In vitro maturation of sheep oocytes using of Moringa Olivera leaf aqueous extract

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Abstract

This study was conducted in the Graduate Studies Laboratory/Department of Animal Production, College of Agriculture, Al-Muthanna University for the period from November 1, 2020 to August 1, 2021, with the aim of knowing the effects of adding Moringa Olivera leaf aqueous extract (MOLAE) to the culture medium for of laboratory maturation of sheep oocytes. The MOLAE was added in concentrations of 0% (control), 5% (T1), 10% (T2), to the culture medium of in vitro maturation (RBMI-1640) of sheep oocytes with and without the cumulus cells**.** It was observed a significant superiority (P≤ 0.05) in the percentage of mature oocytes in the T2(55.51± 1.69) as compared with the control (45.57± 2.67) and T1 (47.14±3.14). On the other hand, there were no significant differences (P≤ 0.05) between the percentages of immature oocytes among the three treatments (control, T1, T2). The result shows a significant effect (P≤0.05) of the interaction between the concentrations of MOLAE and the presence of cumulus cells to the percentage of IVM, a highest percentage was showed in the T2 (10% MOLAE) to the sheep oocytes with cumulus cells was 55.51± 1.69 whereas the lowest percentage was showed in the T1 (5% MOLAE) to the sheep oocytes without cumulus cells was 38.67± 2.98.

Keywords: oocytes, Moringa Olivera, aqueous extract

Introduction

Sheep are one of the most important agricultural animals as they provide basic food such as meat, wool, milk and other important products for humans (Trounson et al., 2005 and Fabjan et al., 2004). (Al-Athab and Abd al-Salam, 2008). The need for sheep production has risen in the world with the continuous increase in population numbers (Zhua et al. 2018). The decline in reproductive efficiency represents one of the causes of economic loss, so improving reproductive efficiency is a goal sought by those interested in animal production. To achieve this, researchers have developed a number of techniques called Assisted Reproductive Technologies (ART). These technologies aims to diagnosis of reproductive problems and increase the number of animal births. In view of the importance of the culture medium in the application of most of the aforementioned reproductive techniques, many researchers have been noticed that to find culture medium that have all the necessary elements for the growth and maintenance of the egg and sperm on the Both include adding different protein sources, hormones and sugars, and all of these aims to create environmental conditions similar to the environmental conditions inside the living body (Son et al., 2008)**.** Medicinal plants have been widely used to enhance or regulate female fertility. The effect of medicinal plants on female fertility is to stimulate the natural response of the pituitary gland to gonadotropin-releasing hormone (GnRH), as well as to improve the secretion of LH and FSH hormones,

release ovulation and increase the secretion of hormones. Steroids in ovarian cells, have effects such as estrogen and progesterone, and directly regulate ovarian function, at least in part, through cytokine secretion (Ushiroyama, 2003; Yasyi et al., 2003). One of this medicinal plant is the Moringa olivera tree , a plant of high value, found in many countries in the tropics and subtropics. Moringa was called the miracle tree for its nutritional, medical and industrial importance as well as its environmental importance (Bhupendra and Neikuozo, 2015). Moringa possesses many pharmaceutical properties as it possesses anti-inflammatory properties. It has anti-bacterial properties, viruses and fungi, as well as containing antioxidant compounds that protect the body from free radicals. It has been used in folk medicine in many countries to treat various diseases (El Sohaimy et al., 2015).Leaves are the most used part in plants because they contain high levels of minerals, proteins and carbohydrates, as well as contain biologically active compounds such as vitamins, carotenoids, polyphenols and alkaloids, in addition to flavonoids, glycosides and plant sterols (Ghosh et al., 2016) .

Material and methods

The current study was conducted in the postgraduate laboratory of the College of Agriculture, University of Muthanna, Department of Livestock, for the period from November 1, 2020 to August 1, 2021:

It included preparing plant extracts and preserving them, then collecting oocytes from the ovaries of slaughtered sheep from the Samawah abattoir , and transferring the ovaries immediumstely after slaughtering to the laboratory an subjected to the procedure of in vitro maturation using culture medium supplemented with Moringa leafs extract.

collection of ovaries

The ovaries were collected from ewes according to Wani and colleagues, (2000) immediately after slaughtering and transferred to the laboratory through one hour using plastic containers containing the physiological solution (0.9%)) reinforced with antibiotics (Penicillin 100IU\ML and Streptomycin100IU\ML) and placed in a preservative vial at a temperature of 37 °C. After being transferred to the laboratory, it was washed three times with warm physiological solution to get rid of From blood and impurities stuck to the ovaries (Rezk, 2009).

Oocyte collection

Sheep oocytes were collected from sheep ovaries by oocyte aspiration method, by withdrawing the vesicular fluid from the large and medium-size located on the surface of the ovary using a 20 mm syringe because the diameter of the needle affects the flow of oocytes through the withdrawal (Smith et al., 1994). And a medical syringe containing 0.5 ml of culture medium with 20 IU/mL of anticoagulant (Heparin) to prevent oocytes from sticking together. On the culture medium for the purpose of washing the oocytes and removing the remnants of cells stuck to them. The oocytes with irregular morphological shape and damaged Atratic oocytes, which were characterized as shrunken and the presence of a wide space between them and the zona pellucida or damage to this area, were removed (Desmedt et al., 1992).

