Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 10 , Issue. 01. 2023
https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/10.1/15

The interaction between GDF9 gene polymorphism and age groups on the in vitro maturation of Arabi sheep Oocytes

Hamood Hatif Zamit and Hadi Awad Hassooni

Animal Production Department, Agriculture College, Al-Muthanna University, Iraq

Received on 25/05/2023 Accepted on 7/06/2023 Published on 15/6/2023

Abstract:

The study was conducted in the graduate laboratory of the Department of Animal Production, College of Agriculture, Al-Muthanna University, from 20/11/2022 to 22/3/2023, it included the collection of ovaries from the female reproductive organs of sheep slaughtered in the Samawa massacre, transferring them immediately after slaughter to the laboratory for the purpose of extracting Oocytes from the ovaries, subject to laboratory maturing using culture media for mature, Molecular investigations were completed at the Marshes Research Laboratory of Thi Qar University, for the purpose of isolation and purification of DNA, with the aim of finding genetic formations and the relationship between genotypes, morphological characteristics, and the ability to mature in the Arabi sheep Oocytes. Oocytes of Arabi sheep were collected by Oocyte aspiration method, then they were classified and examined for their vitality. The results indicated that there were no significant differences between animals carrying the three genotypes resulting from this mutation at position 205 of the studied segment of the GDF9 gene with damaged, immature and mature Oocytes, with no significant effect between the age groups of animals carrying the three genotypes resulting from this mutation at position 205 of the studied segment of the GDF9 gene with damaged, immature and mature Oocytes, there were significant differences in the percentage of mature Oocytes for in vitro maturation in the presence of germinal cumulus cells, the genotype AA of the second age group was superior to the ewes carrying the genotype GG

Keywords: GDF9 gene, polymorphism, age groups, in vitro maturation, Arabi sheep, Oocytes.

Introduction

Orabi sheep are considered one of the three main local breeds in Iraq, located in the southern part of the country, the youngest of the Awassi and Karadi dynasties (Abdelnour, 2011; Ibrahim *et al*., 2019). Arabi sheep are distinguished by different colors, such as brown or white with a black head or white. It is also distinguished by its ability to withstand high temperatures, especially in extremely hot desert areas and dry areas in Iraq and the Gulf (Herdmann *et al*., 2010; Mardenli *et al*., 2019). It is also considered one of the fast-growing sheep, as the weight of females is 4.0 kg at birth, and the average weight of a mature ewe reaches 38.3 kg, and the average body height in ewes is 70.2 cm. It is considered to have moderate reproductive efficiency, as the percentage of twins ranges between 2-8% (Mardenli *et al*., 2020). Female Arabi sheep are also characterized as being hornless, polled or hornless, they are animals with distinct characteristics, high weights, and a wide distribution that descended from very ancient strains that extended from the Arabian Peninsula to the mouth of the Red Sea. Arabi sheep are raised to produce milk, meat and wool, the wool crop is of economic importance because it provides income for sheep breeders in addition to the raw material, which are involved in the carpet and textile industries in the country (Nosratollah *et al*., 2012).

The in vitro maturation of oocytes is a scalable laboratory technology, in which immature Oocytes are isolated from the ovarian follicle, before being fully developed in vivo from the germinal vesicle stage, stage GV, to stage II, stage MII (Gracia and Woodruff, 2012). The in vitro maturation of mammalian oocytes is a major tool for basic research and in the science of assisted reproductive technologies (Smitz *et al*., 2011). In the process of IVM in vitro maturation, germinal cumulus cells play an important role, it transports nutrients directly from the follicle to the egg (Al Saadoon et al. 2014).

During the maturation of the Oocytes, nuclear and cytoplasmic changes occur to the egg, and the maturation ranges in the laboratory for ruminant Oocytes within 24 hours, many of the materials that contribute to this period are added to the culture media into two phases. The first takes 6 hours, during which a slight change occurs in the composition of the egg due to the influence of the cumulus cells, as for the second stage, which lasts 18 hours, and in which the components of the egg are rearranged, the basis for in vitro maturation of the ovum is the spontaneous resumption of division outside the ovarian follicle, mammalian oocyte maturation is defined as the occurrence of successive anaphase events, or the germinal follicle stage to completion of prophase II of meiosis after eruption of oocytes from the ovaries (Mohammed *et al.*, 2019; Saini *et al*., 2022).

In vitro maturation is still inferior to in vivo maturation (Kyasari et al., 2012), due to the difference between in vitro and in vivo conditions, in which

maturation occurs within the female reproductive tract and the ovarian follicle is protected in an ideal environment (Krisher, 2013). Several factors can influence the maturation efficiency of oocytes and are involved in the differences between the maturation process of Oocytes produced in vivo and the process of maturation of Oocytes produced in vitro (Abd El-Aziz *et al*., 2016). The in vitro environment cannot mimic the in vivo environment and leads to advanced stages in which only 30-40% of oocytes develop to important stages after maturation (Camargo *et al*., 2018).

