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RECENT ADVANCES IN GOAT REPRODUCTIVE BIOTECHNOLOGY IN MALAYSIA

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ABSTRACT

Recent advances in reproductive endocrinology and physiology of mammals, molecular biology and genetic engineering have enhanced the expansion of the field of animal reproductive biotechnology. To date, many reproductive techniques have been applied in livestock animals such as cattle, sheep and pigs. Nevertheless, the application of such techniques is still limited in goat. This paper aims to give an introduction to goat farming in Malaysia and some background on the Jermasia goat breed developed by us and our team of researchers at the University of Malaya, Malaysia. A review of the recent developments in the field of reproductive biotechnology in goat, including the various assisted reproductive technologies (ARTs) employed in goat breeding programs such as collection and handling of gametes, estrus control, cryopreservation and manipulation of gametes and embryos, embryo transfer and transgenesis are presented. Finally, the challenges faced and the future direction of reproductive biotechnology in goat in Malaysia is put forward.

ABSTRAK

Kemajuan terkini dalam endokrinologi reproduktif dan fisiologi mamalia, biologi molekular dan kejuruteraan genetik telah membantu mempertingkatkan selanjutnya perluasan bidang bioteknologi reproduktif haiwan. Sehingga kini, pelbagai teknik reproduktif telahpun digunakan dalam haiwan ternakan seperti lembu, kambing biri-biri dan babi. Namun demikian, penggunaan teknik tersebut masih terhad dalam kambing. Makalah ini bertujuan untuk memberi suatu pengenalan kepada ternakan kambing di Malaysia dan sedikit latar belakang mengenai kambing baka Jermasia yang dimajukan oleh kami dan kumpulan penyelidik kami di Universiti Malaya, Malaysia. Penilaian perkembangan terbaru dalam bidang bioteknologi reproduktif dalam kambing, termasuk pelbagai jenis teknologi bantuan reproduksi (ARTs) yang digunakan dalam program pembiakan kambing seperti pengumpulan dan pengendalian gamet, kawalan estrus, krioawetan dan manipulasi gamet dan embrio, pemindahan embrio dan transgenesis adalah dibentangkan. Akhirnya, cabaran yang dihadapi serta halatuju masa hadapan bioteknologi reproduktif dalam kambing di Malaysia dikemukakan.

Key words: *In vitro* embryo production, Jermasia goat, reproductive biotechnology

INTRODUCTION

In Malaysia, the goat industry has been accorded high priority in line with the Third National Agriculture Plan (NAP3), particularly in meeting the country's demand for goat meat. Additionally, approximately 95% (more than15,000 metric tons of goat meat) of the country's demand for goat meat is imported every year to meet the local demand of 700,000 goats (Jamaluddin and Ng, 2005). Nevertheless, the population of goats in Malaysia stands just a little under one-third of this figure. One of the challenges for the government is to lower the cost of agricultural products especially livestock which has been increasing yearly. To overcome this hurdle, the current emphasis is on accelerating the transformation process for the agriculture sector in the country in order to become one of the main countries supplying specific food such as goat meat and milk as well as their products to the world. With appropriate measures being taken to increase the production of quality livestock using reproductive

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biotechnologies available along with modernized farming methods, production of goat meat is expected to increase by 6.7% annually between 2010-2015 and expected to reach 20,000 metric tons in 2015.

