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Recovery and Grading of Goat Oocytes with Special Reference to Laparoscopic Ovum Pick-up Technique: A Review

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Abstract: Oocytes are the main raw materials for *in vitro* embryo production (IVP) experiments. Therefore, the success of any IVP program in goat production, either *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) largely depends on the continuous supply of quality oocytes in optimum quantity. A number of methods are currently used for oocyte recovery (OR) from live or slaughtered goats. Although, abattoir is the most easy and cheapest source of oocytes, however, in certain countries especially in Malaysia, abattoir source is extremely limited due to low slaughtering activities as a consequence of shortage of breeding stock. In Malaysia normally older goats or goats those are culled for breeding are slaughtered for meat. Therefore, the quality of oocytes recovered from ovaries of these goats is generally lower. On the contrary, OR from live goats using laparoscopic ovum pick-up (LOPU) technique provides many advantages, for example, OR can be repeated 3-5 times in the same goat at interval as short as a week or less, OR can be done in prepubertal and aged goats, if prepubertal goat is used for OR then generation intervals will be reduced, LOPU coupled with IVP can become an efficient method of early propagation of valuable goats and LOPU can overcome limitations frequently associated with multiple ovulation-embryo transfer (MOET). Therefore, LOPU can be an alternative and efficient OR method in goat not only for Malaysia but also for other countries. The current study will discuss oocyte recovery methods with special reference to LOPU and grading of recovered oocytes in goats.

Key words: Goat, laparoscopic ovum pick-up, oocyte recovery, oocyte grading, cumulus-oocyte complexes

INTRODUCTION

The success of any *in vitro* production (IVP) system including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) is largely dependant on the continuous supply of good quality oocytes. Oocytes can be collected from live and dead, killed or slaughtered goats. Oocyte recovery (OR) from live goats may be accomplished via ovariectomy, laparotomy, laparoscopic ovum pick-up (LOPU) or transvaginal ultrasound-guided aspiration (TUGA) procedures. On the other hand, oocytes can be obtained from dead goats at post-mortem or from the abattoir at slaughter. While collecting ovaries from dead goats is uncertain and largely depended on the availability; access to abattoir-derived ovaries is easy and provides a cheap and abundant source of oocytes. Although abattoir is an easy and cheapest source of oocyte worldwide, in Malaysia, this source is extremely limited due to low slaughtering activities as a consequence of shortage of breeding stock and when slaughtered, normally older or culled goats are chosen. The quality of the oocytes recovered from these goats is generally lower.

OOCYTE RECOVERY FROM SLAUGHTERED GOATS

Oocytes from ovaries of slaughtered animals are obtained by several methods of which follicle aspiration or slicing the ovary are mostly practiced. Oocyte aspiration from ovarian follicles is performed by simple aspiration of follicular contents using a syringe and a needle of 18 to 22 g (Keskintepe *et al*., 1998; Yadav *et al*., 1998) or aspiration with a constant vacuum source (Velilla *et al*., 2002). The collection by aspiration provides 1.5 to 2.0 cumulus-oocyte complexes (COCs) of acceptable quality per adult ovary (Cognié, 1999). Ovaries have been subjected to dissection and isolation of individual follicles followed by rupture of the latter (Le Gal, 1996; Crozet *et al*., 2000), cutting the surface of the ovary and releasing the oocyte from follicle with a razor blade (Martino *et al*., 1994) or slicing of the ovaries followed by

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simple rinsing of the slices (Pawshe *et al*., 1994, 1996; Ongeri *et al*., 2001; Rho *et al*., 2001). Martino *et al*. (1994) and Pawshe *et al*. (1994) reported that ovary slicing was a simpler and more efficient technique than follicle aspiration in that an average of 6.0 COCs per ovary were obtained by slicing as opposed to between 1.5 to 2.0 oocytes per ovary by aspiration. In their study, Yadav *et al*. (1998) recovered on average 3.0 oocytes per ovary by aspiration. However, oocyte yield can be improved by follicle stimulating hormone (FSH)-priming the female goat before killing or slaughtering (Crozet *et al*., 1993; Mogas *et al*., 1997; Katska-Ksiazkiewicz *et al*., 2004). Using this method, Crozet *et al*. (1993) obtained an average of 9.0 COCs per ovary from FSH-primed goats. In their study, Katska-Ksiazkiewicz *et al*. (2004) retrieved a significantly higher number of oocytes per goat from FSH-primed (24.5) than control (14.7) ovaries after slicing. As mentioned earlier that abattoir is an easy and cheapest source of oocyte worldwide, in Malaysia this source is extremely limited due to low slaughtering activities as a consequence of shortage of breeding stock. The quality of the oocytes recovered from these does is generally lower.

