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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 8 (3): 437-448, 2013
ISSN 1683-9919 / DOI: 10.3923/ajava.2013.437.448

Comparative Study on Goat Oocyte Recovery Methods and Factors Affecting the Quantity and Quality of Oocytes

[1,3]A.A. Anna, [2,3]M.N. Hisham, [3]W.E. Wan Khadijah and [3]R.B. Abdullah

[1]Laboratory of Animal Production, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[3]Animal Biotechnology-Embryo Laboratory, Institute of Biological Science, University of Malaya, Kuala Lumpur 50603, Malaysia

Corresponding Author: A.A. Anna, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

A strategize step in oocyte recovery is important and beneficial in producing successful production of high-quality Cumulus Oocyte-Complexes (COC) for *in vitro* maturation, fertilization and culture (IVMFC), nuclear transfer and other emerging technologies. Experiments were conducted to compare Oocyte Recovery (OR) between Laparoscopic Ovum Pick-Up (LOPU) and ovarian slicing and to evaluate the effects of OR cycle, hormone stimulation, oestrus synchronization, breeds, liveweight and age of goats on the quantity and quality of oocytes obtained. In Experiment 1, oocytes were recovered from abattoir-derived ovaries by the slicing method. The ovaries were transported to the laboratory for immediate harvesting. The slicing method yielded significantly ($p<0.05$) larger number of oocytes per ovary than LOPU (22.53±2.78 vs. 6.10±0.46). However, the proportion of Grade A oocytes was highest from the LOPU technique (30.51±4.14) compared to the slicing method (19.07±3.57). LOPU resulted in good quality Cumulus Oocytes Complexes (COC) with more than 5 layers of cumulus cells. Experiment 2 consisted of three OR cycles (OR-1, OR-2 or OR-3); four durations of oestrus synchronization using CIDR (Day-10, -14 , -17 or -21); three goat breeds (Boer Crossbred, Mixed breed or Katjang); 4 age groups (Young, Mature, Old or Very Old) and 4 levels of body weight (20, 21-29, 30-37 or 38 kg). Goats were synchronized using Control Internal Drug Release (CIDR) combined with 125 μg Estrumate and hyperstimulated with 70 mg FSH and 400 IU hCG after the removal of CIDR 24 h later. There was no significant effect of OR cycle, CIDR removal goat breed, age or body weight on the total number of oocytes recovered per ovary. Katjang goats yielded a higher proportion of Grade A oocytes compared to Boer Crossbreds and mixed breeds which had a higher proportion of Grade C oocytes ($p<0.05$). Age and weight of animals had no significant effect on the quality of oocytes recovered. Grade A and B oocytes were obtained in OR-1, whilst the repetition of LOPU resulted in an increment of Grade C oocytes in OR-2 and OR-3, respectively. Day-10 and -21 gave the highest cumulative percentage of 58 and 64% for Grade A and B oocytes, respectively. Under the conditions of the experiment, LOPU yielded better quality oocytes from the Katjang goats using less or no OR repetition, with oestrus synchronization at 10 or 21-Day.

Key words: Cumulus oocyte-complexes, goat, laparoscopic ovum pick-up, oestrus synchronization, ovarian slicing

INTRODUCTION

Various techniques have been used to obtain oocytes for *in vitro* embryo production (IVP) in goats. These techniques have also been refined to improve recovery rates and oocyte quality, minimize stress and discomfort and complications that might affect the health or future fertility the donors (Abdullah *et al*., 2008).

Goat oocytes are mainly obtained from ovariectomized (Younis *et al*., 1991; Keskintepe *et al*., 1994) or slaughtered does (Pawshe *et al*., 1994). However, the non-invasive Laparoscopic Ovum Pick-Up (LOPU) method has provided an efficient technique for collecting oocytes from small ruminants such as sheep and goats (Abdullah *et al*., 2008; Koeman *et al*., 2003).

Immature oocytes are usually obtained by slicing the surface of ovaries obtained from abattoirs or by the LOPU technique. Both techniques have their advantages and disadvantages. The quantity of oocytes obtained from each ovary are recorded and evaluated for quality by recording the grades of the oocytes based on thickness of cumulus investments of each oocyte (Koeman *et al*., 2003). The number of oocytes obtained from goat ovaries from abattoirs and ovariectomy are generally much higher than via aspiration by LOPU of live donors. However, the number of oocytes recovered showed considerable variation among researchers (Tibary *et al*., 2005; Baldassarre *et al*., 2003).