At the University of Malaya, we are currently involved in a 'Goat Breed Improvement Project' which is an initiative by the Implementation Coordination Unit of the Prime Minister's Department in Malaysia. This project conducted in Kepala Batas in the state of Penang is a systematic arrangement to make goat farming activity as a mechanism to reduce poverty in the rural areas. The goat is an ideal candidate for practical reasons. Goat meat is an attractive choice for farming and production as it carries less taboo connotations unlike other types of meat and is more accessible in a multiracial and multi-religion country such as Malaysia. With an increasingly health conscious society, goat meat is steadily becoming a choice meat as it has significantly lower fat (3%) compared to other meats such as cattle and sheep (16%). It has been reported that broiled goat meat has lower total lipid, phosphorus and vitamin B_{12}, but higher calcium, potassium and thiamin than composite values for beef (Johnson *et al.,* 1995). In terms of monetary gains, the selling price of goat meat is approximately between RM 15-20 (USD 4.7-6.2) per kg where the consumption per capita is estimated to be 0.5 kg. In fact, the National Goat Production policy targets having an estimated 2 million number of goats by 2015. Therefore, based on Malaysia's supply and demand, social and cultural background, the production and commercialization of goat farming promises exciting opportunities and returns.

Jermasia Genotype

At present, the development of goat farming in Malaysia is restricted by the insufficient number of suitable breeds for commercial purposes. In addition, live goats are imported and bred in Malaysia which poses a variety of problems such as the high cost of imported breeds, inconsistent stock quality and inability to adapt to tropical climate. As a result, the goats have low resistance to diseases and thus, higher mortality rate. To ensure success of goat breeding programs, challenges such as selection of goat breeds that are adaptable to tropical climate and offspring that would be economically viable to produce high quality milk and meat all year round have to be overcame. Consequently, the Jermasia goat breed has been the result of the tireless efforts of researchers at the University of Malaya in collaboration with researchers from the Technical University of Berlin, Germany in order to resolve the pressing need for suitable goat breeds in Malaysia (Horst, 1991).

The hybrid goat genotype, Jermasia has been developed through a systematic crossbreeding program between German Fawn and local Katjang goats (Mukherjee, 1991). The German Fawn goat was chosen for meat and milk production while the Katjang goat was selected for its adaptability to the Malaysian climate and resistance to parasites. Studies for the propagation and genetic improvement of the Jermasia breed included utilizing ARTs such as artificial insemination (AI), cryopreservation of sperm, estrus synchronization and superovulation/ovarian stimulation, *in vitro* maturation, fertilization and culture (IVMFC). Additionally, in a concerted effort to rapidly propagate and to improve the Jermasia breed to meet the commercial demand, ARTs such as laparoscopic ovum pick-up (LOPU), intracytoplasmic sperm injection (ICSI), embryo transfer (ET) and nuclear transfer (NT) are being applied as options for the generation of Jermasia kids (Abdullah and Wan Khadijah, 2009).

REPRODUCTIVE BIOTECHNOLOGIES IN GOAT

A more comprehensive review of reproductive biotechnologies in goat can be found in our published review (Rahman *et al.,* 2008). The following are some of the commonly used reproductive biotechnologies described in brief that are currently being adapted in our laboratory.

Artificial Insemination (AI)

AI may be regarded as a first generation ARTs that is most widely used and has made a significant impact on animal production worldwide (Leboeuf *et al.,* 2000; Wilmut *et al.,* 2000). AI is a process by which the sperm from a superior male animal is inseminated into the reproductive tract of a female for the purpose of impregnating the female by using artificial means. The three types of insemination techniques employed in goats are vaginal, cervical and intrauterine. AI can be used to increase male selection intensity, and hence to increase the average genetic merit of offsprings. In a closed population, this serves as a relatively simple method for dissemination of valuable genes. Nevertheless, it can also lead to problems in relation to inbreeding and variability of response. Like other countries, AI in goat has been successfully employed in Malaysia (Abdullah *et al.,* 2002).

