Journal of Animal and Poultry Production

Journal homepage: www.japp.mans.edu.eg
Available online at: www.jappmu.journals.ekb.eg

Comparison of Harvesting Techniques and Corpus Luteum Bearing on Recovery and Quality of Sheep Oocytes

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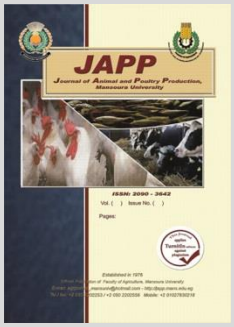

ABSTRACT

The aim of this study was to assess the effects of the slicing and puncture process and CL-bearing on the recovery and efficiency of oocytes from sheep ovaries. In the present study, 100 ovaries with corpus luteum (CL+) and without corpus luteum (CL-) were used. Ovaries weight, length, width, and thickness were measured. Using the slicing and puncture methods, the follicles were counted. The follicles were grouped in small follicles (< 2 mm), medium follicles (2-5 mm), and large follicles (> 5 mm). Oocytes have been categorized as compact cumulus / oocyte complexes (COCs), expanded (EXO), partially denuded (PDO), denuded (DO), and degenerated (DEG) oocytes according to their morphology. Our results revealed that the average weight of sheep ovaries CL+ (0.868 g) were significantly (P>0.05) greater than ovaries CL- 0.727 g, but there were no significant differences in length or width. The average number of total and compact oocytes/ovary when slicing was significantly (P>0.05) higher than puncture techniques were used (6.87 vs. 5.70) and (3.39 vs. 2.46), respectively. CL+ ovaries showed insignificantly (P<0.05) lower oocyte recovery rate in total, compact (COCs) and denuded oocytes, while the opposite of this trend in partial denuded and degenerated oocytes as compared to CL- ovaries. In conclusion, the cumulus-oocyte complexes obtained from sheep ovaries without CL and from the slicing rather than puncture method can be used for *in vitro* embryo production of sheep.

***Keywords*:** Sheep ovary; corpus luteum bearing; harvesting techniques; oocytes.

INTRODUCTION

Reproductive technologies such as super-stimulation, immature oocyte collection, *in vitro* maturation (IVM), *in vitro* fertilization (IVF), artificial insemination (AI), and embryo transfer (ET) have been used to increase the number of young of selected females and reduce generation intervals in Bovidae spp. (Suresh, *et al*., 2009; El-Sharawy, *et al*., 2021). Either surgical or laparoscopic procedures *in vivo* matured oocytes are used (Baldassarre, *et al*., 1994). These procedures are costly and the amount of oocytes per ovary is tiny (Pawshe, *et al*., 1994).

In vitro embryo development has become a popular way of growing embryos with minimum costs from slaughtered ovaries (Hoque, *et al*., 2011). Slaughterhouse ovaries represent an efficient oocyte source. This make available large and cost-effective embryo development (Sianturi, *et al*., 2002). The rate of recovery of oocytes from the ovaries without CL obtained from abattoir was substantially higher (Merton *et al*., 2003). Ovaries with CL yields were less oocyte upon assortment; large follicles were modest in ovaries CL+ (Jamil, *et al*., 2008). The bovine oocytes obtained from CL bearing ovaries were smaller in proportion (P<0.001) than the ovaries not CL-bearing in their collections (Hajarian *et al*., 2016).

The negative impact of CL on bovine oocyte production depending on the follicle size in which the oocytes number was affected (Shabankareh, *et al*., 2015). Wang *et al*. (2007) evaluated three methods of slashing, aspiration and slicing obtained from the goats' ovaries directly harvested from abattoirs; their findings showed that slice techniques away from the development grade generate a high number of oocytes as opposed to the other two methods. Bovine oocytes were harvested by using four methods: aspiration, slashing, slicing, and slicing after aspiration (Saleh, 2017), who assumed that slicing processes produce more oocytes of moderate quality and embryos, whereas aspiration techniques produce moderate oocytes of high quality and embryos. Follicular dissection was utilized to extract ovine oocytes for the first time (Crosby *et al*.,1981; Fukui *et al*., 1988). Also, a significant factor is the number of superior oocytes harvested from ovaries (Wang *et al*., 2007). The goal of this study was to determine the effects of the slicing and puncture process and CL-bearing on the recovery and efficiency of oocytes from ewe ovaries.