Oocytes collected through LOPU are subjected to oestrus synchronization and ovarian stimulation. LOPU is becoming a convenient oocytes recovery method for *in vitro* production of embryos in goats, with appropriate timing for maturation of oocytes and allows for repeatable aspiration of oocytes from donor animals (Baldassarre *et al*., 2003). The quality and quantity of oocytes plays an important role in establishing a more practical IVP for the production of goat embryos and in developing efficiency of *in vitro* maturation, fertilization and culture (IVMFC) systems for further improvement in goats. In addition to the influence of the oocyte recovery method, other factors may also affect the quality and quantity of Cumulus-Oocyte-Complexes (COC) harvested through LOPU. Therefore, the objectives of the present study were to: (a) compare the number and quality of oocytes obtained by slicing ovaries and LOPU method and (b) determine the effect of OR cycle, goat breeds, oestrus synchronization, age and live weight of goats on the quantity and quality of oocytes obtained through LOPU.

MATERIALS AND METHODS

Experimental animals: The ooctyes and ovarian samples for the study were obtained by LOPU from live animals and abattoir samples from slaughtered animals, respectively. The study was conducted for a duration of 3 years between September 2003 until September 2006.

Goats for LOPU: The study was conducted at the Mini Farm of the Institute of Biological Science, University of Malaya, Malaysia. The goats were fed daily with commercial pellet, Napier grass, clean water and mineral lick and managed indoors under an intensive system. A total of 82 goats were used in this study and consisted of three different breeds (Boer crossbreeds, Katjang and Mixed breed), aged 8 to 96 months old and weighing 8.5-45.5 kg each.

Abattoir source: Ovaries were collected from local abattoirs in Shah Alam, Senawang and Kuala Pilah and at the University of Malaya Mini Farm. Ovaries were collected within 30 min of slaughter, kept in sterile saline (0.9% NaCl) supplemented with penicillin G (100000 IU) and streptomycin sulfate (100 mg L^{-1}) and transported to the laboratory at 35-37°C within 2-3 h of collection.

Oestrus synchronization and superovulation: Oestrus was synchronized by using an intravaginal controlled internal drug release device (CIDR, 0.3 g progesterone, Pharmacia and Upjohn Limited, Auckland, New Zealand) for 10, 14, 17 or 21 days with cloprostenol (Estrumate, 125 μg; Schering-Plough, NSW, Australia) 24 h prior to removal of CIDR. After the removal of CIDR, the does were superovulated by injecting with Ovagen (FSH, 70 mg; ICPbio Limited, Auckland, New Zealand) and Profasi (hCG, 400 IU, Laboratories Serono, Switzerland). Two days after the removal of CIDR, the does were prepared for LOPU. Oestrus was detected by visual observation of tail twitching and the use of a teaser buck.

Anesthetization and preparation of animals: The does were fasted for 24 h before LOPU. Anesthetic was induced through intra-muscular treatment with xylazine (Troy Laboratories Pty Ltd, NSW, Australia) at 0.22 mg kg^{-1} followed by ketamine hydrochloride (Ketmav 100, Mavlab, Australia) at 11 mg kg^{-1} b.wt. The sedated animal was then placed and maintained in dorsal recumbency on a cradle at a 45° angle by tying its legs on to each end of the surgery table. Hibiscrub was applied to clean up the animal's abdominal area. The animals were then shaved using a razor and iodine was applied.

Oocyte retrieval method

Follicle flushing and oocyte aspiration medium: Dulbecco phosphate-buffered saline (DPBS, Dulbecco A., BR0014G, Oxoid Limited, Hampshire, UK) supplemented with gentamicin sulfate (50 μg mL^{-1}, G1271, Sigma), heparin (100 μg mL^{-1}, H0777, Sigma) and 10% steer serum was used as follicle flushing and oocyte aspiration medium.