Oocyte classification

The oocytes were isolated and classified according to their external appearance (after being collected and washed three times using the culture medium) as the mature oocytes were excluded, which were distinguished by the presence of the first polar body and (Atratic oocyte), which was characterized as shrunken and the presence of a wide space between them and the transparent area. As for the viability of the oocytes, after collecting and washing the oocytes three times by using Trypan blue dye, the oocytes that accepted the dye were diagnosed as dead and the oocytes that did not stain as live (Nogueira et al., 2009).

Preparation of the MOLAE

The moringa leaves were obtained directly from the tree, and they were washed and then dried under the sun shines with continuous stirring, individually. Continuous shaking by the shaking device and then filtered in two stages, the first stage by layers of medical gauze, then the second stage was filtered by filter paper and then placed in the incubator at a temperature of 37C˚ in order to evaporate the water and obtain a concentrated extract (Handa et al., 2008).

Preparation of culture medium for laboratory maturation

100 ml of RPMI 1640 standard culture medium was prepared by adding 5 g of RPMI 1640 to 100 ml of distilled water at a temperature of 37 °C and then placed in a shaker to mix the mixture. The mixture was well filtered and the mixture was re-filtered using a 22 mm filter. Then the PH was adjusted to a degree of 7. The solution was divided into 750 ml, which was kept at a temperature of 25 ° C and 250 ml were added to the hormones (Serum albumin 5%, 10 IU/ml hcg and PMSG 5 IU). /Ml and 1ug/Ml Estradiol).

laboratory maturation

The obtained oocytes were subjected to the laboratory maturation process, each group individually. The oocytes maturated in three culture mediums control treatment(0% MOLAE) , T1(10% MOLAE), and T2(15% MOLAE). The three mediums were formed from RBMI-1640 supplemented with hormones (Serum albumin 5%, 10 IU/ML hcg, PMSG 5 IU/Ml and 1ug). The immature oocytes ware placed in the Four Wall dishes according to the treatments, and covered by a layer of paraffin oil and incubated in CO2 incubator for 24 hours (5% CO2 , with a temperature of 38.5 and a relative humidity of 95%) according to Mrton and colleagues (2008).

Results and discussion

The effect of MOLAE on the percentage of in vitro maturation of sheep oocytes:

The results of in vitro maturation of sheep oocytes with cumulus cells were presented in the Figures (1) .It was observed a significant superiority ($P \leq 0.05$) in the percentage of mature oocytes in the T2(55.51± 1.69) as compared with the control (45.57± 2.67)and T1(47.14±3.14) .

On the other hand, there were no significant differences (P≤ 0.05) in the percentages of immature oocytes among the three treatments (control, T1, T2).whereas The results noticed a non-significant differences between the three treatments in the percentage of in vitro maturation of sheep oocytes without cumulus cells , figure (2).

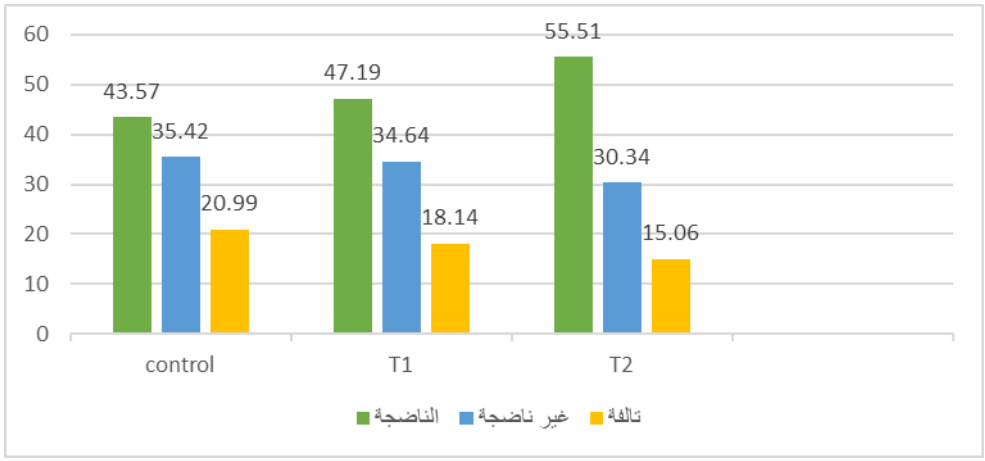

Figure (1) the effect of adding Moringa Olivera leaf aqueous extract (MOLAE) on the percentage of laboratory maturation of sheep oocytes with cumulus cells.

These plants showed their beneficial properties in folliculogenesis and steroidogenesis through their antioxidant properties and regulation of some steroids leads to improve the maturation of oocytes. (Telefo PB et al., 1998; Jha U et al. 2010).

