Effect of repeated laparoscopic ovum pick-up on yield and quality of oocytes in goats

Nor Farizah, A.H., Rahman, M.M., Wan Khadijah, W.E. and Abdullah, R.B.*
Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*Corresponding author: ramli@um.edu.my

Abstract

Laparoscopic ovum pick-up (LOPU) is a less invasive surgical technique for oocyte recovery from antral follicles seen as 'pimple-like' protrusion on the ovarian surface. LOPU can be repeated several times without ovarian damage or reducing the doe's fertility. This study investigated the effect of LOPU cycles on yield and quality of oocytes in goats. Three repeated cycles (oocyte retrieval 1 (OR1), oocyte retrieval 2 (OR2) and oocyte retrieval 3 (OR3)) of LOPU procedure were performed on 16 donor Boer crossbred does. The oocytes obtained were classified based on cumulus oocytes complexes (COC) layer criterion into 5 grades: grades A, B, C, D and E. OR1 gave a higher number of quality oocytes ($P<0.05$), followed by OR2 and OR3 of 5.47, 3.94 and 3.09 oocytes per ovary, respectively. Number of oocytes with grades B and D was not significantly different ($P>0.05$) among the OR cycles. The number of grade A oocytes obtained in OR1 and OR2 was significantly higher ($P<0.05$) than that in OR3 whereas the number of grade C oocytes obtained in OR1 was significantly higher ($P<0.05$) than those in OR2 and OR3. Grade E oocytes were significantly higher ($P<0.05$) in OR2 and OR3 compared to OR1. Percent recovery of grade C oocytes was higher ($P > 0.05$) than for grades A and B oocytes with mean oocyte recovery rate of 153 versus 106 and 91%, respectively. In conclusion, LOPU is a reliable procedure to provide consistently high quality oocytes of grades A, B and C and its usage can be repeated on the same donor doe for up to 3 times. However, the number of oocytes is reduced following each oocyte retrieval cycle.

Keywords: laparoscopic ovum pick-up, goat oocytes, superovulation

Introduction

Laparoscopic ovum pick-up (LOPU) is an alternative procedure to obtain consistent quality oocytes for *in vitro* production programme. It is minimally invasive with faster post-operative recovery compared with standard laparotomy (Koeman *et al.*, 2003). The LOPU procedure is reported to be an efficient method for oocyte provider (Abdullah *et al.*, 2007; Kong, 2010). It allows repetition of the laparoscopic procedure during the reproductive life of a valuable female (Baldassare *et al.*, 2007), production of embryos and offspring from animals not capable of reproducing by multiple ovulation–embryo transfer and artificial insemination, including pre-pubertal (Baldassare and Karatzas, 2004) and aged goats (Baldassare *et al.*, 2007). Baldassare and Karatzas (2004) stated that the LOPU technique avoids several causes of the poor results related with superovulation, such as poor ovulation rate, early regression of corpus luteum and poor fertilisation.

Compared to laparotomy the LOPU procedure is also less traumatic and results in fewer surgical adhesions thus this procedure could be repeated several times without

ovarian damage or decrease in the donor fertility (Baldassarre and Karatzas, 2004). According to Pierson *et al.* (2004), LOPU could be repeated up to 5 times in the same donor with minimal surgical adhesions and no major changes in overall response, and that donor could become pregnant and kid after artificial insemination or natural mating. Therefore, this present study was undertaken to determine the effect of LOPU cycles on the yield and quality of oocytes in goats.

Materials and Methods

The experimental goats were sourced from Institut Sains Biologi (ISB) Mini Farm, University of Malaya. Sixteen Boer crossbred female goats were selected as oocyte donors were used. The age of the animals ranged from 12 to 42 mo. The selected donor goats underwent oestrus synchronization, superovulation and LOPU procedure repeated 3 cycles of 3-mo intervals designated as oocyte retrieval 1 (OR1), oocyte retrieval 2 (OR2) and oocyte retrieval 3 (OR3). For oestrus synchronization, controlled intravaginal drug release (CIDR) was left in the vagina for 14 d starting at 0900 h on day 0. Cloprostenol (Estrumate® 125 µg) was administered intramuscularly at 0900 h on day 13 to regress the corpus luteum. For superovulation, pregnant mare's serum gonadotrophin (PMSG; Folligon®; 1500 IU) was administered intramuscularly at 1600 h on day 14 before the CIDR was removed. Human chorionic gonadotrophin (hCG; Ovidrel®; 250 IU) was administered intramuscularly at 2100 h on day 14, upon the removal of CIDR. The onset of oestrus behaviour was observed about 48 h after PMSG administration. The presence of oestrus indicated successful stimulation of ovaries, thus facilitating the subsequent LOPU procedure to be carried out.