LOPU: Oocyte collections by LOPU were conducted at NaTuRe Laboratory, Institute of Postgraduate Studies, University of Malaya, Malaysia. The LOPU procedure involved administration of anesthetics, surgery and ovum pick-up. The Storz laparoscope (Karl Storz Endoscopes GmbH and Co., Tuttlingen, Germany) was attached to a washed and sterilized 7 mm light source cable before being attached to the light source machine. Carbon dioxide gas was supplied by connecting the CO_2 tube, which has been exposed to UV light overnight and the automatic CO_2 gas insufflator unit, while the other end of the CO_2 tube was connected to the veress needle. A small incision (3-5 mm) was made on the lower abdominal wall of the goat to insert the veress needle. The endoscope was checked for its white balance using sterile gauze. Two more small incisions were made using a cannula and trocar for the endoscope to go through the abdominal area to detect the ovaries and view it on a TV monitor and for the pediatric grasper to grasp the ovaries. One end of the ovum pick-up needle was attached into the air filter on the IVF Ultra Quiet Aspiration System (V-MAR 5100, Cook Australia, Eight Mile Plains, Queensland, Australia).

Aspiration media (PBS-DulbeccoA, Ovoid Limited, England) in 50 mL syringe was removed from the incubator as soon as the surgery started. The other end of the pick-up needle was attached to the 50 mL syringe containing the flushing medium. The syringe was connected to the Flushing System (V-MAR 4000, Cook Australia, Eight Mile Plains, Queensland, Australia) and the laparoscopic pump and flush tubes were then checked for viability. Video camera was set-up to record the whole procedure. The surgery was finally conducted and oocytes were retrieved by follicle flushing and aspiration method. Oocytes were flushed into sterile plastic tubes and counted with the aid of a stereo microscope (Olympus, Japan). The total number of oocytes flushed from each ovary was recorded.

After the surgery, the incised skin was sutured using a size 2 surgical silk suture (B. Braun, Germany) and the animal was then given antibiotic injection containing

200 mg Oxytetracycline mL^{-1} (Terrmycin/LA, Pfizer, New York) at a dose of 1 mL per 10 kg b.wt. every 4 days for 2 weeks to avoid post surgical infection. Later, the animal was returned back to the farm.

Slicing technique: Ovaries recovered from abattoirs were immediately transported to the laboratory and washed with the same aspiration medium used in LOPU procedure. Slicing of ovaries was conducted under sterile conditions in a laminar flow chamber where the surface of ovaries were thinly sliced with a sterile razor blade to obtain the oocytes.

Classification of oocytes: Recovered Cumulus Oocyte-Complexes (COCs) were allocated to different grades depending on the status of the cumulus cells and homogeneity of the cytoplasm (Blondin *et al.*, 1997). Grade A oocytes are the best oocyte in terms of quality oocytes with having more than 5 compact layers of cumulus cells and a cytoplasm that was either homogeneous or showed a dark zone around the periphery, followed by Grade B showing 3 to 5 layers of cumulus cells, Grade C with 1-2 layers of cumulus cells and Grade D consisting of naked oocytes or COCs with incomplete cumulus.

Statistic analysis: Statistical analysis was performed on the number and quality of oocytes recovered. Effects of various factors on the parameters measured were analysed using SPSS (Statistical Packages for Social Sciences) one-way ANOVA and significant differences between means were tested with Duncan's Multiple range test. The level of significance was observed at the 5% level ($p<0.05$). All data are presented as Mean±SE.

RESULTS

Effect of recovery technique: Oocytes obtained from the abattoir ovaries using the slicing method showed a significantly higher number of oocytes per goat compared to the LOPU method from live donors ($p<0.05$) (Table 1). However, the LOPU method resulted in a higher percentage of Grade A oocytes compared to the slicing method which had a higher percentage of Grade D oocytes ($p<0.05$) (Table 2).

Table 1: Number of oocytes obtained from slicing and LOPU methods

		Ovary location		
Oocyte collection method	No. of goats	Oocytes per left ovary	Oocytes per right ovary	Oocytes per goat
Slicing (abattoir samples)	26	$11.30\pm1.45^{a,x}$	$11.23\pm1.44^{a,x}$	22.53 ± 2.78^{x}
LOPU	30	$2.86\pm0.40^{b,y}$	$03.23\pm0.36^{b,y}$	06.10 ± 0.46^{y}

^{a,b}Means within rows with different superscripts are significantly different ($p<0.05$, DMRT), ^{x,y}Means within columns with different superscripts are significantly different ($p<0.05$, DMRT)