MATERIALS AND METHODS

Oocyte recovery

The present study was performed at the International Livestock Management Training Centre, Sakha, Agricultural Research Centre with cooperation of the Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University. Ovaries collected from the sheep slaughterhouse in Mahla district, were placed in physiological saline NaCl (9 mg / mL) encompassing antibiotics (penicillin, 100 IU / mL, 100 µg /mL streptomycin-sulfates) and transferred to a laboratory within 1-2 hours of slaughter. The ovaries were thoroughly washed in a freshly prepared salt and then in distilled water. All observable antral follicles were measured and determined based on their diameters on ova surfaces (CL+ or CL-), in small follicles (SF < 2 mm), medium follicles (MF 2-5 mm), and large follicles (LF > 5 mm), as well as in weight, length and width of ovary in each category.

Ovaries were individually processed (CL+ or CL-), and one of two techniques was used to harvest oocytes. In puncture technique, ovaries were put with forceps in a Petri dish having 5 mL of oocyte harvesting medium. The entire ovarian surface was

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DOI:10.21608/jappmu.2021.109280.1024

punched by an 18 gauge hypodermic needle. Meanwhile, in slicing technique, ovaries put in a Petri dish carrying 5 mL of the oocyte harvesting medium. Incisions were produced on the ovarian surface utilizing a scalpel blade. The Petri dishes were stayed intact for 5 minutes, enabling the oocytes to settle down, by means of punching and slicing methods. A syringe removed excess media without disrupting the oocyte.

Oocyte categories

The total number of oocytes collected was counted under a stereomicroscope. The oocytes cleaned in the harvest medium three times after collection, weighted, evaluated using an inverted microscope, and categorized into five groups. 1- Compact oocytes cumulus cells (COCs), oocytes with homogenous cytoplasm, and ≥ three cumulus cell layers 2- Expanded oocytes, either with homogenous or heterogeneous cytoplasm and expanded cumulus cells 3- Partial denuded, cumulus cells are incompletely surrounding the oocytes 4- Denuded, cumulus cells completely free of oocytes 5- Degenerated, oocytes were vaculted or cytoplasmic shrinking.

Provision of medium for harvesting

Phosphate buffer saline (PBS) medium has been prepared based on Gordon (1994). PBS was applied as two mg / mL of bovine serum albumin (BSA). The pH value of the medium was set to 7.2-7.4 by pH-meter and the osmolarity to 280-300 mOsmol / kg by osmometer. The medium was then filtered with a 0.22 µm millipore filter (milieux GV, millepore, Cooperation Bedford MOA).

Rate of yield and oocyte recovery

The oocyte production was accounted and the oocyte numbers / ovary was determined. In proportion to the total number of visual follicles on the ovarian surface of each doe, the recovery rates were estimated using the following formula (RR percent):

RR (%) = (Number of recovered oocytes/number of follicles) x 100.

Data analysis

Data have been analyzed using ANOVA for t-testing of ovarian biometry, as well as for variance analysis (2 harvesting techniques x 2 CL bears). All analyses were performed using statistical analysis system software (SAS, 2004). The medium variations were assessed using the Multiple Range Test of Duncan (Duncan, 1955).

RESULTS AND DISCUSSION

Results

Ovarian biometry and frequency of follicles

The average weight of sheep ovaries was 0.795g. In contrast, the average value of sheep ovary CL + (0.868 g) was significantly greater (P<0.05) as compared to ovary CL- (0.727 g). But, no substantial differences (P>0.05) between them in length and width were observed in Table 1.

Table 1. The main value of ovarian parameters as affected by CL bearing.