OOCYTE RECOVERY FROM LIVE GOATS

Oocytes from live goats can be retrieved following superovulation or without hormonal stimulation using various procedures. These include follicle aspiration or slicing of ovariectomized ovary, follicle aspiration through laparotomy, LOPU or TUGA. Laparotomy or standard surgery method of OR is invasive, frequently causing adhesion of ovaries and other viscera and other surgery related complications, even death (Melican and Gavin, 2008). In their experiment, using superovulated goats Samaké *et al*. (2000) retrieved 18.0 oocytes per goat through aspiration of follicles after ovariectomy and 8.3 oocytes per goat through aspiration of exteriorized ovaries by laparotomy. The LOPU procedure is recognized as less traumatic than the standard laparotomy (Koeman *et al*., 2003; Tibary *et al*., 2005). Recently, TUGA technique has been introduced in goat OR which is non-invasive in nature (Graft *et al*., 1999; Melican and Gavin, 2008).

In TUGA technique, the goat is placed in dorsal recumbence and a 5 MHZ transvaginal transducer, attached to the ultrasound unit, is positioned vaginally for oocyte aspiration (Graft *et al*., 1999; Melican and Gavin, 2008). This approach has proved to be a safe, practical alternative to surgical or laparoscopic oocyte collection. However, efficiency of TUGA-derived oocytes for IVP is not well studied like LOPU yet (Melican and Gavin, 2008). The TUGA is commonly used in large domestic livestock, particularly cattle and not much practiced in small ruminants such as goats. This technique can be problematic in the goat, due to premature luteal regression, low fertilization rates and variability in the superovulatory response to exogenous hormones (Cognié, 1999). In a study, number of follicles detected and oocytes harvested using TUGA (9.5 and 4.3, respectively) was less than for goat obtained by LOPU (17.4 and 14.4, respectively) (Graft *et al*., 1999). The percentage of oocytes recovered from goats subjected to the TUGA (68%), however, was similar to those subjected to the LOPU (69%). It has been reported that repeated TUGA in prepubertal calf caused histological lesions in the ovary which may alter normal ovarian function and could possibly affect future fertility (Snel-Oliveira *et al*., 2002). This technique could offer an alternative, compared with traditional breeding, for progeny development from female transgenic founder dairy goats in near future (Melican and Gavin, 2008).

LAPAROSCOPIC OVUM PICK-UP OR LOPU IN GOATS

The LOPU is one of the best techniques for oocyte recovery from live goats. The LOPU followed by IVP has been proposed as an efficient method for the propagation of sheep and goat of high genetic value (Baldassarre *et al*., 1996, 2002; Tervit, 1996; Cognié *et al*., 2004). The LOPU is advantageous than standard laparotomy-based method which allows repetition of the laparoscopic procedure more frequently and more times during the reproductive life of a valuable female (Baldassarre *et al*., 2007). Another advantage of LOPU is the possibility of producing embryos and progeny from animal categories those are not able to reproduce by multiple ovulation-embryo transfer (MOET) and artificial insemination (AI), including prepubertal (Baldassarre and Karatzas, 2004) and aged goats (Baldassarre *et al*., 2007). The LOPU technique obviates several causes of the poor results observed with superovulation, such as poor ovulation rate, early regression of corpus luteum (CL) and poor fertilization (Baldassarre and Karatzas, 2004). Therefore, collection of oocytes after exposure of the ovary by laparotomy has been gradually replaced by LOPU technique in small ruminants.