Table 2: Grades of oocytes recovered by LOPU and slicing methods

		Oocyte quality (%)			
Oocyte collection method	No. of oocytes	A	B	C	D
Slicing (abattoir samples)	586	$19.07\pm3.57^{b,x}$ (111)	$22.44\pm4.73^{ab,x}$ (132)	$22.13\pm3.24^{ab,x}$ (133)	$35.67\pm5.45^{a,x}$ (210)
LOPU (Control)[n]	183	$30.51\pm4.14^{b,y}$ (57)	$21.52\pm3.37^{ab,x}$ (40)	$31.99\pm4.79^{b,x}$ (57)	$15.97\pm3.63^{a,y}$ (29)

^{a,b}Means within rows with different superscripts are significantly different ($p<0.05$, DMRT), ^{x,y}Means within columns with different superscripts are significantly different ($p<0.05$, DMRT), Values in parenthesis refer to the actual number of oocytes in each category

Effect of donor characteristics

Goat breeds: There were no significant differences (p>0.05) between breeds in the number of oocytes recovered per ovary (Table 3). However, in terms of oocyte quality, the Katjang goats showed a relatively higher percentage of Grade A oocytes compared to Boer crossbreds and Mixed breed goats which had a higher percentage of Grade C oocytes (Table 4).

Age of goats: The numbers of oocytes recovered per ovary from young, mature, old and very old goats were 3.20±0.37, 2.77±0.42, 3.12±0.98 and 3.30±0.25, respectively (Table 5). There were no significant differences (p>0.05) in oocyte recovery rate between goats of different ages. All age groups showed an average of 3.0 oocytes per ovary. The quality of oocytes produced among goats of different ages is shown in Table 6. There were no significant differences (p>0.05) in the quality group of oocytes analysed between the young, mature, old and very old goats. However, within the goats of more than 60 months (very old) of age the recovery rate of Grade C oocytes was significantly higher than Grade D oocytes (p<0.05).

Table 3: Effect of goat breeds on oocyte recovery

Breeds	No. of goats	Total No. of ovaries	Total No. of oocytes	Oocytes per ovary
Boer cross breeds	9	18	59	3.27±0.44^{x}
Katjang	9	18	54	3.00±0.44^{x}
Mixed	10	20	50	2.60±0.27^{x}

^{x}Means are not significantly different (p>0.05, DMRT)

Table 4: Effect of goat breeds on quality of oocytes harvested (%)

			Oocyte quality (%)			
Breeds	No. of goats	Total oocytes	A	B	C	D
Boer crossbred	9	59	26.18±7.01^{a,x} (18)	16.90±3.86^{a,x} (10)	49.78±10.37^{b,y} (26)	07.12±3.08^{a,x} (5)
Katjang	9	54	38.11±7.52^{b,x} (20)	14.85±5.16^{a,x} (9)	23.16±6.58^{ab,x} (13)	23.86±8.66^{ab,x} (12)
Mixed	10	50	26.70±8.63^{a,x} (12)	28.14±6.80^{a,x} (15)	27.40±8.66^{a,xy} (13)	17.74±5.86^{a,x} (10)

^{a,b}Means within rows with different superscripts are significantly different (p<0.05, DMRT), ^{x,y}Means within columns with different superscripts are significantly different (p<0.05, DMRT), Values in parenthesis indicate the number of oocytes recovered

Table 5: Effect of goat age on oocyte recovery

Goat age	No. of goats	Total of ovaries	Total of oocytes	Oocytes per ovary
Young (<18 months)	10	20	65	3.20±0.37^{x}
Mature (19-36 months)	11	22	61	2.77±0.42^{x}
Old (36-60 months)	4	8	25	3.12±0.98^{x}
Very old (>60 months)	5	10	33	3.30±0.25^{x}

^{x}Mean are not significantly different (p>0.05, DMRT)

Table 6: Effect of goat age on quality of oocytes produced

			Oocyte quality (%)			
Goat age	No. of goats	Total oocytes	A	B	C	D
Young (<18 months)	10	65	36.28±8.66^{a,x} (22)	26.58±6.81^{a,x} (17)	19.25±5.04^{a,x} (14)	17.88±5.53^{a,x} (12)
Mature (19 months-36 months)	11	61	28.69±6.66^{a,x} (19)	16.44±6.25^{a,x} (10)	38.94±9.62^{a,x} (23)	16.01±6.79^{a,x} (9)
Old (36-60 months)	4	25	26.25±9.43^{a,x} (21)	19.50±7.02^{a,x} (10)	33.33±12.47^{a,x} (16)	20.91±15.85^{a,x} (9)
Very old (>60 months)	5	33	26.40±9.17^{ab,x} (9)	24.20±3.33^{ab,x} (8)	41.10±12.43^{b,x} (12)	8.10±3.31^{a,x} (4)