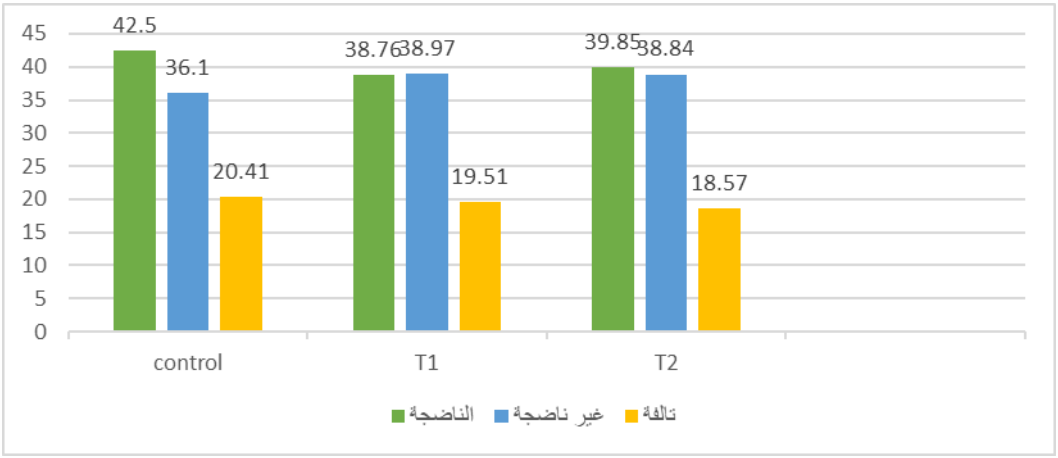

Figure (2) .The effect of adding MOLAE on the percentage of laboratory maturation of sheep oocytes without cumulus cells.

Granulocytes are directly or indirectly essential to oocyte development and dynamic oocyte survival (Juengel JL et al. 2002). Despite the importance of a critical amount of reactive oxygen species for physiological activities, the excess amount causes oxidative stress (Engel RH et al., 2006), and this causes damage to mitochondria and the cytoskeleton of the lipid membrane, as well as damage to amino acids and nucleoproteins (Gosta Rap et al. 2011). Therefore, it becomes necessary to use antioxidants, and one of these antioxidants is the medicinal plant Moringa. The prevention of oxidative stress is vital to maintain normal reproductive function. ATR actions can be either endogenous from gametes or by external

factors from the environment. However, if measures are not taken The task of reducing the production of reactive oxygen, both internal and external sources, will eventually lead to the development of oxidative stress, which will adversely affect the growth and maturation of oocyte.

The effect of interaction between the concentrations of MOLAE and the cumulus cells in the percentage of in vitro maturation of sheep oocytes.

The results in Table (1) showed that there was a significant effect ($P \leq 0.05$) of the interaction between the concentrations of MOLAE and the presence of cumulus cells to the percentage of IVM, a highest percentage was showed in the T2 (10% MOLAE)to the sheep oocytes with cumulus cells was 55.51± 1.69 whereas the lowest percentage was showed in the T1 (5% MOLAE)to the sheep oocytes without cumulus cells was 38.67± 2.98 , also the T2 (5% MOLAE) with cumulus cells was recorded a lowest percentage of immature oocytes 30.34±1.95 whereas the highest percentage of immature oocytes was showed in the T1 (5% MOLAE)to the sheep oocytes without cumulus cells was 38.97± 2.51.

compared to the oocytes that do not contain the cumulus, and this confirms the necessity of the cumulus in maturation due to the presence of terminal nutrient supplied with the cumulus, which have a crucial role in the maturation, fertilization and implantation processes (Paramio, 2010). According to the results of this study we can conclude that adding a concentration of 10% of MOLAE to the medium of in vitro maturation of sheep oocytes to improve the percentage of in vitro maturation of sheep oocytes and the preferences to the oocytes with cumulus cells .

Table (1) The effect of interaction between the concentrations of MOLAE and the cumulus cells in the percentage of in vitro maturation of sheep oocytes. (mean ± standard error).

Treatments	cumulus cells (CC)	Mature	Immature	Abnormal
Control	**CC$^+$**	± 2.6743.57 A b	2.42±35.42 ba B	±20.99 1.91 C a
	CC$^-$	3.63±42.50 A b	2.99 ± 36.10 ba B	1.65 ± 21.41 C a
T1	**CC$^+$**	3.14±47.14 A b	34.64 2.85± B ba	± 18.14 1.20 C a
	CC$^-$	2.98±38.67 A b	2.51 ± 38.97 A a	2.13 ± 19.31 C a
T4	**CC$^+$**	1.69 ±	1.95 ±	1.90 ±

		55.51 A a	30.34 B b	15.06 C a
	CC^{-}	2.03 ± 39.85 A b	3.06 ± 38.84 A a	2.97 ± 18.57 C b

Similar small letters in (one column) represent that there are no significant differences between mean below the significant level of 0.05. According to the (Duncan) polynomial test (Duncan, 1955), a test of CC + and CC- separately .The capital letters (in the same row) represent that there are significant differences between means below the level of significance 0.05 (between mature, immature and damaged oocytes).

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