The oocyte recovery through LOPU from live donors in goats and sheep has been performed by puncture of follicles and then aspiration of the follicular content with a sharp needle guided by laparoscopy. Snyder and Dukelow (1974) first conducted LOPU in small ruminants where 6.0 oocytes were retrieved from 21 aspirated follicles from a ewe. However, the potential of the LOPU technique was not fully realized until IVP technologies

were developed (Baldassarre *et al*., 1994, 2002). Subsequently, LOPU becomes an efficient method for oocyte collection both in sheep (Baldassarre *et al*., 1994; Kühholzer *et al*., 1997) and goats (Baldassarre *et al*., 2002; Pierson *et al*., 2004). In LOPU procedure, donor goats are restrained on a standard laparoscopy table using general anesthesia and follicles are aspirated under laparoscopic observation using an 18 to 22 g needle mounted in a plastic pipette connected to a collection tube and a vacuum line. The procedure takes less than 30 min for each goat by an experienced surgeon depending on the number of follicles to be aspirated which also reduces animal stress (Kühholzer *et al*., 1997). The LOPU almost always results in >5 oocytes aspirated per donor (Baldassarre and Karatzas, 2004). The LOPU can be repeated several times without ovarian damage or decrease in the donors' fertility (McKelvey *et al*., 1986; Stangl *et al*., 1999; Alberio *et al*., 2002; Pierson *et al*., 2004). The LOPU, after multi-dose hormonal treatments, may be repeated at intervals as short as a week, even 4 days (Alberio *et al*., 2002; Gibbons *et al*., 2007), without diminishing good quality oocytes harvest (Stangl *et al*., 1999; Alberio *et al*., 2002). However, repeated LOPU in unstimulated goats also provided oocytes (4-6 per doe per session) (Cognié *et al*., 2003). If the quality of these oocytes for IVP is confirmed, this method could provide a way to produce offspring from genetically valuable females without using hormones (Cognié *et al*., 2003).

In superovulated goats, oocyte recovery rate ranged from 33% to 80% with an average yield that ranged between 5.6 and 13.4 oocytes per animal (Kühholzer *et al*., 1997; Alberio *et al*., 2002; Baldassarre *et al*., 2002; Baldassarre *et al*., 2003; Baldassarre and Karatzas, 2004; Pierson *et al*., 2004; Gibbons *et al*., 2007). It has been found that LOPU can be repeated up to 5 times in the goats at different intervals and in different seasons with little or no important change in overall response (Pierson *et al*., 2004; Rahman *et al*., 2007a). Like other mammals, goat oocyte acquires the ability to mature and accomplish its developmental competence during oogenesis and folliculogenesis. Previously it has been shown that the proportion of developmentally competent oocytes increases with follicular size (Humblot *et al*., 2005; Han *et al*., 2006; Khatir *et al*., 2007). Consequently, the timing of ovum pick-up affects oocyte developmental competence. In humans (Thornton *et al*., 1990; Mansour *et al*., 1994), monkeys (Ng *et al*., 2002; Chen *et al*., 2006) and pigs (Ratky *et al*., 2003) ovum pick-up was performed 36 h after human chorionic gonadrotrophin (hCG) injection. Majority of the oocytes collected at this interval are at the mature or metaphase II stage and are developmentally and meiotically competent. However, in goats, oocytes collected 36 h after hCG injection are still at the immature stages and are matured *in vitro* for 27 h before reaching meiotic competence (Baldassarre *et al*., 2003; Rahman *et al*., 2007b, 2008). Keeping this in mind our recent study showed that delaying LOPU post-FSH plus hCG treatments can improve oocyte retrieval rate and oocyte quality (Abdullah *et al*., 2008). Therefore, LOPU at 60 and 72 h post FSH plus hCG treatment could be the preferred protocol to optimize yields of good quality oocytes for IVM and ICSI embryos in goats. As already mentioned earlier, abattoir ovary source is very limited in Malaysia, therefore, LOPU could be an alternative procedure for goat OR to carry out IVP, especially ICSI study. Timeline of significant finding of LOPU experiments in goats is depicted in Table 1.

