Effect of Follicle Diameter and Culture Medium on The in Vitro Maturation of Sheep Oocytes Using Culture Media Supplemented With Different Concentrations of Sucrose in Local Sheep (Ovis Aries)

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Abstract

The current study was conducted in the postgraduate laboratory of the college of Agriculture / University of Al-Muthanna / Department of Animal Production, which aimed to find out the effect of follicle diameter and the culture medium on the percentage of *in vitro* maturation of sheep oocytes (Ovis Aries). The experiment included *in vitro* maturation of sheep oocytes aspirated from large, medium and small follicles in different culture media (named A,B and C). *In vitro* maturation of oocytes was carried out using three culture media that differed only in the concentration of sucrose, which were 0. M, 0.25 M and 0.5 M for A, B and C culture media respectively. The results of the current study showed significant superiority ($P \leq 0.05$) of culture medium C than the two media A and B in the percentages of *in vitro* maturation, which were to 38.0 ± 1.71 %, 27.37±1.47 % and 21.902±0.76 % for C, A and B respectively. The results also indicated significant effect ($P \leq 0.05$) of follicle diameter in the percentages of *in vitro* maturation of sheep oocytes which were 38.0±1.71 %, 29.57±2.06 % and 18.5± 0.27 % for large, medium and small follicles respectively. It could be conclusion from the present study that the follicle diameter and culture medium had a significant effect on the percentage of in vitro maturation of sheep oocytes.

Keywords: Follicle diameter, In vitro maturation, Sheep.

1. Introduction

Sheep have a great economic importance because they are inexpensive animals to raise and have the ability to convert low-value materials into high-value materials such as meat, milk and wool, as well as having a relatively fast capital cycle [1]. [2,3], indicated that the decline of fertility rates of Iraqi sheep affects their reproductive efficiency, and the increase in fertility rates in local sheep is reflected in improving the efficiency of sheep production.
There are many important and necessary modern techniques to improve the reproductive efficiency of sheep, such as in vitro fertilization, embryo transfer and genetic selection [2,4]. [5], indicated that in vitro matured oocytes are able to play a role in improving productivity of sheep, so that the process of oocytes maturation has a strong positive correlation with the diameter of the follicle [6], and the growth and development of oocytes are completed with an increase in the size of the follicle [7,8]. The aim of the current study is to study the effect of follicle diameter and culture media on the percentage of in vitro maturation of oocytes and to know the effect of the interaction between culture media and follicle diameter on the percentage of in vitro maturation of sheep oocytes.

2. Materials and Methods

The current study was conducted in the laboratory of postgraduate studies and included the maturation of sheep oocytes after collecting the follicular fluid from the ovaries, which collected immediately after slaughter from the Samawa slaughterhouse.

2.1 Ovaries collection

The ovaries were collected immediately after slaughter according to the method of [8] and transported to the laboratory inside a plastic container, containing warm physiological solution (0.9% Nacl at 37°C) supplemented with antibiotics (streptomycin 100 IU/ml, pencillin 100 IU). /ML), then the samples were placed inside a thermos bottle and transported within less than an hour after slaughter to the laboratory, washed at least three times with warm physiological solution at a temperature of 35-30 (C) to remove the clotted blood and reduce contamination on the ovarian surfaces and to get rid of the impurities suspended in the ovaries.

2.2 Oocytes collection

Sheep Oocytes were collected from ovaries by oocyte aspiration method, through aspiration of follicular fluid using a 5ml medical syringe, from large, medium and small follicles each individually, and before oocytes aspiration, 0.5 ml of the culture medium was added to a medical syringe with 20 IU/ML of anticoagulant (Heparin) to prevent the oocytes from sticking together, then the oocytes were placed in a petridish in SMART Medium, then placed under the dissected microscope, transported by a Pasteur pipette to the culture medium three times for washing the oocytes and removing the remains of suspended cells according to the method [9].

2.3 Oocyte classification

The oocytes were classified according to their external appearance after washing three times in the Smart culture medium, to mature, immature and atretic oocytes. Oocytes with ooplasm shrunken away from the zona pellucida or not filling the zona pellucida , these types and mature oocytes were removed from the experiment after conducting a viability examination using a dye Trypan blue also classified the oocyte that accept the dye as dead, and those that do not accept the dye as live, according to the method ([10].

2.4 In vitro maturation

The recovered oocytes were subjected to the *in vitro* maturation program, each group individually in three different culture media A, B, and C. The three media consisted of smart medium supplemented with hormones, A medium was left without sucrose as a control group, while both B and C medium contained sucrose at a concentration of 0.25 M and 0. 5 M respectively. The oocytes were cultured in in four-well Petri dishes containing 0.5 ml the culture medium, covered with a layer of paraffin oil, and incubated in a 5 % co_2 incubator for 24 hours at a temperature of 38.5 and relative humidity 95%.