Laparoscopic Oocyte Pick-up (LOPU) for Oocytes Retrieval

A day before performing the LOPU, the donor goats were deprived of feed and water (1730 h on day 16). On the day prior to LOPU surgery (at 0800 h on day 17), the donor goats were anaesthetized with xylazine hydrochloride (0.22 mg/kg body weight), followed by ketamine hydrochloride (11 mg/kg body weight) through intramuscular injection. Surgical instruments and accessories were disinfected. The follicles visible on the surface of ovaries were punctured and fluids were aspirated with an aspiration needle. The fluid was collected in collecting tubes (3 ml) and searched for oocytes under microscope. After searching the cumulus-oocyte complexes (COCs) during the LOPU, the COCs were washed to remove the debris. Subsequently, the COCs were placed into another *in vitro* maturation microdroplet (10-15 COCs/80 µl). The oocytes obtained were classified based on COCs layer criterion into 5 grades: grades A, B, C, D and E as described by Rahman *et al.* (2007). After the completion of oocyte retrieval, the donor goat underwent post-surgical treatment in order to prevent possible post-surgical infection.

Statistical Analysis

The effect of LOPU cycles (OR1, OR2 and OR3) on yield and quality of oocytes were analysed using one-way analysis of variance. The differences among the means were determined using Duncan's Multiple Range Test (DMRT) at 5% significant level. The analysis was performed using SPSS statistical analysis programme.

Results and Discussion

Table 1 shows the number and grades of oocytes obtained for different

oocyte retrieval cycles. Generally, the number of oocytes recovered decreased as the cycle increased with the values of 5.47, 3.94 and 3.09 for OR1, OR2 and OR3, respectively. The total number of oocytes retrieved in OR1 was significantly higher (P<0.05) than in OR2 and OR3. This might be due to the repeated follicular punctures during the LOPU procedure which might have altered the endocrine profiles slightly and subsequently causing minor morphological changes in the ovaries (Petyim *et al*., 2001). The number of grade A oocytes obtained in OR1 and OR2 was significantly higher (P<0.05) than in OR3, which were 1.50 and 1.25 versus 0.56, respectively. For grade C oocytes the number obtained in OR1 was significantly higher (P<0.05) than in OR2 or OR3, which were 2.41 versus 1.25 and 1.06, respectively. The number of grade E oocytes was significantly higher (P<0.05) in OR2 and OR3 compared with OR1 (0.22 and 0.34 versus 0.03, respectively). When analysed by oocyte recovery rate, grades A, B, C and D oocytes were not significantly different (P>0.05) between the OR cycles, except in grade E oocytes in OR3 showed significantly higher (P<0.05) oocyte recovery rate (10.35) compared to OR1 (0.26). However, OR2 recovery rate was not significantly different (P>0.05) from OR1 or OR3. This is in agreement with Pierson *et al.* (2004) and Rahman *et al.* (2007) who stated that LOPU can be repeated up to 5 times in goats at different intervals and in different seasons with little or no important change in overall response. Therefore, LOPU can be repeated more than 3 times.

Table 1: Number (mean ± SEM) of oocytes per ovary obtained after laparoscopic ovum pick-up (LOPU) according grades for different oocyte retrieval (OR) cycles in goats