^{a,b}Means within rows with different superscripts are significantly different (p<0.05, DMRT), ^{x}Mean within columns with same superscripts are not significantly different (p>0.05, DMRT), Values within parenthesis indicate the number of oocytes recovered

Liveweight of goats: The number of oocytes per ovary obtained from goats weighing less than 20, 21-29, 30-37 and more than 38 kg was 3.00±0.41, 3.05±0.34, 3.00±0.70 and 3.33±0.44, respectively (Table 7). In Table 8, significant differences were detected ($p<0.05$) between groups of oocytes for goats weighing more than 38 kg, where the mean percentage of Grade C oocytes obtained was highest (46.78%) ($p<0.05$).

Oocyte recovery (or) cycle: There were no significant differences in number of oocytes per ovary produced between OR cycles (Table 9), except for Grade C oocytes which showed significant differences ($p<0.05$) between OR cycles (Table 10). Significantly higher percentage of Grade C oocytes was obtained in OR-2.

Table 7: Effect of goat weight on number of oocytes per ovary

Goat weight	No. of goats	Total of ovaries	Total of oocytes	Oocytes per ovary
<20 kg	13	26	81	3.00±0.41^{x}
21-29 kg	9	18	56	3.05±0.34^{x}
30-37 kg	5	10	30	3.00±0.70^{x}
>38 kg	3	6	21	3.33±0.44^{x}

^{x}Means are not significantly different ($p>0.05$, DMRT)

Table 8: Effect of liveweight of goats on quality of oocytes harvested

			Quality of oocytes (%)			
Goat liveweight	No. of goats	Total oocytes	A	B	C	D
<20 kg	13	81	28.10±6.50^{a,x} (23)	24.94±5.22^{a,x} (22)	28.46±5.64^{a,x} (22)	18.56±4.62^{a,x} (14)
21-29 kg	9	56	35.95±7.99^{a,x} (21)	17.46±7.98^{a,x} (10)	32.10±11.61^{a,x} (17)	14.47±7.85^{a,x} (8)
30-37 kg	5	30	29.51±12.18^{a,x} (11)	25.03±2.75^{a,x} (7)	29.25±13.57^{a,x} (9)	16.20±12.98^{a,x} (3)
>38 kg	3	21	26.42±7.45^{a,x} (6)	13.09±7.24^{a,x} (3)	46.78±6.78^{b,x} (9)	13.69±8.26^{a,x} (3)

^{a,b}Means within rows with different superscripts are significantly different ($p<0.05$, DMRT), ^{x}Mean within columns with same superscripts are not significantly different ($p>0.05$, DMRT), Values in parenthesis indicate the number of oocytes produced

Table 9: Effect of OR cycles on number of oocytes produced per ovary

OR cycle	No. of goats	Total of ovaries	Total of oocytes	Oocytes per ovary
OR-1	13	26	79	3.03±2.80^{x}
OR-2	12	24	75	3.12±0.47^{x}
OR-3	5	11	29	2.90±0.48^{x}

^{x}Mean are not significantly different ($p>0.05$, DMRT)

Table 10: Effect of OR cycles on quality of oocytes produced

			Oocyte quality (%)			
OR cycle	No. of goat	No. of oocytes	A	B	C	D
OR-1	13	79	37.77±6.16^{b,x} (23)	30.51±6.2^{ab,x} (26)	16.93±3.97^{a,x} (12)	14.78±2.22^{a,x} (11)
OR-2	12	75	23.45±7.02^{a,x} (17)	13.16±3.5^{a,x} (12)	46.00±8.70^{b,y} (31)	17.28±3.94^{a,x} (15)
OR-3	6	29	28.60±7.67^{a,x} (9)	18.20±5.59^{a,x} (5)	37.50±10.18^{a,xy} (12)	15.90±13.00^{a,x} (3)

^{a,b}Means within rows with different superscripts are significantly different ($p<0.05$, DMRT), ^{x,y}Means within columns with different superscripts are significantly different ($p<0.05$, DMRT), Values in parenthesis indicate number of oocytes produced