Cryopreservation of Sperm

The use of sperm cryopreservation methods have revolutionized AI in livestock breeding and within the present context has served to further the

advancement of goat production albeit at a much slower rate compared to cattle and sheep (Abdullah *et al.,* 1997). Using cryopreservation methods, it is possible to store sperm indefinitely, allowing extensive usage and easy transport. Cryopreserved sperm facilitates international exchange of genetic material of valuable animals and extends the effective reproductive life beyond that of its own. It also invariably means that AI is able to be carried out at times beyond the determined reproductive seasons. As sperm cells vary between species in terms of size, shape and lipid composition (Purdy, 2006), hence optimization of cryopreservation protocols unique to the species of interest is necessary. Nelson and Mukherjee (1982) used different diluents and storage conditions to determine the quality of fresh semen in goats. Noran *et al.* (1998) studied in detail semen quality of local Katjang and crossbred (Katjang x German Fawn) bucks. The sperm freezing protocol developed in our laboratory which has produced much success constitutes a Tris-egg yolk citrate extender (Abdullah *et al.,* 1997, 2002). Nevertheless, usage of similar types of cryopreservation diluents (Salamon and Ritar, 1982) to those omitting biological components (Hinsch *et al.,* 1997; Gil *et al.,* 2003) and which are commercially available (Baldassarre and Karatzas, 2004) have been reported for goat.

Estrus Synchronization and Superovulation

In any genetic improvement programs, detection of estrus is important to ensure the success of artificial insemination. Estrus is controlled by reproductive hormones. Thangavelu (1987) showed that progesterone levels in the Katjang and crossbred were comparable. Estrus synchronization serves as a labor saving tool for capitalizing superior genes available through the use of AI. Estrus synchronization plays a key role in fixed time breeding, AI, LOPU and ET. Estrus synchronization in goats involves the treatments of progesterone for 9-11 days delivered through an intravaginal sponge, a controlled internal drug release (CIDR) device or a subcutaneous implant (Evans and Maxwell, 1987; Freitas *et al.,* 1997) and followed by a luteolytic dose of prostaglandin 1.5 days prior to removal of the intravaginal sponge (Baldassarre and Karatzas, 2004). To elicit a superovulatory response, ovarian stimulation with gonadotrophins, for example, equine chorionic gonadotrophin (eCG) and follicle-stimulating hormone (FSH) are generally used in higher doses, each of which has its advantages and disadvantages. The eCG is preferred due to its lower cost and easy availability; it can be more easily administered than FSH, usually as a single injection of up to 1500 to 2000 IU, but the superovulatory response to eCG can be quite variable and is usually lower than in a FSH-induced superovulation (Amoah and Gelaye, 1990). Problems associated with eCG-induced superovulation are high number of non-ovulated follicles, early regression of corpus luteum, short or irregular estrous cycles and potential risk of embryo expulsion (Amoah and Gelaye, 1990). Like in cow and ewe, a number of experiments have been performed in goat to compare the superovulatory response between FSH and eCG, with evidence favouring the use of FSH rather than eCG (Tsunada and Sugie, 1989; Pendelton *et al.,* 1992). It has been reported (Tsunada and Sugie, 1989) that the average number of oocytes recovered was significantly higher in FSH-treated goats (9.4) compared with those eCG-treated (5.7). Evaluating all the superovulation protocols in use to date none can be singled out to satisfy all expectations concerning predictability and reliability of the response. Administration of gonadotrophins around the time of estrus enables ovulation to be synchronized with better accuracy in goat (Pierson *et al.,* 2003). Exogenous FSH is administered towards the end of the luteal phase of the cycle (days 9-11) or around 2 days before the end of the synchronizing treatments. Superovulation with gonadotrophins increases the number of follicles available for collection which also potentially enables use of juvenile or prepubertal animals as oocyte donors. Nevertheless, variability in number of ovulations and yield of viable oocytes remains a major drawback with a host of intrinsic and extrinsic factors contributing to the variability (Nuti *et al.,* 1987; Mahmood *et al.,* 1991; Holtz, 2005). In the past, pregnant mare serum gonadotrophin (PMSG) alone (Samsul, 1997) or in combination with human chorionic gonadotrophin (hCG) (Mohd Noor Hisham, 2006) was used in our laboratory to superovulate does. However, due to higher variability of stimulation and lower oocyte retrieval (OR) rate, a combination of recombinant ovine FSH (Ovagen™; ICPbio Limited, New Zealand) and hCG (Ovidrel; Laboratories Serono, Switzerland) as single doses was later introduced in our laboratory (Mohd Noor Hisham, 2006; Phua, 2006; Amir, 2007), which has been improvised further (Rahman *et al.,* 2007a,b,c, 2008; Abdullah *et al.,* 2008; Rahman, 2008) and is still in use.