CL bearing	Ovarian parameters			
	No. ovary	Weight of ovary (g)	Length of ovary (cm)	Width of ovary (cm)
CL-	52	0.727±0.032b	1.0±0.049	0.769±0.039
CL+	48	0.868±0.037a	1.05±0.026	0.817±0.027
Overall mean	100	0.795±0.025	1.02±0.027	0.794±0.023

a and b: Values in the same column with different superscripts are significantly different at p < 0.05.

CL-: Ovary without Corpus Luteum; CL+: Ovary with Corpus Luteum

The average number of different types of follicles is shown in Table 2. Variance analysis revealed that the experimental ovarian sheep (CL-) had a significant increase in total follicle number, small and large follicles per ovary (P>0.05) when compared to ovary (CL+). However, there were no significant differences in medium follicle (3.59 and 3.25, respectively) between these follicles per ovary (Table 2).

Table 2. The average number of follicles per ovary as affected by CL bearing.

CL bearing	No. of ovary	No. of follicles categories			
		LF/ ovary	MF/ ovary	SF/ ovary	Total follicles /ovary
CL-	52	0.634±0.07a	3.59±0.26	4.59±0.32a	8.83±0.37a
CL+	48	0.354±0.07b	3.25±0.33	3.52±0.26b	7.13±0.47b
Overall mean	100	0.50±0.05	3.43±0.21	4.08±0.21	8.01±0.31

a and b: Values in the same column with different superscripts are significantly different at p < 0.05.

-SF: Small Follicles (< 2 mm), MF: Medium Follicles (2-5 mm), LF: Large Follicles (> 5 mm)

Effect of CL bearing and type of harvesting techniques on oocytes categories

Data in Table 3 showed that the average oocytes number categories of ovary was affected by CL bearing and type of harvesting techniques. The median number of total and compact oocytes per ovary from sheep ovary without CL bearing were substantially (P<0.05) greater as compared to those from ovary with CL (6.85 vs. 5.72) and (3.39 vs. 2.46) respectively, (Table 3). However, this trend's opposite is the average number of expanded oocytes (0.65 vs. 0.83). Meanwhile, there is no a significant difference in the average number of other oocytes categories from the ovary either with or without the CL bearing.

Table 3. The average number of oocytes categories per ovary as affected by harvesting techniques and CL bearing.

Oocytes categories	CL bearing		Harvesting technique		Overall
	Oocytes per ovary		Oocytes per ovary		
	CL-	CL+	Slicing	Puncture	
Total oocytes	6.85^{a}	5.72^{b}	6.87^{A}	5.70^{B}	6.33
Compact (COCs)	3.39^{a}	2.46^{b}	3.39^{A}	2.46^{B}	2.96
Partial denuded	0.83	0.80	0.76	0.89	0.82
Denuded	1.09	0.80	0.96	0.96	0.96
Expanded	0.65^{b}	0.83^{a}	0.78	0.67	0.73
Degenerated	0.89	0.83	0.96	0.74	0.86

The values of A and B or a and b are different across rows (P < 0.05).

In comparison with CL+, the proportion of compact oocytes originating from CL- ovary was significantly greater (P<0.05). While, the other types of oocytes were not (P<0.05) in terms of the regeneration of oocytes from CL- ovaries. Ovarian bearing CL also showed that the harvest techniques did not interact. This effect of results in the highest per ovary oocyte of CL- ovaries with slicing techniques (Fig. 1). In comparison with the type of techniques of oocyte collection, the average number of complete and compact oocytes for an ovary with slicing technique was significantly (P<0,05) greater than puncture, (6,87 vs. 5,70) and (3,39 vs. 2,46), respectively. However, the total number of other types of oocytes using slicing and puncture techniques did not vary significantly as shown in Table 3.

As affected by CL bearing, CL+ ovaries were insignificant lower oocyte recovery rate in total, compact (COCs), and denuded oocytes, while the opposite of this trend in partial denuded and degenerated oocytes as compared to CL- ovaries (Table 4). As affected by the type of harvesting techniques, the present results demonstrated that the slicing technique was a significantly higher oocyte recovery rate in total and compact oocytes (COCs) as compared to puncture technique. While there was no difference between two harvesting techniques in recovery rate of other oocytes categories (Table 4).

