanning (Raja Ili Airina, 2009) and hormone analysis using RIA techniques (Mashitah, 2006).

Sexing of embryos prior to transfer has commercial application especially in dairy goats. The established technique for sex determination of embryos in domestic species is the application of Polymerase Chain Reaction (PCR) to amplify sex-specific gene(s), followed by electrophoresis. An embryo biopsy is performed whereby the biopsied portion is used for the sex determination assay and the remaining portion for further culture. Although bovine embryo sexing has been commonly reported, there is still a paucity of available information in goat embryo sexing (Phua *et al.*, 2003).

Nuclear transfer or transgenesis has the potential to play a crucial role in accelerating and facilitating genetic improvement. Transgenesis in the goat is important for developing and propagating founder animals which will produce valuable recombinant pharmaceutical or biomedical proteins in their milk. In goats, births have been produced from embryos obtained by transfer of either adult or foetal cell line nuclei into enucleated ova, with subsequent transfer of reconstituted embryos into recipients at the 2-4 cell stage (Yong, 1998; Baguisi *et al.*, 1999; Keefer *et al.*, 2002). Genes that regulate growth and milk production or pharmaceutical proteins can be inserted into embryos to obtain transgenic goats that produce desirable traits of economic and medical importance. Subsequently, pharmaceutical proteins produced in the transgenic goats will then be extracted from the milk. Very soon our laboratory will start a goat NT programme.Currently we are culturing the caprine ear fibroblast and cumulus cell obtained from various passages. The cleavage rates obtained from cloningusing these somatic cells are encouraging (Goh, 2009, personal communication; Kwong, 2009, personal communication; Soh, 2009, personal communication)

In summary, reproductive techniques have been actively researched in goats under

Malaysian condition, mainly at the ABEL laboratory, University of Malaya. These techniques include sperm freezing, AI, oestrus synchronization and superovulation, LOPU, IVMFC, ICSI, embryo sexing, cryopreservation, embryo transfer as well as nuclear transfer and stem cell research. The established techniques are goat sperm freezing using TCAYE extender, AI technique, LOPU technique as well as oestrus synchronization and superovulation. Other techniques such as IVMFC, ICSI, vitrification, embryo sexing as well as embryo transfer are still being perfected before it can be used routinely in our laboratory. Currently, we are initiating the cloning and stem cell research in goat and the progress is encouraging with production of cleaved cloned embryo from the ear fibroblasts and cumulus cells. In the future, it is hoped that our laboratory will be the reference center for goat reproductive technologies in Malaysia as well as in Asian region.

In conclusion, research and development in assisted reproductive technologies in goat shows a great deal of promises both in increasing scientific information on reproductive processes and mechanisms in goat as well aspractical application complementing conventional goat farm management to ensure sustainability and profitability of goat industry in Malaysia in the future.

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