Table 11: Effect of duration of CIDR implantation on number of oocytes per ovary

Days of CIDR implantation	No. of goats	Total no. of ovaries	Total no. of oocytes	Oocytes per ovary
Day-10	7	14	38	2.71±0.39[x]
Day-14	4	8	27	3.37±0.89[x]
Day-17	5	10	26	2.60±0.55[x]
Day-21	6	12	36	3.00±0.34[x]

[x]Means are not significantly different (p>0.05, DMRT)

Table 12: Effect of duration of CIDR implantation on quality of oocytes produced

			Oocytes quality (%)			
Days of CIDR implantation	No. of goats	Total oocytes	A	B	C	D
Day-10	7	38	33.85±8.56[a,x] (13)	25.71±3.34[a,xy] (9)	23.28±8.54[a,xy] (17)	17.14±9.06[a,x] (11)
Day-14	4	27	22.91±11.47[a,x] (5)	34.16±10.89[a,y] (9)	34.58±16.71[a,xy] (9)	08.33±8.33[a,x] (4)
Day-17	5	26	30.22±10.85[ab,x] (8)	04.00±4.00[a,x] (1)	55.55±17.05[b,y] (14)	10.22±7.74[a,x] (3)
Day-21	6	36	37.41±12.83[a,x] (14)	27.91±10.37[a,y] (10)	17.08±6.46[a,x] (7)	17.58±6.80[a,x] (6)

[a,b]Means within rows with different superscripts are significantly different (p<0.05, DMRT), [x,y]Means within columns with different superscripts are significantly different (p<0.05, DMRT), Values in parenthesis indicate number of oocytes produced

Oestrus synchronization: The number of oocytes per ovary obtained with 10, 14, 17 and 21 day CIDR implantation were 2.71±0.39, 3.37±0.89, 2.60±0.55 and 3.00±0.34, respectively (Table 11). There were no significant (p>0.05) differences in the number of oocytes per ovary obtained between duration of CIDR implantation. However, the quality of oocytes on Day-17 CIDR treatment showed significant differences in the percentage of Grade B and C oocytes (Table 12). There was a lower percentage of Grade B oocyte and a higher percentage of Grade C oocytes.

DISCUSSION

The harvesting of oocytes using the LOPU and slicing method from abattoir sourced ovaries are suitable methods for obtaining oocytes. However, there were significant differences in the total number of oocytes per goat and per ovary (p<0.05). Abattoir-derived oocytes obtained by using slicing method showed significantly higher average total number of oocytes per goat as compared to LOPU for OR in goats (22.53 vs. 6.10). Slicing method had a higher capability in terms of number of oocyte and the results were in agreement with Keskintepe *et al.* (1998) who obtained higher number of oocytes Toggenburg, Nubian and Saanen goat ovaries. Keskintepe *et al.* (1998) also reported that mincing of ovaries gave more oocytes compared to follicular aspiration but the total of 12 oocytes per goat reported was a combination of mincing and aspiration techniques and no data on separate technique was shown in his report. Meanwhile Wani *et al.* (2000) obtained 6 to 11 oocytes per ovary in ewes and slicing technique resulted in the production of more debris (Wani *et al.*, 1999). However, the rate of oocyte recovery in the present study succeeded in resulting higher number with an average of 22.53 oocytes per goat. Collection of oocytes is progressively being replaced by the laparoscopic technique in small ruminants. The technique is performed under general anesthesia or heavy sedation after standard surgical preparation and can be completed in <20 min (Baldassarre *et al.*, 1996; Graff *et al.*, 1999). The shorter time consumed and repeatable cycle on the same animal showed that LOPU is relatively a simple and efficient technique compared to the slicing method (Koeman *et al.*, 2003; Baldassarre *et al.*, 2002). Baldassarre and Karatzas (2004) observed an average yield of 13.5 oocytes per goat using the "one shot" (unpublished) regime, which was about twice the number obtained in the present study

(6.10 oocytes per goat). These results suggested that higher number of oocytes can be obtained using LOPU method with suitable hormone stimulation and oestrus synchronization, depending on breeds, weight and age of goats.

