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Effect the age and Cumulus Cells on morphological characteristics of bovine oocytes before *in vitro* maturation

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Abstract

The current study was conducted in the postgraduate laboratory of the Department of Animal Production, College of Agriculture, Al-Muthanna University during the period from 11/11/2023 to 10/3/2024 to study the effect of age and the Cumulus Cells on the morphological characteristics of bovine oocytes in vitro maturation. The study included 416 cow ovaries in different ages distributed into two age groups with 1181 oocytes as follows: The first group 1-3 years included 216 ovaries, the second group 3 years and over included 200 ovaries .The cows ovaries were collected from the slaughterhouse in Al Samawah city immediately from slaughtered animals. The ovaries were placed in plastic boxes containing physiological solution and transferred to the lab within a period less than one hour after slaughtering of animals.

The oocytes were collected from the ovaries by the aspiration method and assessed from the Morphological measurements . All oocytes were subjected to IVM using the culture medium 1640 RPMI. The results of the study showed that there were no significant differences ($P<0.05$) between oocyte groups according to the number of germinal aggregate layers in the percentages of immature, damaged and live oocytes IVM. The interaction between age groups and the number of germinal aggregate layers had a significant effect ($P<0.05$) on the percentages of immature, damaged, live and dead oocytes IVM

Keywords: Age, Cumulus Cells, morphological, , bovine oocytes, IVM .

Introduction

Reproduction and fertility play a very important role in the economy of livestock farming[1]. Fertility is a primary biological and productive trait that affects meat and milk production in cattle[2], it is the decisive factor in milk and meat production[3], for farm animal husbandry, this means the ability to produce morphologically and physiologically healthy animals. Indicators of good

reproduction in a cattle herd are obtaining one calf from one cow per year[4]. Maintaining reproductive capacity until 8-12 years of age [5]

The use of new developments or new biotechnologies in animal husbandry as a powerful tool to improve the genetic structure of animal herds and increase the selection of animals rapidly[6]. Assisted reproductive techniques (ART) play an important pivotal role in increasing the spread of superior genotypes, enhancing productivity as well as reducing the interval between two births in small ruminants[7].

In vivo fertilization is defined as the series of events resulting from the fusion of two parental gametes, the sperm from the male and the oocyte from the female, when they meet in the upper third of the fallopian tube in mammals. Fertilization involves several sequential steps including acrosome interaction, penetration of the zona pellucida, sperm-oocyte fusion, and membrane fusion[8].

Fertilization triggers a series of subsequent events that result from the fertilized oocyte to develop a new individual[9]. Robert Edwards was the first to develop this technique and was awarded the 2010 Nobel Prize in Physiology or Medicine[10].

The term "in vitro" refers to an environment outside the body where oocytes are matured and fertilized in a petri dish[11]. In vitro embryo production is an effective technique for obtaining large numbers of embryos for transfer to recipient mothers[12].

The germinal cumulus cells are connected to the oocyte by TransZonal[13]. The germinal cumulus cells contain vacuoles through which the germinal cumulus cells are capable of transporting amino acids and nutrients resulting from metabolism that are essential for the growth and development of the oocyte[14]. The germinal cumulus cells are responsible for the confinement of the germinal vesicle (GV) nucleus during growth and completion of meiosis, by raising the level of cAMP between cells[15].

The aim of this study is to knowing the effect of age and the Cumulus Cells on the morphological and biological characteristics of cow oocytes before in vitro maturation.

Material and methods

The current study was conducted in the postgraduate laboratory of the College of Agriculture, Al-Muthanna University, Department of Animal Production, during the period from 11/11/2023 to 10/3/2024, to study the effect of age and the Cumulus Cells on the morphological and biological characteristics of cow oocytes after in vitro fertilization. The study included 1181 oocytes aspirated from 416 cow ovaries of different ages distributed into two age groups. The ovaries were distributed into the age groups as follows:

The first group 1-3 years old included 216 ovaries, the second group 3 years old or older included 200 ovaries. The experiment included collect the ovaries from the slaughtered cows immediately after slaughtering in the slaughterhouses of Al-Samawah and Al-Rumaitha than transferring the ovaries to the laboratory to aspirate the oocytes[16], distributed according to the age and the type of cumulus cells before subjected to the laboratory maturation using the culture medium Rbmi-1640.

Viability test:

The cows oocytes was subjected to the Viability test using the tryban blue dye before in vitro maturation tryban blue dye according to [17].

***In vitro* maturation:**

The oocytes were washed three times using SMART containing 5% HSA to remove substances in follicular fluid .The immature oocytes were directly distributed in average of 5 oocytes per droplet (0.5mL) from culture medium ,supplied with 10 IU/mL hCG, 5 IU/mL PMSG and 1µg/mL estradiol and cultured in four well dish and covered with liquid paraffin, then incubated for 24 h in CO2 incubator (5% CO2) at 38.5°C with 100% humidity [18]; [19]

Results and Discussion

Table (1) shows no significant effect (P≤0.05) on the percentage of mature oocytes among the oocyte categories classified according to the germinal cumulus layers. There was a significant effect (P≤0.05) on the percentage of immature oocytes among the germinal cumulus categories. The percentage was higher among the oocytes of the first category A classified according to the germinal cumulus layers than the second categories B, the third C and the fourth D, the averages were (75.3±1.19), (73.6±1.50), (71.7±2.61), (60.6±5.78). This may be attributed to the important role of the germinal cumulus layers in regulating the accumulating cells and the growth and development of oocyte maturation[20].[21] Aindicated that the maturation rate of oocytes containing germinal cumulus cells was higher compared to oocytes that do not contain these cells. This is due to factors affecting the quality of oocytes, which include ovary collection, oocyte extraction process, animal age, and reproductive season. It is also affected by the environment during oocyte collection, ovary condition at extraction, and nutritional status. As for the percentage of damaged oocytes, the current study recorded significant differences (P≤0.05). The fourth category, D, recorded the highest average of (34.5±9.40).

