How to Cite:
Al-Shammari, R. F. H., & Almeeni, I. M. (2022). Effect of oocytes collection methods on oocytes quantity and quality in local Iraqi ewes. *International Journal of Health Sciences*, *6*(S1), 14560–14567. https://doi.org/10.53730/ijhs.v6nS1.10148

Effect of oocytes collection methods on oocytes quantity and quality in local Iraqi ewes

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***Abstract*---**This study was designed to investigation the effect of oocytes collected methods (aspiration and slicing) to oocytes recovery per ovary and the quality oocytes (normal and abnormal) in local Iraqi ewes. The female genitalia was brought from Al-shulla abattoir from the animals after slaughtered, the samples was collected and transport to reproductive techniques laboratory with 1-3 hrs in thermal box containing normal saline (0.9%) supplemented with 100 IU/ml penicillin, and 1mg / ml streptomycin. In laboratory the ovaries washed in phosphate-buffered solution with antibiotics three times. Each time the oocytes collection in different method aspiration or slicing to compared in recovery rate and quality of oocyte collected after classification four group according number of cumulus layers and uniform the cytoplasm to (A + B) when contained multiple layer of cumulus cells with homogenous cytoplasm considered normal oocytes and (C+D) when absent cumulus cells or degenerated with heterogeneous cytoplasm considered abnormal oocytes in two different methods. The result of this current study showed the high significant effect (P≤0.01) in recovery rate to slicing than aspiration (4.91, 3.23) oocyte /ovary respectively. But when comparing the total number of normal oocytes rate (A+ B) between the two methods of oocyte collection, was found a significant difference (P≤0.05) between aspiration than slicing (65.19% ,49.08%) respectively. Also in total abnormal oocytes collected, when compared in two different methods we found significant effect (P≤0.05) between two collection method to slicing than aspiration (50.90%, 34.80%) respectively. Conclusion the aspiration oocytes collection method was low in quantity of oocytes harvested but was high quality (normal oocytes) when compared with slicing, it high in quantity of oocytes harvested but the oocytes was low quality when compared with aspiration. Also the slaughterhouse

sample is abundant and cheap source of oocyte collection .The aspiration follicle fluid and slicing ovary, both was useful methods of retrieval of oocytes in local ewes. recommended the oocytes collected by aspiration when high number genitalia to get good quality oocyte , also the oocytes collection by slicing when low number of genitalia to get high number oocyte to use in IVEP technology.

***Keywords*---**sheep Oocyte, oocyte collection method, graded oocyte.

Introduction

Sheep breeding is an important enterprise in animal production yielding foods such as meat and milk which are very important for people's nourishment all over the world (Rather *et al.*, 2020) there has been meat consumption increased throughout the course of recent years and this growth attributed to increases in per person consumption of sheep meat (Godfray *et al.,* 2018). In vitro embryo production (IVEP) systems as new reproductive technology may play an important role for the production of sheep in the future to accelerate sheep breeding and improve the efficiency of production (Botigelli *et al.*, 2017; Gharban, 2021). Also to increase the productivity in the small ruminant industry the genetic material of these species should be improved so, the in vitro embryo production could be important technology to reach this goal by combining selected male and female gametes (Paramio *et al.*, 2016). The IVEP allows the production of a high and cheap number of embryos essential for studies which need a high number of embryos using ovaries recovered from slaughterhouse as a source of oocyte such as sexing, stem cells, cloning and in general studies needing a high number of embryos (Paramio and Izquierdo, 2014). There are currently facing many technical challenges in improving the efficiency of the ovine IVEP systems such as low efficiency and poor quality of embryos, so need to find solutions to overcome the problems for that the system (Botigelli *et al.*, 2017).

The method of oocyte harvest for in vitro maturation is the first and most important step toward effective in vitro embryo development (Hegab *et al.*, 2018). The main goal of an oocyte recovery method which can be used for in vitro maturation, in vitro fertilization and in vitro culture is to maximize the total number of oocytes and the yield of high-quality oocytes (Shirazi *et al.*, 2005). To produce good embryos in IVM must be recovered a large number of good quality and high developmental competence oocytes from ovaries (Contreras *et al.*, 2008). The oocyte is a gamete that provides the zygote with not only half of the genetic material but also nearly all of the cytoplasm as well as the transcripts and proteins required for early embryonic development (Schultz, 2002).