Although, abattoir-derived oocytes were higher in quantity, the quality of oocytes obtained with the LOPU technique was much better. The LOPU technique yielded a higher percentage of Grade A oocytes compared to abattoir-derived-oocytes collected using the slicing method. The results of the present study indicated that LOPU was an efficient technique for the recovery of high quality oocytes compared to abattoir-derived-oocytes obtained through slicing method. Koeman *et al.* (2003) obtained oocytes from prepubertal and adult goats comprising of around 82-87%, Grade A and Grade B oocytes through the LOPU method, while in the present study 84% of the oocytes were of similar grades.

In this study, there is no significant difference on the quantity of oocytes between breeds of goats through LOPU. It can be suggested that all breeds (Boer crossbreeds, Katjang and Mixed breed) in this study have been adaptable to the local environment of high temperature and humidity in Malaysia which contribute to no differences on the quantity of oocytes between the studied breeds. There were significant differences in the quality of the oocytes between the breeds by which the Boer Crossbreds and Mixed breeds showed a higher percentage of Grade C oocytes compared to Katjang. However, for the quality of oocytes between breeds, Katjang goats showed a numerically greater percentage of Grade A oocytes compared to the other breeds, although, this was not significant. Although, all breeds have been considered well adaptable and reared widely in Malaysia, there might be a possibility that the qualities of the reproductive system/organ are different between breeds which contributed to no differences of good quality of oocytes except for Grade C between breeds. However, in depth investigation is required in future study to conclude this suggestion. A study conducted by Fair *et al.* (2006) on ewes found that the differences in embryo development of immature oocytes following IVMFC (*in vitro* maturation, fertilization and culture) between Suffolk (multiparous) and purebred Belclare ewes cannot be explained by the differences in oocyte competence. It was also speculated that differences in uterine development maybe the principal basis for differences between breeds. In the same study, the differences in the oviduct or uterine environment post Day 1 of *in vitro* culture (IVC) resulted with lower development rate of fertilized oocytes in Suffolk ewes (Fair *et al.*, 2006). A delayed elevation of progesterone in Suffolk compared to Belclare ewes following ovulation may have deleterious effect on the embryo development on Suffolk ewes (Fair *et al.*, 2007). An earlier study by Fair *et al.* (2005) also found that a significantly lower proportion of *in vivo* fertilized oocyte from Suffolk than Belclare developed to morula/blastocyst stage. Thus, the differences in the oocyte quality between breeds cannot be explained by the different breeds itself, thus more studies are needed to examine other factors such as the uterine environment, hormonal level of the animal and also the development of the animal's reproductive organ.

In this study, we have demonstrated that the quantity and quality of oocytes obtained was not dependent on age of goats, although, there was a trend of better quality of oocytes in the younger goats compared to the older goats. The good quality oocytes mainly classified as Grade A and B is required to produce a good *in vitro* production of embryos in goats. In our study, 62% of good quality oocytes consists of Grade A and B were obtained from the young goats, compared to very old (50%), old (45%) and mature (44%), respectively. Previous study reported a significant follicular response and higher oocyte yields recovered by LOPU from stimulated prepubertal goats less than 3 months of age (Baldassarre *et al.*, 2002). This study have showed that recovery of oocytes by

LOPU from prepubertal goats resulted in a significantly higher yield from 2-3 months old goats than from 3-5 months old goats. Meanwhile, Koeman *et al.* (2003) showed that more follicles were stimulated and more oocytes recovered from prepubertal than adult goats. The prepubertal animals were approximately 90 days of age at the time of the first collection and close to 200 days old at the final collection session. Above studies strongly indicated that LOPU was an efficient technique for the recovery of high quality oocytes from young or prepubertal goats. Our finding suggested that good quality oocytes and high number of oocytes can be obtained through LOPU from goats of all ages. However, this study also demonstrated that although the same quantity of oocytes can be recovered from goats of all age groups, the quality of oocytes maybe compromised slightly.

In the present study, no significant differences in oocyte quality and quantity were observed between four different categories of live weights (Table 7 and 8). This was in disagreement with a study done Abdullah *et al.* (2005) found that there was an interaction between age and liveweight of LOPU goats, where young goats (= 1 year 6 month) with body weight = 20 kg had optimum number of oocytes recovered by LOPU. In this present study, ages were separated into four groups compared to other studies with significant results between prepubertal and adult goats. We concluded that more numbers of animal and smaller numbers of groups can be used in future studies. A study conducted by Koeman *et al.* (2003) on prepubertal and adult goats showed that the recovery of oocytes from prepubertal goats declines substantially as the prepubertal animal increase age. At the University of Malaya goat farm, female goats come to puberty at approximately 8 month of age with a body weight of more than 20 kg [unpublished].