Table 1: Timeline of significant finding of LOPU experiments in the goats

Follicle aspiration (FA)/ oocyte recovery (OR)	Significant findings	References
-	LOPU coupled with IVP demonstrated great potential for the production and propagation of transgenic goats.	Baldassarre *et al.* (2002)
FA = 32 versus 18 and OR = 25 versus 16, respectively, from adult and prepubertal goats	LOPU is an efficient technique for the recovery of high quality oocytes from both adult and prepubertal goats.	Koeman *et al.* (2003)
FA = 42.5/goat OR = 33.4/goat	LOPU in combination with IVP is an efficient technique for early propagation of valuable does by SCNT.	Baldassarre and Karatzas (2004)
-	LOPU may be repeated up to 5 times in goats at different intervals and in different seasons with little or no important change in overall response.	Pierson *et al.* (2004)
OR = 6.7/goat	LOPU after 36 h of FSH plus hCG treatment provided minimum oocytes.	Rahman *et al.* (2007b)
FA = 27.0/goat OR = 21.9/goat	LOPU could provide a higher number of oocytes in goats.	Cox and Alfaro (2007)
FA = 17.9/goat OR = 15.7/ goat	Oocyte recovery from aged (7-8 years) goats of high genetic value.	Baldassarre *et al.* (2007)
FA = 12.4-18.6/goat OR = 5.6-8.0/goat	Repeated OR in goats as short as 4 days interval can provide quality oocytes.	Gibbons *et al.* (2007)
OR = 4.2, 11.4 and 16.1/goat, respectively, when LOPU performed at 36, 60 and 72 h post-hormone treatment	Prolonging the interval from ovarian superstimulation to laparoscopic ovum pick-up improves oocyte yield, quality and developmental competence in goats.	Abdullah *et al.* (2008)

BASIC LOPU TECHNIQUE

Appropriate technique is a must for efficiency in LOPU and finishing the procedure in a minimum time with minimum stress and discomfort to the animal. Proper positioning and restrain of the goat is very important for achieving the optimum access and examination of the reproductive tract (Edey, 1983). First of all, the goat should be anaesthetized with proper anesthetic agent and the anesthesia should be maintained until finishing the surgery. This can be achieved with intramuscular administration of Xylazine hydrochloride (Ilium Xylazine-20; Troy Laboratories Pvt. Ltd., Australia) at a dose rate of 0.22 mg kg^{-1} body weight followed by intramuscular administration of Ketamine hydrochloride (Ketamil; Troy Laboratories Pvt. Ltd., Australia) at a dose rate of 11 mg kg^{-1} b.wt. (Rosnina *et al*., 1992). The anaesthetized goat should be restrained on a standard laparoscopy table with tilting apparatus at 40 degree angle which was first developed by Hulet and Foote (1968) with head-down in a dorsal recumbent to allow the gut and *Omentum majus* to move cranially.

LOPU must be performed in a clean, dust-free area. Following the induction of anesthesia and restraining of the goat, it should be shaved aseptically using a razor and disinfected by applying ethanol (90%) over the shaved part of the abdomen. Insufflations of carbon dioxide, nitrous oxide and filtered air should be utilized from the automatic gas insufflating unit and then connected to the abdomen of the goat to create a pneumoperitonium for visualization of viscera including uterus and ovaries and to maintain a constant intra-abdominal pressure and air space. The pneumoperitonium should be created carefully to avoid over inflation which can cause discomfort of the goat (Chemineau and Cognié, 1991). From experience, it is seen that approximately 4 to 6 L of carbon dioxide gas is required for an average adult goat (30 kg b.wt.).

The critical part in laparoscopy is the site of the trocar-canula insertion. The trocar-canula insertion site should be approximately 2 cm cranial to the mammary gland and 4 cm away from the midline in each side of the abdomen in an average sized goat. Once properly inserted into the inflated abdominal cavity, the trocar should be removed from the canula sheath and replaced by the telescope. Visualization of the urinary bladder indicates that the operator is proceeding well.

Laparoscopy is started by the appointed surgeon and oocytes are retrieved by follicle flushing and aspiration method. After recovery of required oocytes, the LOPU is terminated by cautiously removing laparoscope and the pair of forceps from the respective canula sheath. The insufflating gas is evacuated from the abdominal cavity by manually pressing the abdominal wall through the canula hole. After the laparoscopic surgery, the incised skin is sutured and antiseptics are spread over the suture. LOPU procedure has been shown in Fig. 1a-c.

Fig. 1: Oocyte recovery from oestrus synchronized and superovulated goat ovaries through LOPU procedure. (a) Surgeons are conducting LOPU procedure, (b) follicle puncture and oocyte aspiration and (c) collection of follicular contents including oocyte in the collection tubes