OR cycle	No. of ovaries	No. of oocytes recovered per ovary	No. of oocytes recovered per ovary				
			Grade A	Grade B	Grade C	Grade D	Grade E
OR1	32	5.47±0.67^{b} (n=175)	1.50±0.34^{by} (n=48)	1.25±0.27^{ay} (n=40)	2.41±0.36^{bz} (n=77)	0.28±0.13^{ax} (n=9)	0.03 (n=1)
OR2	32	3.94±0.44^{a} (n=126)	1.25±5.27^{bz} (n=40)	0.94±0.22^{az} (n=30)	1.25±0.23^{az} (n=40)	0.28±0.08^{ay} (n=9)	0.22±0.07^{ay} (n=7)
OR3	32	3.09±0.50^{a} (n=99)	0.56±0.13^{ayz} (n=18)	0.66±0.20^{ayz} (n=21)	1.06±0.31^{az} (n=36)	0.41±0.15^{ay} (n=13)	0.34±0.13^{by} (n=11)
Average (Total)	32 (n=96)	4.17±0.33 (n=400)	1.40±0.14^{yz} (n=106)	0.95±0.13^{y} (n=91)	1.57±0.19^{z} (n=153)	0.32±0.07^{x} (n=31)	0.20±0.05^{x} (n=19)

OR1, oocyte retrieval 1; OR2, oocyte retrieval 2; OR3, oocyte retrieval 3; n, number of oocyte. ^{ab} Mean values within column with different superscripts were significantly (P<0.05) different. ^{xyz} Mean values within row with different superscripts were significantly (P<0.05) different.

Table 2 shows the oocyte recovery rate for different grades of oocytes. Grade C oocyte recovery rate was highest among the oocytes grades from the LOPU procedure. In OR1, the recovery rates of grades D and E oocytes were not significantly different(P>0.05) (4.16 and 0.26, respectively). Recovery rates of grades A and B oocytes of 24.78 and 21.39, respectively, were not significantly different (P>0.05) but they were significantly higher (P<0.05) than those of grades D and E oocytes. The recovery percentage of grade C oocytes of 40.04% was significantly higher (P<0.05) compared to the other grades of

oocytes, In OR2 cycle, the oocyte recovery percentage for grades A (29.36%), B (21.95%) and C (26.50%) was significantly higher($P<0.05$) than those of grades D oocytes (7.69%) and E oocytes (8.30%). In OR3 cycle, there was no significant difference($P>0.05$) for all grades of oocytes recovered. Grade C oocytes showed the highest recovery rate followed by grades A, B, D and E oocytes. This might be due to the oocytes were still immature during the time of oocyte pick-up. It has been known that the cumulus cells could be easily detached when under going maturation. In addition, needle size and vacuum pressure during LOPU procedure could cause the loosening of cumulus cells. Therefore, the preservation of cumulus vestment in recovered oocytes was reported to be good (Baldassare *et al.*, 2007). Number of grade E oocytes tended to increase with increasing OR cycle. This could probably be due to the effects of repeated hormonal treatments for ovulation induction that generally is followed by decreasing fertility in goats. It is also linked to the presence of anti-hormone antibodies such as eCG and PMSG + hCG antibodies, that might have shown immune response to previous treatments (Drion *et al.*, 2001).

Table 2: Percentage (mean ± SEM) of oocytes obtained after laparoscopic ovum pick-up (LOPU) according grades for different oocyte retrieval (OR) cycles in goats

OR cycle	Grade A	Grade B	Grade C	Grade D	Grade E
OR1	24.78 ± 4.73^{ay} (n=48)	21.39 ± 4.31^{ay} (n=40)	40.04 ± 5.22^{az} (n=77)	4.16 ± 1.90^{ax} (n=9)	0.26 (n=1)
OR2	29.36 ± 5.27^{az} (n=40)	21.95 ± 5.01^{az} (n=30)	26.50 ± 4.37^{az} (n=40)	7.69 ± 2.40^{ay} (n=9)	8.30 ± 3.64^{ay} (n=7)
OR3	23.38 ± 6.38^{az} (n=18)	18.95 ± 6.40^{az} (n=21)	24.01 ± 5.56^{az} (n=36)	13.37 ± 5.37^{az} (n=13)	10.35 ± 4.37^{az} (n=11)
Average (Total)	25.84 ± 3.15^{yz} (n=106)	20.76 ± 3.04^{y} (n=91)	30.18 ± 2.99^{z} (n=153)	8.40 ± 2.07^{x} (n=31)	6.10 ± 1.70^{x} (n=19)

Conclusion

LOPU could be repeated up to 3 times in the same hormonal treated goat donor (60 h of post-PMSG+hCG) without any detrimental effect. The OR1 cycle gave higher quantity and quality oocytes, followed by OR2 and OR3 cycles. Higher number of grades A, B and C oocytes were obtained compared to grades D and E.

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