The procedures for collecting oocytes from the ovaries of slaughtered animals include slicing, ovary puncture and follicle aspiration (Wani *et al.*, 2000). Because the use of these oocytes allows for the acquisition of a large number of ovaries at a minimal cost (Udin *et al.*, 2020; Gharban and and Al-Shaeli, 2021). It's also critical to improve IVEP technology's efficiency in the development of embryos derived from slaughterhouse animals' ovaries, as well as procedures that promote the harvesting of a large number of high-quality oocytes per ovary (Mohammadi,

2010). The presence of cumulus cells enclosing the zona pellucida (ZP), the absence of cracked zona pellucida and the absence of vesicles in the ooplasm are all features of normal oocytes, it is preferable if there are more compact layers of cumulus cells (Rahman *et al.*, 2008). The author Landeo *et al.* (2022) classified oocytes into four groups based on their compaction, the amount of cumulus cell layers, and the homogeneity of the ooplasm into grade I (≥4 layers of compacted granulosa cells tightly surrounding the oocyte and homogeneous ooplasm), grade II (partial layers of granulosa cells scattered around the oocyte and homogeneous ooplasm), grade III (denuded, mostly absence of granulosa cells but homogeneous ooplasm) and grade IV (expanded/degenerated, granulosa cells and vacuolated ooplasm).Multiple intrinsic and extrinsic factors can influence the quality of cumulus-oocyte complexes (COCs), the intrinsic factors include breed, age, reproductive status, metabolism, nutritional status, hormone levels and estrous cycle stage (Moussa *et al.*, 2015). The period between slaughter and oocyte withdrawal from the ovary, morphology and methods of collecting COCs, storage temperature of the ovaries, collection media and the operator's micromanipulation abilities are all important extrinsic factors (Tello *et al.*, 2020).For this reason, this study decided to investigate the effect of the oocytes collection methods and its effect on the quantity and quality of oocytes harvesting by using two collection methods slicing and aspiration in local Iraqi sheep.

Material and methods

Female genitalia collection

The experiment was conducted in the reproduction techniques laboratory in College of Veterinary medicine of Baghdad University, for the period of (1/1/2022 – 1/5/2022). The female genitalia 56 in number was collection from alshulla slaughterhouse belong to adult animal (3-6) years according to dental formula (Ruggeri *et al.*, 2015). The female gonads samples were collection and transport to reproductive techniques laboratory in veterinary medicine College Baghdad University with 1-3 hrs in thermal box containing normal saline (0.9%) supplemented with 100 IU/ml penicillin and 1 mg / ml streptomycin (AL-Maeeni, 2012).

Oocyte collection

In laboratory the ovaries washed in phosphate-buffered solution with antibiotics three times. Oocytes were collected using the aspiration or slicing technique described by Wang *et al.* (2007). In the slicing method the ovaries slice to small parts by surgical scalpel in a graded plastic petri dish containing a collection medium (TCM199 with an antibiotic). In aspiration method the visible follicles was aspirating using a 18 gauge hypodermic needle attached with a sterile disposable syringe containing 1ml harvesting medium. The cumulus oocyte complexes (COCs) was isolated under light microscope and transferred to 35 mm petri dish containing oocyte collection medium and wash three times to isolate oocytes from debris. Then the oocytes collected stained with trypan blue to detect the viability of oocytes before used in study. The petri dish contained viable oocytes were examined under an inverted microscope to determine the number of oocytes harvest and collection rate .The oocytes collected categorized to 4 grades

depending the layer cells cumulus moreover the homogenous of cytoplasm designated by Wani *et al.* (2013) the grades A when oocytes enclosed by cumulus cells completely and homogenous the cytoplasm, grade B when oocytes enclosed by cumulus cells partially with homogenous the cytoplasm, grade C when oocytes without the cumulus cells with homogenous cytoplasm and grade D when oocytes degeneration with heterogeneous cytoplasm . Grade A with B considered the normal oocytes use in the study, while grade C with D considered abnormal oocytes so rejected

Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study percentage. Chi-square test was used to significant compare between percentages in this study.

Result and Discussion

The effect of oocyte collection method on oocytes recovery rate

The ovaries brought from abattoir are cheap and simple source to oocytes collecting when used in IVF, also the collection oocytes methods is important technique to get high number oocytes used in IVEP stage, the effect of oocyte collection method on oocytes recovery rate explained in (table 1) when used two methods oocytes collection aspiration and slicing in local Iraqi ewes. In the present study we brought 112 ovaries divided equally randomly to collected (181 and 275) oocyte in aspiration or slicing method respectively. After used statically methods we found the high significant effect ($P \leq 0.01$) in recovery rate to slicing than aspiration (4.91, 3.23) oocyte per ovary respectively. Also the result in present study, when comparing the two method of oocyte collection (aspiration and slicing) shows the superiority of ovarian slicing method than aspiration through the greater number of oocytes collected per ovary in slicing compared with the aspiration method .This result agreement with Wani *et al.* (2000) when study the effect of oocyte collection techniques in sheep to in vitro fertilization, the authors used three methods in oocytes collection puncture, slicing and aspiration, he confirmed the three methods can be applied in IVF but high significant in recovery rate between these three methods to puncture and slicing than aspiration, he explore the low number in oocytes recovery rate in aspiration because some follicle found deeply in cortex not prominent on the surface of the ovary.Also agreement with Davachi *et al.* (2014) when study the effect of oocytes recovery methods and season in ewes, when used four methods to oocytes harvesting (aspiration, centrifugation, puncture and slicing) he confirmed the oocytes recovery rate using the slicing and centrifugation technique was high significantly than the oocytes recovery rate by aspiration and puncture in both breeding seasons and non- breeding season. Also, the superiority of the oocytes slicing collection was confirmed by Wang *et al.* (2007) in goats. Also agreement with Saleh (2017) when study the effect of oocytes recovery methods to type of oocytes collected in cow, he confirmed the slicing methods yield more oocytes count with a moderate quality while aspiration methods yield a moderate oocytes count with elevated quality. But Shirazi *et al.* (2005) when used ewe lamb did not found significant effect between slicing and aspiration and this result different