DETERMINATION OF QUALITY AND GRADING OF RETRIEVED OOCYTES

In any *in vitro* oocyte manipulation protocols including ICSI require the assessment of an oocyte's potential to undergo fertilization and further development prior to subjecting it to the procedure of interest. This generally involves evaluation of the morphology of the cumulus investment, the ooplasm and the COC as a whole. Visual assessment of morphological features remains the most important criterion for selection of COCs before maturation to select developmentally competent goat oocytes during oocyte retrieval. Therefore, oocyte classification or grading and selection become an important and essential part of any IVP program. Like other domestic or ruminant species, goat oocytes are also classified before being cultured in the IVM medium. However, classification or grading of oocytes varies depending on the type of experiments and interest of the researchers. While some researchers grade oocytes according to the cumulus cell (CC) layers or compactness of the CCs; others grade them on the basis of morphology of the oocytes or ooplasm, homogeneity of the ooplasm, diameter of the ovarian follicles from where oocytes were originated or diameter of the ooplasm, or combination of CC layers or compactness of CCs plus morphology of the ooplasm. In cattle, good results were achieved from an oocyte having compact, complete and multilayered CCs, a finely granulated and homogenous ooplasm and an oocyte diameter measuring more than 120 μm (Gandolfi *et al*., 1997). In a recent study in our laboratory, it was found that oocyte quality improved with mean percentages of good quality oocytes (Grade A) from 29.7 to 37.6% when LOPU was performed at 36 and 60 h post FSH plus hCG treatments; delaying LOPU to 72 h post FSH plus hCG did not improve the mean percentage of good quality oocytes. Oocyte quality and developmental competence can also be related to follicle size and/or the composition of follicular fluid or FF (Madison *et al*., 1992; Pavlok *et al*., 1992; Crozet *et al*., 1995; Hazeleger *et al*., 1995; Arlotto *et al*., 1996). However, the determination of these parameters is labored intensive and hardly compatible with the routine production of a high number of embryos. In fact, the assessment of follicle diameter requires their dissection from the ovary prior to the isolation of COC and the analysis of FF is generally based on radioimmunoassay procedures (Gandolfi *et al*., 1997). Some important oocyte grading-related studied in goat have been summarized in Table 2. Although, after retrieval oocytes are graded, however, they are not always subjected to IVM, IVF/ICSI or IVC according to grades. In their study, Jiménez-Macedo *et al*. (2006) graded the prepubertal goat oocytes according to diameter after IVM and studied the developmental competence through ICSI and IVC. The relationship between follicle and oocyte size (diameter) predicts the further developmental competence of an oocyte. Oocytes retrieved from <2 mm sized follicles are smaller in size and less competent than those of 2 to 6 mm follicles (Crozet *et al*., 1995, 2000). In most of the *in vitro* studies, although cumulus-free oocytes (CFOs) are graded but discarded and only COCs with few layers of CCs are used for IVM. Till to date, there is no report of IVM, ICSI and IVC in goat according to oocyte grades. Different grades of LOPU-derived goat oocytes have been shown in Fig. 2a-g.

Although, uncommon in animals, with few exceptions, some researchers working with human IVF or ICSI classify oocytes with dysmorphic ooplasm or other

Table 2: Oocyte grading experiments in goat

Oocytes grading system	References
Goat oocytes were graded into 3 based on the size of the follicle from where oocytes were retrieved as 1) Small follicles: follicles of 2.0-3.0 mm in diameter, 2) Medium follicles: 3.1-5.0 mm and 3) Large follicles: >5.0 mm follicles.	Crozet *et al*. (1995)
LOPU-derived oocytes from adult or prepubertal goats were graded into 4 as 1) Grade A: ≥3 CC layers, 2) Grade B: 1-2 CC layers, 3) Grade C: denuded oocytes and 4) Grade D: oocytes with expanded CC layers.	Koeman *et al*. (2003)
LOPU-derived oocytes were graded into 3 as 1) Grade I: ≥3 CC layers with homogenous ooplasm, 2) Grade II: 1-2 CC layers with homogenous ooplasm and 3) Grade III: oocyte with heterogeneous ooplasm or denuded oocytes.	Wang *et al*. (2003)
Goat oocytes were graded into 4 groups after IVM based on the diameter of the ooplasm as 1) Group A: <110 μm, 2) Group B: 110-125 μm, 3) Group C: 125-135 μm and 4) Group D: >135 μm.	Jiménez-Macedo *et al*. (2006)
Abattoir-derived goat oocytes were graded into 4 according to the surrounding CCs and quality of oocytes as 1) Grade A: four or more CC layers, 2) Grade B: one to three layers of CCs, 3) Grade C: completely or partially denuded and 4) Grade D: all other oocytes including those with expanded CCs or degenerating oocytes.	Han *et al*. (2006)
Goat oocytes were graded into 2 groups as 1) normal: oocytes completely surrounded by CCs and 2) abnormal: oocytes partially surrounded by CCs or completely denuded.	Islam *et al*. (2007)
LOPU-derived goat oocytes were graded into 5 based on CC investment and oocyte morphology as 1) Grade A: COCs with >5 complete layers of CCs, finely granulated homogeneous ooplasm and normal morphological features, 2) Grade B: COCs with 3-5 complete layers of CCs, finely granulated homogeneous ooplasm and normal morphological features, 3) Grade C: COCs with 1-2 complete layers of CCs or COCs with 3-5 partially invested CC layers, finely granulated homogeneous ooplasm and normal morphological features, 4) Grade D: CFOs or oocyte with incomplete investment of CCs (1-2 layers), finely granulated homogeneous ooplasm and normal morphological features and 5) Grade E: degenerating oocyte or oocyte with abnormal, size, shape and heterogeneous ooplasm, or apoptotic oocytes in jelly-like CC investment or very small oocytes.	Rahman *et al*. (2007b)