Laparoscopic Ovum Pick-Up (LOPU)

LOPU is a swifter method where it can be generally completed in less than 30 minutes by an experienced surgeon, as well as cost effective and can be repeated several times on the same animal without the complications that accompany laparotomy or surgical oocyte collection. It is 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 to be employed for prepubertal or aged goats which would be unable to reproduce using AI or MOET. LOPU is performed under general anesthesia after standard surgical preparation and ovarian follicles are aspirated under laparoscopic observation.

Like in human (Thornton *et al.,* 1990; Mansour *et al.,* 1994), monkey (Ng *et al.,* 2002; Chen *et al.,* 2006) and pig (Ratky *et al.,* 2003), LOPU is also performed after 36 hours of FSH plus hCG treatment in goat (Baldassarre *et al.,* 2002). However, unlike in human, monkey and pig, oocytes recovered after 36 hours time interval between FSH plus hCG treatment and LOPU in goat are still at the immature stages and require IVM for 27 hours before becoming meiotically competent (Baldassarre *et al.,* 2003). While some groups (Baldassarre *et al.,* 2002; Baldassarre *et al.,* 2007) obtained optimum OR rates (13.4-15.7 oocytes per doe) after performing LOPU at 36 hours of FSH plus hCG treatment, OR rates in our laboratory (Mohd Noor Hisham, 2006; Phua, 2006; Amir, 2007; Rahman *et al.,* 2007a,c) was consistently less than 7 oocytes per goat. However, one study (Gibbons *et al.,* 2007) using a shorter time interval of 24 hours between FSH plus eCG and LOPU also obtained low OR rates (5.6-8.0 oocytes per goat). Therefore, in our laboratory we increased the time interval between FSH plus 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 interval) (Rahman *et al.,* 2007c; Abdullah *et al.,* 2008). With slight modification of the superovulation 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 was performed 60 hours post-FSH-hCG treatment (Rahman, 2008) which was similar to another group (Baldassarre *et al.,* 2002, 2003, 2006) who used a slightly different protocol consisting of a single dose of FSH combined with a moderate dose of eCG (e.g., 80 mg FSH and 300 IU eCG). Therefore, LOPU at 60 or 72 hours post-FSH plus hCG treatment is suggested to be the preferred protocol in our laboratory to optimize yields of good quality oocytes for IVM and ICSI embryos in goat and provide flexibility in the time interval.

In Vitro Maturation, Fertilization and Culture (IVMFC)

In vitro maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) which are collectively known as IVMFC or *in vitro* production (IVP) of embryos is important in enabling optimization of ARTs and other existing biotechnologies for the improvement in the number of offspring produced by genetically valuable does and the subsequent possibilities of animal cloning and gene transfer. Nevertheless, some of the main limitations of MOET in goat particularly are the often low and variable embryo numbers. Therefore, one way to circumvent this limitation is by non-surgical retrieval of ova from females (ovum pick-up, OPU) and the subsequent optimized IVMFC which can potentially yield large numbers of transferable embryos. Additionally, IVMFC are favored techniques in instances such as for the production of offspring from sub-fertile males and females, to increase the number of progenies from selected mature or juvenile females and salvage oocytes or sperm from valuable dead or dying animals. Currently, our laboratory is engaged in goat IVMFC using oocytes mainly obtained from LOPU and up till now morula stage embryos have been successfully produced (Phua, 2006; Amir, 2007).