The average yield from ovaries without CL with the slicing technique was recorded the highest value. Before the cumulus expansion, the oocytes stay fixed to the small and medium follicles. However, by using the slicing process, the

oocytes with expanded cumulus investment can be recovered easily from the tiny follicles (Fig. 2).

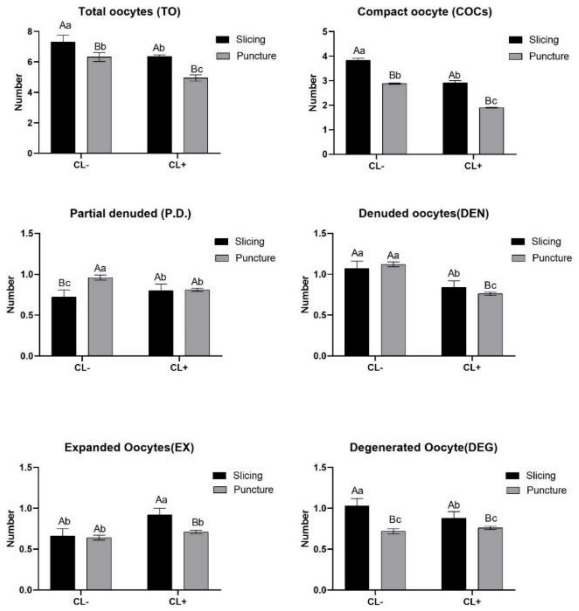

Fig. 1. Average number of oocytes categories per ovary as affected by harvesting techniques and CL bearing. Different lower-case letter show a significant difference among different groups and different capital letters indicate a significant difference between the two CL-and CL+ of Slicing and Puncture procedure. Data expressed as Mean± SEM, Significant difference between at (P<0.05).

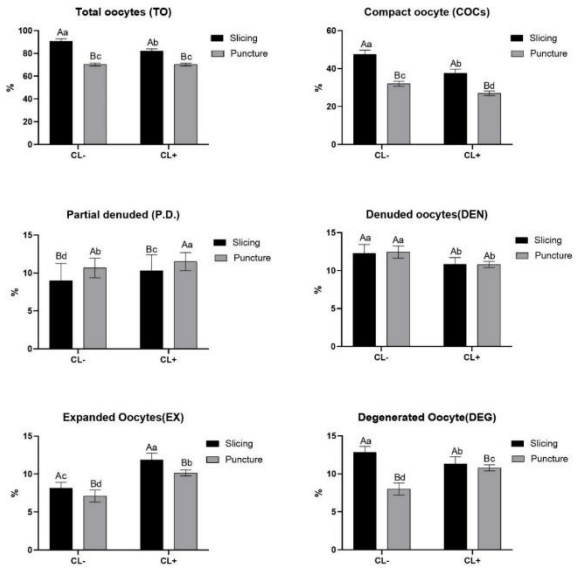

Fig. 2. Recovery rats of total oocytes and their categories as affected by harvesting techniques and CL bearing. Different lower-case letters indicate a significant difference among different groups and different capital letters indicate a significant difference between the two CL-and CL+ of Slicing and Puncture procedure. Data expressed as Mean± SEM, Significant difference between at (P<0.05).

Table 4. Recovery rates of total oocytes and their categories as affected by CL bearing and harvesting techniques.

Oocytes categories	CL bearing		Harvesting technique		Total
	Recovery rate %		Recovery rate %		
	CL-	CL+	Slicing	Puncture	
Total follicles (No.)	459	342	428	373	801
Total oocytes	80.61	76.9	86.68^{A}	70.24^{B}	79.03
Compact (COCs)	39.87	33.04	42.99^{A}	30.00^{B}	36.95
Partial denuded	9.8	10.82	9.58	10.99	9.11
Denuded	12.85	10.82	12.15	11.8	10.24
Expanded	7.63	11.11	9.81	8.31	11.99
Degenerated	10.46	11.11	12.15	9.12	10.74

- **Values in each row with different superscripts are statistically different at P<0.05.**
- **No.– number follicle**

Discussion

Applications have implemented to upsurge the amount of offspring from certain females, as were assisted reproductive technologies, including ovarian super-stimuli (superovulation), immature oocyte selection, *in-vitro* maturation (IVM), *in vitro* fertilization (IVF), artificial insemination (AI) and embryo transfer (ET), and to cut back generation intervals (Suresh *et al*., 2009).