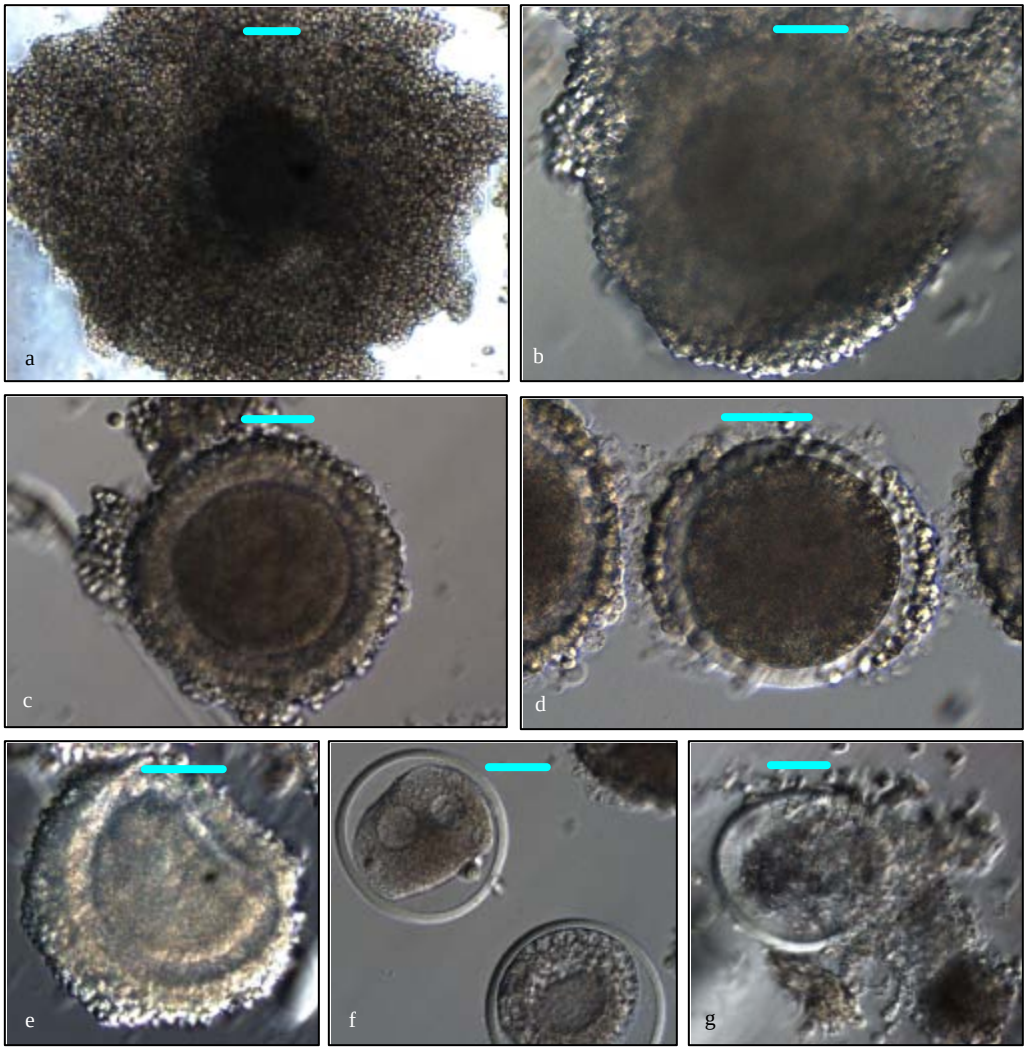

Fig. 2: Goat oocyte grading according to cumulus cell (CC) investment and morphology of the oocyte. (a) Grade A, (b) Grade B, (c) Grade C, (d) Grade D and (e-g) Grade E. Grade A to D are selected for IVM and Grade E are discarded. Scale bar represents 50 μm