Our study found that the repeated cycle of LOPU procedure up to three times, retained the quantity of oocytes retrieved in goats. This was in an agreement with Pierson *et al.* (2004) where repeated LOPU up to five times and repeated gonadotrophin stimulation in goats showed no differences on the quantity of the oocytes and the follicular responses. However, this finding was in disagreement with a study conducted in the same laboratory by Hisham (2006) which observed that the number of oocytes was decreased with increased number of repeated LOPU in goats. It has been suggested that the production of antibodies against the repeated superovulation treatments and physical damage in ovaries during LOPU cause the ovarian dysfunction, thus decrease the quantity of oocytes obtained via LOPU (Abdullah *et al.*, 2005). In the same study, plasma oestradiol concentrations in goats were found to be greater in OR-1 which suggests that increasing levels of oestradiol indicates more matured and dominant follicles were developed in ovaries. Thus, this could explained the first cycle of oocyte recovery (OR-1) resulted in an utmost number of oocytes recovered via LOPU with majority of the oocytes recovered were classified as Grades A and B (Hisham, 2006). Meanwhile, on the oocyte quality, we also found that Grade A and B oocytes were higher in OR-1, whilst Grade C was higher in OR-2 compared to OR-1 and OR-3 cycles. Good quality oocytes were obtained in OR-1 as it was the first OR cycle for the goat and the ovary was free from any puncturing resulting in better oocyte quality. This was in agreement with Kuhholzer *et al.* (1997), who manage to obtained good quality oocytes which decreased steadily from 60% in OR-1-40% in OR-5. Our finding suggested that LOPU may be repeated up to 3 times in goat with an acceptable quantity and quality of oocytes. LOPU was an effective and minimally invasive technique, which offered the possibility of repeated ovum pick-up and less traumatic than surgical method used in oocyte recovery (Baldassarre and Karatzas, 2004). Repeated production of embryos from a single donor can be done and does not cause permanent damage to the donor ewe's reproductive health (Koeman *et al.*, 2003).

Synchronization in breeding is one of the advantages of livestock farmers. Our finding demonstrated that the duration of CIDR implantation has a significant effect on oocyte quality. Day-17 CIDR implantation resulted in a lower percentage of Grade B oocytes and a higher percentage of Grade C oocytes compared to Day-10 and -21. There is no significant differences in the percentage of Grade A oocytes observed between all the duration of CIDR implantation. Baldassarre and Karatzas (2004) mentioned that oestrus synchronizations were mostly carried out using intravaginal sponges containing medroxyprogesterone acetate (60 mg) (Veramix®, Upjohn, Canada) inserted 10 days prior to LOPU, representing an average recovery rate of around 80%. However, in the our study, CIDR implantation for 10 or 21 days gave a higher number of Grades A and B oocytes with a total percentage of 58 and 64%, respectively. Oocyte recovery via LOPU for different selected days of oestrus cycle for two times oestrus synchronization using CIDR in goats was studied by Hisham (2006). The first CIDR implantation was used to synchronize the oestrus cycle of the female doe for later second CIDR implantation. The quantity and quality of oocytes obtained were numerically greater on Day 3 and 5 of oestrus using 10-day CIDR implantation.

CONCLUSIONS

The present study illustrated that laparoscopic ovum pick-up was a reliable method to obtain high quality immature oocytes, while slicing method from abattoir samples was a very useful tool for increasing the number of oocytes. The abattoir ovaries were cheap source of oocyte for research in need of high number of oocyte material. There was no difference regarding the number of oocytes collected through LOPU on the OR cycle, CIDR duration, goat breeds, age and liveweight. A higher yield of quality oocytes from LOPU was obtained from Katjang goats, with less repetition and oestrus synchronization at 10 and 21-Days. The results from this research indicated the factors affecting goat oocyte recovery in Malaysia. The successful production of good quality oocytes from goats may facilitate the application of these techniques in *in vitro* production (IVP) and emerging biotechnologies such as nuclear transfer and transgenesis.

ACKNOWLEDGMENTS

The authors would like to thank the ABEL members for their kind assistance and cooperation. This work was supported by MOSTI Special Grant (01/02/03/0696).

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