explore to age of female ewe used in this study and procedure technique used in slicing method.

Table (1): Effect of oocyte collection method on oocytes recovery rate

Type of collection method	No. of ovaries	No. of Oocytes recovery	Recovery rat %
Aspiration	56	181	3.23
Slicing	56	275	4.91
P-value	--	--	0.0001 **
** (P≤0.01).			

The effect of oocyte collection method on type of oocytes

The type of oocytes or oocyte quantity is important factor in the in vitro embryo production, in the present study investigation the effect of oocyte collection method (aspiration and slicing) on type of oocytes illustrate in (table 2) when the oocytes classified four grad (A,B,C and D) according the number of cumulus cells around oocyte and type of cytoplasm to oocytes , when consideration the grad (A and B) normal oocytes and used in this present study, conversely (C and D) abnormal and rejected. In the present study we show the high significant effect (P≤0.01) between grad A oocyte rate in two oocytes collection in method aspiration than slicing (47.51% , 24.72%) respectively. while we did not find any significant effect in grad B oocyte rate in two collection method aspiration than slicing (17.67%, 24.36%) respectively .But in the overall, when comparing the total number of normal oocytes rate (A+ B) between the two methods of oocyte collection , was found a significant difference (P≤0.05) between aspiration than slicing (65.19% ,49.08%) respectively. Also the table 2 illustrate the comparative between two oocyte collection methods in quality abnormal oocytes (C and D), we showed non-significant effect between oocytes collected rate in grad C in two type collecting aspiration and slicing (19.88%, 24%) respectively, but in oocyte collected in grad D we showed significant effect (P≤0.05) between two collection method to slicing than aspiration (26.90%,14.91%) respectively. Also in total abnormal oocytes collected, when compered in two different methods we found significant effect (P≤0.05) between two collection method to slicing than aspiration (50.90% ,34.80%) respectively these results agreement with Wani *et al.* (2000) when study the effect of oocytes collection on maturation and fertilization in ovine, this result demonstrated the good oocytes quality grad A was high in aspiration collection method than slicing and puncture method, while not significant different between slicing and puncture in quality oocytes recovery. Other agreement with Sianturi *et al.* (2002), when study the effect of oocytes collection and type of grading oocytes to in vitro maturation and fertilization in cow this result indicate the oocyte collection by aspiration method the normal oocytes (A+ B) high significant than abnormal (C+D) .Also agreement with Wang *et al.*,(2007) he explore the high number of grad D in slicing oocytes collection attributed to degeneration oocytes during chopping the ovary to small parts in scrapple surgery. Other Hoque *et al.* (2011) and AL-Nuaimi *et al.* (2020), they got the same result in goats when these results indicated the high significant in grad (A +B) in aspiration method compared with slicing method so, the aspiration of follicle low effect on oocyte and yield normal oocytes than slicing method.

Table (2): Effect of oocyte collection method on type of oocytes

Type of collection method	No. Oocytes recovery	Type of oocytes					
		Normal oocytes			Abnormal oocytes		
		Grad A	Grad B	Total	Grad C	Grad D	Total
Aspiration	181	86 (47.51%)	32 (17.67%)	118 (65.20%)	36 (19.88%)	27 (14.91%)	63 (34.80%)
Slicing	275	68 (24.72%)	67 (24.36)	135 (49.10%)	66 (24%)	74 (26.90%)	140 (50.90%)
P-value		0.0081 **	0.079 NS	0.037 *	0.217 NS	0.041 *	0.027 *
* (P≤0.05), ** (P≤0.01).							

Acknowledgments

We express our sincere gratitude to Prof. Dr. Nasr Al-Anbari, Faculty of Agriculture, Department of Animal Production for helping to analyze the results statistically and to provide support for the success of this research.

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