The results showed significant differences (P≤0.05) among the categories of oocytes classified according to the layers of the germinal aggregate between the second category and the third category in the percentage of live oocytes with an average of (83.3±1.45), (70.4±4.08), and between the second category and the fourth category with an average of (83.3±1.45), (70.0±9.57), as for the dead oocytes, the results showed no significant differences (P≤0.05) between the first category and the second category and the third category and the fourth category classified according to the layers of the germinal aggregate.

Table (1) Effect of bacterial aggregate on morphological and biological characteristics of cow oocytes before in vitro maturation (mean ± standard error).

Cumulus	Mature oocytes%	Immature oocytes%	Atretic oocytes %	Viable oocytes%	Dead oocytes %
3-4 layers	0.98±7.5	75.3±1.19 a	16.6±0.97 a	83.3±1.45 a	16.6±1.45
2-3 layers	1.39 ±7.5	73.6±1.50 a	19.1±1.34 b	79.1±2.93 ab	19.5±2.90
1-2 layers	1.53±6.08	71.7±2.61 a	22.1±2.92 b	70.4±4.08 b	29.5±4.08

Denuded	3.49±5.95	60.6±5.78 b	34.5±9.40 b	70.0±9.57 b	30.0±9.57
Sig.	N. S	*	*	*	N. S

Table (2) shows that there was no significant effect of the interaction between age and bacterial aggregate on the percentages of mature oocytes before in vitro maturation, while there was a significant effect (P≤0.05) of the interaction between age and bacterial aggregate on the percentages of immature oocytes before in vitro maturation, as the first group classified according to bacterial aggregate layers of type A outperformed the fourth group for the first age and the fourth group type D for the second age with an average of (78.1±1.65), (66.6±6.66), (62.1±8.36) respectively, while there were significant differences (P≤0.05) for the interaction between age and germinal aggregate in the percentages of damaged oocytes before in vitro maturation. It decreased in the oocytes of the first category classified according to germinal aggregate layers A and the fourth category C for the second age was superior with an average of (27.9±1.33) over the other categories for the first and second ages with averages of (13.8±1.59), (20.0±1.61), (22.4±5.08), (26.5±66.1), (19.3±0.93), (17.9±2.27), (21.7±2.42).

The results of the current study indicated that there were significant differences (P≤0.05) for the interaction between age and germinal aggregate before in vitro maturation in the percentages of live oocytes, as the first group of type A was superior for the first age with an average of (86.6±3.33) and there were no significant differences between the first group and the second, third and fourth groups for the first age with an average of (82.6±20.67), (75.8±5.70), (71.6±4.18) and the first and second groups for the second age with an average of (84.0±2.04), (82.6±3.95).

The results of the current study showed that there were no significant differences in the interaction between the first age group and the bacterial aggregate in the percentages of dead oocytes before in vitro maturation, while the second age group and the fourth group D of the bacterial aggregate showed the highest significant difference (P≤0.05) with an average of (38.3±13.27), and significant differences (P≤0.05) appeared between the second age group and the fourth group of the bacterial aggregate with the first and second groups of the first age and the first and second groups of the second age with an average of (13.3±3.33), (17.3±20.67), (17.4±3.95), (15.9±2.04) respectively.

Table (2) Effect of age and bacterial accumulation on the morphological and vital characteristics of cow oocytes before in vitro maturation (mean ± standard error).

Age group	Cumulus cells	Immature oocytes%	Mature oocytes%	Atretic oocytes %	Viable oocytes%	Dead oocytes %
1	3-4 layers	7.38±1.49	78.1±1.65 a	13.8±1.59 c	86.6±3.33 a	13.3±3.33 c
	2-3 layers	8.16±2.10	71.7±1.92 abc	20.0±1.61 bc	82.6±2.06 ab	17.3±2.06 bc
	1-2 layers	7.63±2.12	69.9±4.47ab c	22.4±5.08 bc	75.8±5.70 abc	24.2±5.70 abc
	Denuded	6.76±6.61	66.6±6.66 bc	26.5±6.61 b	71.6±4.18 abc	21.7±4.29 abc
2	3-4 layers	7.73±1.32	27.6±1.59	19.3±0.93	84.0±2.04 a	15.9±2.04 c

			abc	bc		
	2-3 layers	6.76±1.74	75.9±2.34 ab	17.9±2.27 bc	82.6±3.95 ab	17.4±3.95 bc
	1-2 layers	4.23±2.16	74.0±2.21 ab	21.7±2.42 bc	64.0±5.41 bc	36.0±5.41 ab
	Denuded	4.44±10.0	62.1±8.36 c	27.9±1.33 a	61.6±1.32 c	38.3±1.32 a
Sig.		S. N	*	*	*	*

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