Intracytoplasmic Sperm Injection (ICSI)

ICSI is a process where 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 not be possible due to male factor infertility. It has potential values for the study of interspecies embryos within the caprine family. ICSI can be applied for the production of transgenic goats for the production of biopharmaceuticals in their milk (Wheeler, 2003; Wheeler *et al.,* 2003). Therefore, ICSI has very real potential and can be a useful method to employ for the generation of transgenic animals (Perry *et al.,* 2001). Our laboratory is actively involved in goat ICSI studies using oocytes retrieved from both LOPU and abattoir source. Embryos were produced both from normal (Rahman *et al.,* 2006a,b; Rahman *et al.,* 2007a,c; Abdullah *et al.,* 2008; Rahman *et al.,* 2008) and dysmorphic oocytes (Rahman *et al.,* 2007b) using this technique.

Embryo Transfer (ET)

Pre-implantation embryos obtained from IVP or cryopreservation are transferred into the foster mother (recipient) to undergo a period of pregnancy until birth. In goats, the use of ET technique in breeding programs is limited compared to cattle. This is probably due to the excessive cost when compared to the value of the animal. ARTs such as AI and MOET have been introduced to overcome reproductive inefficiencies in goats, and accelerate genetic gain. Nevertheless, although MOET may be unlikely to replace AI as a routine reproductive technology due to its high cost, it can be applied to allow extra genetic gain through production by embryo transfer of males with positive indexes used for AI. The application of IVP and interspecific embryo transfer has been proposed as a strategy for the rescue of some endangered species. Embryo transfer in goats has the potential to facilitate safe

worldwide movement of germplasm, provided that management and handling of the animals and embryos are conducted according to satisfactory sanitary standards. Our laboratory is actively engaged in goat ET by minor surgery using embryos derived from IVMFC, ICSI, AI or natural mating.

Nuclear Transfer (NT) and Transgenesis

Nuclear transfer and gene transfer or transgenesis has the potential to play a crucial role in accelerating and facilitating genetic improvement. Transgenesis of 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 fetal 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.,* 2001, 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 could then be extracted from the milk. In our laboratory, cloned goat embryos were produced using both intraspecies- and interspecies nuclear transfer techniques (Abdullah *et al.,* 2011).

FUTURE DIRECTIONS

It is evident that technologies based on IVP of embryos will influence animal breeding strategies. Therefore, there is an urgent need for a concerted and sustained investment in research to improve technologies for embryo production *in vitro*. Focused initiatives need to be further driven towards greater involvement of scientific organizations and research scientists in developing and refining embryo technologies available. ARTs applied in the field of agriculture play a significant role in enhancing genetic gain in nucleus breeding programs as well as reducing the lag between the breeding population (the nucleus) and the commercial production population in goats.

At our research centre at the University of Malaya, we have undertaken various strategies for Jermasia commercialization. Amongst the strategies undertaken are conducting intensive research to propagate the nucleus herd at the University of Malaya with application of ARTs. The dedicated and ongoing research conducted by our team of scientists to develop the Jermasia genotype serves not only for teaching and research purposes, but also as an earnest contribution to society through commercialization. This is hoped to be achieved by applying advanced reproductive biotechnologies to continuously propagate and further refine the Jermasia goat breed. Future improvements in Jermasia development potentially opens up opportunities in the pharmaceutical and medical sectors.

Hence, embryo technologies applied to animal breeding have a role of increasing the impact of superior genotypes in the population. However, a more widespread and competent use of the available techniques is required in order to gain the most benefit from their application. Future developments linked to the fast emerging areas of research such as somatic cloning and embryo genotyping are expected to find a role in advanced animal breeding. Together with the requirement for continuous scientific progress there is also the need to address public concerns over new ARTs. In this respect, more studies are needed to demonstrate the safety of embryo biotechnologies and the suitability of the derived products that enter the food chain.

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