forms of dysmorphism to study their developmental competence and capacity to produce offspring using ICSI techniques. A survey of available literature could not generate any data regarding classification or grading of dysmorphic oocytes. This may be due to the fact that as good quality goat oocytes are readily available from abattoir source, therefore, researchers are not much interested to study the developmental competence of dysmorphic goat oocytes after IVF or ICSI. It could be noted here that unlike other countries, abattoir-derived goat oocytes are not readily available in Malaysia due to a huge shortage of does for slaughter and the quality of abattoir-derived oocytes are also lower due to slaughter of low quality does. Therefore, there is a good scope to study the developmental competence of dysmorphic goat oocytes derived from abattoir source. Keeping this in mind, we produced the first ICSI derived goat preimplantion embryos from dysmorphic goat oocytes (Rahman *et al*., 2007c). Except a recent one in goat, a number of reports describing classification of dysmorphic oocytes have been published in human and a very few in bovine. Some of the important studies regarding classification of oocytes having ooplasmic dysmorphism mainly based on human and cattle oocytes are presented in Table 3. Different types of dysmoprhic goat oocytes have pictured in Fig. 3a-d.

Table 3: Grading of dysmorphic oocytes in human, cattle and goat

Species	Oocyte grading system	References
Human	Graded oocytes into 7 categories as 1) subtle change in organelle distribution, 2) small region(s) of intracellular cytolysis, 3) massive aggregation of smooth endoplasmic reticulum (SER) tubules, 4) accumulation of vesicles presumed to be of SER origin, 5) change in the structural organization of the cortical ooplasm and overlying plasma membrane, 6) sudden and rapid internalization of perivitelline fluid by means of endocytosis and 7) premature and partial exocytosis of cortical granules.	Van Blerkom (1990)
Human	Graded oocytes into 9 categories as 1) Type I: dark, granular or vesiculated, 2) Type II: central clustering of organelles or very dark appearing central area, 3) Type III: saccules of SER, 4) Type IV: regions of intracellular necrosis, 5) Type V: polarized or contracted ooplasm, 6) Type VI: ooplasmic vacuoles, 7) Type VII: variation in the size and integrity of PB-1, 8) Type VIII: nonspherical oocyte or anomalies of ZP and 9) Type IX: multiple anomalies.	Alikani *et al.* (1995)
Human	Graded oocytes into 3 categories as 1) normal ooplasm: having clear ooplasm with uniform texture and homogeneous fine granularity, 2) excessive ooplasmic granularity: dark oocytes with granularity either homogeneous affecting whole ooplasm or concentrated as a dark mass in the central portion of the oocytes with a clear peripheral ring and 3) ooplasmic inclusions: comprised of vacuoles presumed to be of endocytotic origin, accumulations of saccules of SER or refractile bodies containing lipid materials and dense granules.	Sehral *et al.* (1997)
Human	Graded oocytes into 9 categories as 1) normal morphology, 2) dark ZP, 3) dark ooplasm, 4) ganular ooplasm, 5) large perivitelline space, 6) refractile body, 7) irregular oocyte shape, 8) double anomaly and 9) triple anomaly.	Balaban *et al.* (1998)
Cattle	Graded oocytes into 2 categories as 1) Category 1: normal oocytes with homogeneous ooplasm and multilayered CC investment and 2) Category 2: oocytes having heterogeneous ooplasm with dark clusters and multilayered CC investments.	Nagano *et al.* (1999)
Human	Graded oocytes into 7 categories as 1) normal morphology, 2) varying degrees of organelle clusters or central granularity, 3) aggregation of SER, 4) ooplasmic inclusions, 5) ooplasmic vacuoles, 6) organelle clusters with fragmented PB and increased perivitelline debris and space and 7) combination of ooplasmic and extra-ooplasmic dysmorphism.	Meriano *et al.* (2001)
Cattle	Graded oocytes into 7 categories as 1) brown and homogenous ooplasm, 2) brown and homogenous ooplasm with dark zone around the periphery, 3) brown and heterogeneous ooplasm with dark clusters, 4) pale and homogeneous ooplasm, 5) pale and heterogeneous ooplasm with dark clusters, 6) black and homogeneous ooplasm and 7) variable ooplasmic features and a diameter of <115 μm.	Nagano *et al.* (2006), Nagano *et al.* (2007)
Goat	Graded oocytes into 4 categories according to ooplasmic morphology as 1) Normal: COCs with 1-5 or more than 5 complete or incomplete layers of CCs and CFOs with finely granulated homogeneous ooplasm, golden, golden-yellow or brownish in color and evenly distributed dark fat globules, 2) Pale: COCs with 1-5 or more than 5 complete or incomplete layers of CCs and CFOs having a paler or semi-translucent ooplasm, central portion of some of those have clear appearance due to lack of ooplasmic organelles, 3) Big fat globules: COCs with 1-5 or more than 5 complete or incomplete layers of CCs and CFOs having big fat globules of different sizes in any part of the ooplasm and 4) Darker: COCs with 1-5 or more than 5 complete or incomplete layers of CCs and CFOs having a darker or black zone or appearance at one side or end of the ooplasm.	Rahman *et al.* (2007c)