2.5 Statistical analysis

The data were statistically analyzed by using the complete random design in global experiments and the analysis of variance (Anova) test was used in the study of the significant differences and to study the significant differences between the means, the statistical program spss [11] was used in the statistical analysis with a significant level ($p \leq 0.05$).

3. Results and Discussion

The results of the current study showed a significant effect of follicle diameter on the percentage of *in vitro* maturation, the oocytes aspirated from large diameter follicle were significantly superior than the oocytes aspirated from small and medium diameter follicle in the percentage of *in vitro* maturation. The percentages were 38.0 ±1.71, 29.57±2.06 and 18.5 ±0.27 % for oocytes aspirated from large, medium and small diameter follicle respectively (figure 1).
The results were in agreement with [12,13] they found that the oocytes of large follicles contain more layers of cumulus cells, which are considered a link between the oocytes and its external surroundings and increase the contact between oocytes and the components of the culture medium for transfer of nutrients and factors for oocytes growth and development, therefore, the cumulus cells contribute to supporting nuclear and cytoplasmic maturation, and the percentage of *in vitro* maturation of oocytes in small follicles decreases due to the low rate of development in small follicles and the lack of protein synthesis needed in the development of oocytes. The results also agreed with [14,15,16] in sheep and cattle, where they found that the superiority of oocytes in large follicles due to that the large follicles containing a high level of 17-B estradiol as well as the presence of influence factor within the cytoplasm of aspirated oocytes from large follicles has a role in the oocyte developmental competence, since the development competence of oocytes depends on the size of the follicles and that the oocytes must acquire integral development to reach *in vitro* maturation.

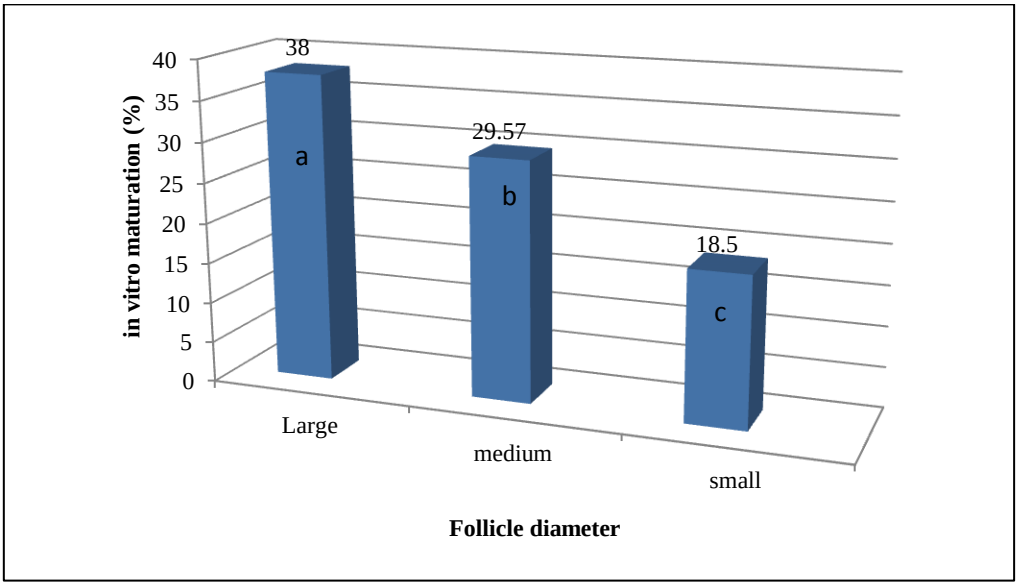

Figure 1. The effect of follicle diameter on the percentage of *in vitro* maturation of sheep oocytes.

The results of the study also showed a significant effect of the culture media on the percentage of *in vitro* maturation of sheep oocytes, and the superiority of C and B mediums than A, the percentages of *in vitro* maturation were 35.46 ± 2.9, 27.37±1.47 , 21.902±0.76 for matured oocytes in C, B and A medium respectively (figure 2). The results were agreed with ([17].in sheep, when it was found that the reason for the superiority is due to the role of sucrose in the C and B culture medium. And sucrose contributes in the activation of the oocytes and increases the rate of division and development, sucrose also plays an important role in the development of the efficiency of the oocytes to reach maturity [18].while this result did not agree with [19].in cows and where they found that sucrose only maintains the normal morphology of the oocytes. [20].reported that the culture media supplemented with hormonal additives increases the nuclear maturation of the oocytes, activates the cells and leads to the extension and expansion of the cumulus cells.