Our results showed that the average weight value of sheep ovaries CL + were significantly higher, (0.868 g) compared to ovaries CL – (0.727 g). The current study is in agreement with those reported by Alsafy and El-Shahat, (2011), who reported that the presence of CL was affected by the ovary weight significantly (P > 0.05). So, with CL bearing ovary weight being higher than non-CL bearing.

In the same trend, Islam *et al.* (2007) demonstrated that the mean weight of goat ovary was significantly greater (P<0.05) and width comparatively more elevated CL (0.71±0.03 g) in ovaries and (0.76±0.03cm) in the same ovaries as in ovaries CL- (0.64±0.01 g) and (0.75±0.01 cm) correspondingly. In contrast, in ovaries CL-, the average lengths were found higher (1.14±0.02 cm). In this respect, Jablonka-Shariff *et al.* (1993) reported that the luteinized granulose cell hypertrophy, fibroblasting hyperplasty of the connective tissue and vascularity support to increased CL size.

Various methods have been used to collect ovaries from goats (Nieto *et al*., 1992; Pawshe *et al*., 1994) and sheep (Wani *et al*., 1999). Established on the present results, the processes of slicing resulted in more oocyte per ovary than the process of puncture (P<0.05). In conflict with the findings of this results, Wani *et al*. (1999) suggested that no significantly differences (P<0.05) between slicing (9.5 ± 0.4) and puncture (9.5 ± 0.4) in producer of oocytes per ovary in sheep.

Nada *et al*., (2008) stated that the average percentage of oocytes regained from sheep ovary was considerably greater (P<0.05) by slicing (84.60±1.01) than the aspiration method (67.60± 1.37). However, the proportion of mature oocytes of superior quality was greater in the aspiration procedure (40.57 ± 0.90) than in slicing methods (37.40±0.27). Higher number of good-quality oocytes recovered per ovary by slicing (5.2 ± 0.2) and puncture (5.2 ± 0.2) related to aspiration (4.4 ± 0.2) (Ramsingh *et al*, 2013). Unlike the findings of this study, the amount of oocytes per ovary for slicing (4.0) and aspiration (3.7) did not differ substantially in lambs (Shirazi *et al*., 2005), large oocytes number per ovary were collected in sheep (Martino *et al*., 1992) using the slicing technique.

As affected by CL bearing, ovaries CL+ showed insignificantly (P>0.05) lower oocyte recovery rate in total, compact (COCs), and denuded oocytes, while the opposite of this trend in partial denuded and degenerated oocytes as associated to CL- ovaries was obtained. In terms of the adverse effect of CLs on oocyte recovery in bovine animals, this result showed a marked effect of ovarian status on oocyte recovery rate (El-Ratel *et al*., 2017).

In this regard, Singh *et al*., (2012); Nandi *et al*.(2000) and Kadoom *et al*., (2014) described bovine and buffalo oocytes recovery rates declined when ovaries had CL+, correspondingly. This pattern was assigned to limited follicular growth, since CL's lutein cells occupy most ovaries (Kumar *et al*., 1997). Also, in the CL+ ovaries, the dominant follicle is usually observed alongside other small follicles (Gasparrini *et al*., 2000). Penitente-Filho *et al*., (2015), on the other hand, found that ovaries with CL had a higher number of superior quality oocytes than ovaries without CL.

In terms of harvesting methods, the slicing technique recovered more total oocytes and COCs with compact cumulus investments than the puncture technique. Wani *et al*., (2000) reported that the percentage of healthy oocytes contained in the suction process was greater (64.4 %) related to the puncture (54.7%) or slicing procedures (54.3 %). Shanthi *et al*., (2012) informed that the slicing technique extracted 708 oocytes from 120 sheep's ovaries with a mean recovery rate of 5.9 oocytes/ovary. The average number of grades A, B and C of the oocyte sliced was 47.17±1.64, 54.17±1.85 and 6.67±0.61 respectively.