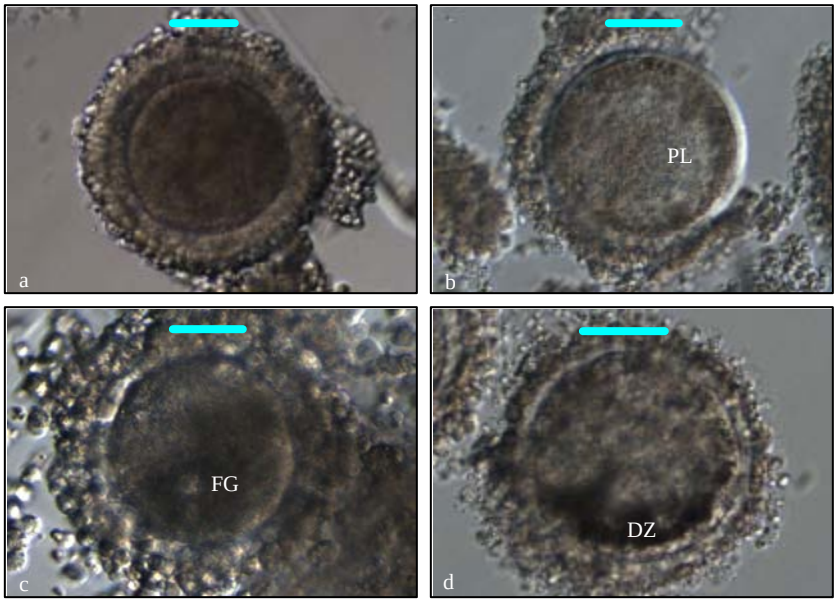

Fig. 3: Goat oocyte grading according to morphology of the ooplasm. (a) Normal oocyte, (b) pale oocyte, (c) oocyte with big fat globules and (d) dark oocyte. PL = Pale ooplasm, FG = Fat globule, DZ = Darker zone. Scale bar represents 50 μm

CONCLUSIONS

Like cattle, goat IVP including IVF and ICSI will become first emerging ART in the livestock industry in near future. The present state of goat IVP shows a great deal of promise. Through improved nutrition and veterinary assistance, dramatic changes have been brought in the management of goat breeding during the last two decades. However, despite these improvements, AI is the only ART widely applied in selection programs till now. The other important techniques related to goat IVP for example superovulation and estrus synchronization, which are essential requirements for LOPU, still have a large margin for improvement. As both quantity and quality of recovered oocytes are crucial for IVP outcome in goats, therefore, more research should be focused on LOPU technique for OR from live goats which will in turn improve the efficiency of these *in vitro* technologies. Although abattoir-derived ovaries are a cheapest source of oocyte, however, in Malaysian situation LOPU is the most reliable and efficient method of OR. Because LOPU is performed in the live donor, therefore, OR rate and quality of oocytes are much higher than abattoir ovaries as same goat can be used for several times.

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