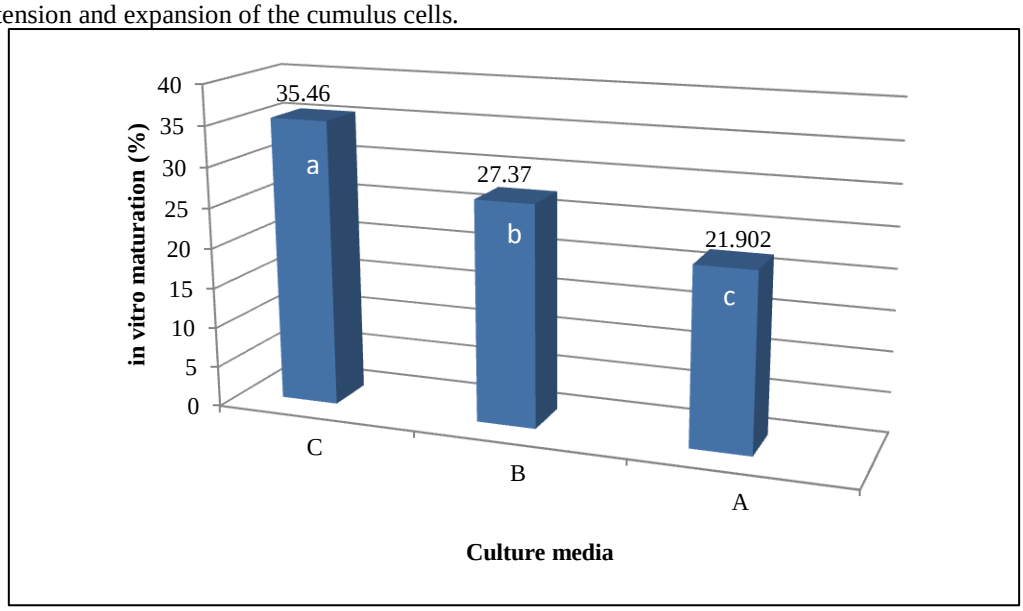

Figure 2. The effect of culture media on the percentage of *in vitro* maturation of sheep oocytes.

The results of the study (table 1) showed that there was no significant effect in the interaction between culture medium A and follicle diameter on the percentage of *in vitro* maturation of sheep oocytes in all follicles, which were 24.87±1.6 , 20.0±1.1 , 20.0 ±1.1 for oocytes aspirated from large, medium and small follicles. In the medium B, the oocytes aspirated from the large diameter were significantly ($P \leq 0.05$) superior in the percentage of *in vitro* maturation, as the percentages were 26.67±3.25 , 40.0±0.3, 18.86±0.62 for the oocytes aspirated from large, medium and small follicles.

Table 1. The effect of the interaction between culture media and follicle diameter on the percentage of *in vitro* maturation of sheep oocytes (mean ± standard error).

Culture media	The percentages of *in vitro* maturation of oocytes		
	Follicle diameter		
	Large	Medium	Small
A	c 24.87±1.6 A	c 20.0±1.1 B	a 20.0±1.1 B
B	b 40.0±0.3 A	b 26.67±3.25 B	ab 18.86±0.62 C
C	a 50.0±2.11 A	a 41.25±4.24 B	b 17.2±0.47 C

*small letters within column differ significantly at the level of probability ($P \leq 0.05$).
*capital letters within row differ significantly at the level of probability ($P \leq 0.05$).

These results are in agreement with [21]. where they found that large-sized follicles complete their development inside the body, and this results in highly developed oocytes in these follicles. And the oocytes in the large-sized follicles produce highly developed embryos. The results were in agreement with [22] which achieves the that sucrose plays a great role in *in vitro* maturation, as it helps to increase the percentage of maturation in the oocytes, including the oocytes obtained in the large follicles. Also, this result was in agreement with [23, 24] in sheep and cattle, where they found that the maturation rate of oocytes are higher in large-diameter follicles compared to oocytes in small-diameter follicles, since the large follicles are containing oocytes surrounded by the largest number of cumulus cells layers and has a high ability to develop, Therefore, the large follicles produce high rates of oocyte maturation *in vitro* and the increasing the follicles size gives a better indication of the maturation of the oocytes.
It could be conclusion from the present study that the follicle diameter and culture medium had a significant effect on the percentage of *in vitro* maturation of sheep oocytes.

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