The retrieved oocytes in the slicing procedure (5.52 ± 0.40) were considerably higher, chased by dissection (2.52 ± 0.28) and aspiration (1.35 ± 0.18) (El-Ratel *et al*., 2017). A changeable number or percentage of oocytes found in ovaries of small ruminants. Ovarian bearing CL was also shown to interfere with harvesting techniques. This effect contributes to the highest oocyte recovery rate of CL- ovaries with slicing techniques compared with CL+ ovaries with puncture techniques (Fig., 2). The average yield with the cutting technique was the largest. Until cumulus expansion, the oocytes are securely connected to the small and medium-sized follicles and that cannot aspired but simply recover from the small follicles when cutting. Ovarian slicing is a quick and effective method to recover superior quality oocytes, whereas the aspiration procedure is time consuming and tedious (El-Ratel *et al*., 2017).

CONCLUSION

Based on the above mentioned results, it is possible to conclude that a higher number of total and COCs oocytes were obtained from sheep ovaries without CL- than from ovaries with CL+, as well as the harvesting technique slicing than a puncture.

ACKNOWLEDGEMENTS

We appreciate and thank Prof. Dr. Sherief M. Shamiah, Animal Research Institute, Agriculture Research Center, for his technical support during the study and his manuscript revision.

Conflicts of Interest Declaration*:* The authors declare no conflicts of interest.

Ethical Animal Research: The experimental procedure was approved by the Faculty of Agriculture, Kafrelsheikh University, Egypt.

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مقارنة فعالية تقنيات جمع البويضات و تأثير وجود الجسم الأصفر على معدل إسترداد وجودة بويضات الأغنام

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الهدف من هذه الدراسة هو تقييم فعالية تقنيات جمع البويضات (التقطيع أوالثقب) وكذلك تأثير وجود الجسم الأصفر من عدمه بالمبيض على جودة ونوعية بويضات المبيض بالأغنام. في هذه الدراسه، تم استخدام 100 مبيض يحمل جسم أصفر أو لا يحمل جسم اصفر كما تم قياس وزن، طول، عرض وسمك المبيض. تم عد الحويصلات بإستخدام طريقتي التقطيع و الثقب وتقسيمها الي حويصلات صغيره (أقل من 2مم)، حويصلات متوسطه (2-5 مم) و حويصلات كبيره (أكبر من 5مم). تم تصنيف البويضات الي مجمعات ركامية / بويضات مضغوطة (COCs)، وموسعة او مفككه (EXO) وجزئية الاحاطه (PDO)، وعاريه (DO) وبويضات متحللة (DEG) وفقًا لشكلها المورفولوجي. من خلال النتائج التي توصلنا إليها خلال هذه الدراسه، كان متوسط وزن المبيض المحتوي علي جسم أصفر أعلي (P<0.05) معنوياً 0.868 جم مقارنة بالمبيض غير المحتوي علي جسم أصفر 0.727 جم وأظهرت النتائج عدم وجود إختلافات معنويه عند وجود الجسم الأصفر في طول وعرض المبيض. بينما كان متوسط عدد البويضات الاجمالي و البويضات كاملة الاحاطه لكل مبيض عالية المعنويه عند استخدام طريقه التقطيع مقارنة بالثقب (6.87 و 5.7) و (3.39 و 2.46) علي الترتيب. كما أظهرت نتائج المبايض المحتوية علي الجسم الأصفر انخفاضًا طفيفًا (P >0.05) في معدل استرداد البويضات الكلية والكاملة الاحاطه والبويضات المتعرية، بينما العكس في البويضات المتعرية جزئيًا والمتحللة مقارنة بالمبايض غير المحتوية علي الجسم الأصفر.يمكن إستنتاج أنه يمكن إستخدام البويضات كاملة الإحاطة التي تم الحصول عليها من مبيض الأغنام بدون جسم أصفر وبطريقة التقطيع بدلاً من طريقة الثقب وذلك لإنتاج الأجنة معملياً في الأغنام.