The current study aims to demonstrate The interaction between GDF9 gene polymorphism and age groups on the in vitro maturation of Arabi sheep Oocytes.

Materials and Methods:

Experimental location:

The study was conducted in the Graduate Studies Laboratory, Department of Animal Production, College of Agriculture, Al-Muthanna University, from 20/11/2022 to 22/3/2023. It included the collection of ovaries from the female reproductive organs of sheep slaughtered in the Samawa massacre, transferring them immediately after slaughter to the laboratory for the purpose of extracting Oocytes from the ovaries, subject them to laboratory maturing using culture media for maturing, molecular investigations were completed at the Marshes Research Laboratory of Thi Qar University, for the purpose of isolation and purification of DNA, with the aim of finding genetic formations and the relationship between genes, morphological characteristics, and the ability to mature in the Arabi sheep Oocytes.

Oocytes collection:

Sheep oocytes were collected from the ovaries of medium and large follicles on the surface of the ovaries by the method of Oocyte aspiration, by withdrawing the follicular fluid with a medical syringe measuring 18 mm because the diameter of the needle affects the flow of Oocytes during the aspiration process containing 0.05 ml of the culture medium RBMI-1640, the Oocytes were placed in a Petri dish after retrieval, Oocytes collection was carried out under a microscope using a manually designed Pasteur pipette, the Oocytes were washed three times with RBMI-1640 culture medium through a Petri dish, for the purpose of removing the remnants of cells attached to the Oocytes, damaged and irregularly shaped Oocytes have been removed, which was characterized by being shrunken and having a wide space between it and the zona pellucide or damage to this area.

Oocytes Classification:

The Oocytes were classified after being isolated in a Petri dish and washed three times in the RBMI-1640 culture medium on the basis of their external appearance according to the method of Nogueira *et al*. (2009), containing layers of bacterial aggregates or not, and according to their being mature or immature, as

mature, damaged and abnormally shaped were excluded, as well as according to the homogeneity of the cytoplasm from the heterogeneity.

Ability test:

Examination of the Oocytes ability before their maturation, because it is necessary to know it, Oocytes were stained with tryban blue (0.05%), to find out the live Oocytes from the dead ones, the Oocytes that can be dyed tryban blue are classified, they were dark blue, as if they were dead Oocytes, as for the Oocytes that were not amenable to the tryban blue dye, they are considered alive (Abd-Allah, 2010: Al-Jubouri, 2019).

In vitro maturation of oocytes:

After washing the Oocytes three times with culture medium (RBMI-1640), fortified with some hormones IU/mL PMSG 5, hCG mL/10 IU and 1µg/ml Estradiol for the purpose of using it as a medium for in vitro maturing, then the Oocytes were transferred in a petri dish called four well divided into five sections, it lays evenly 4-6 Oocytes, in each well contain standard culture medium (RBMI-1640), then covered with paraffin oil, and put it in a CO2 incubator at 95% relative humidity and a temperature of 38.5°C for 24 hours, then the Oocytes were examined under a microscope to distinguish between mature and immature Oocytes.

Results and discussion

Polymorphic relationship of the studied segment of the GDF9 gene to in vitro maturation of Arabi sheep

The results of Table (1) showed that there were no significant differences between animals carrying the three genotypes resulting from this mutation at position 205 of the studied segment of the GDF9 gene, with each of the damaged Oocytes, immature Oocytes, and mature Oocytes. In the presence and absence of bacterial aggregates, as the results of the genotypes were GG, GA, AA. As follows in the case of the presence of bacterial aggregates, 12.11, 16.55 and 13.88 for damaged Oocytes. 15.59, 22.11 and 18.26 for immature Oocytes. 72.25, 61.33 and 67.84 for mature Oocytes. In the absence of bacterial aggregates 28.14, 23.97 and 25.75 for damaged Oocytes. 30.80, 33.97 and 36.17 for immature Oocytes. 41.05, 42.03 and 38.06 for mature oocytes, respectively.

Table (1) The relationship between the genotypes of the GDF9 gene (G<A) and the in vitro maturation of oocytes in the case of the presence and absence of germinal cumulus of Arabi sheep.

Genotype	Animal No.	Presence of germinal cumulus cells			Absence of germinal cumulus cells		
		damaged Oocytes	immature Oocytes	mature Oocytes	damaged Oocytes	immature Oocytes	mature Oocytes
GG	62	1.29±12.11	2.01±15.59	3.83±72.25	2.30±28.14	2.20±30.80	2.80±41.05
GA	8	2.70±16.55	3.52±22.11	5.02±61.33	2.92±23.97	4.80±33.97	5.93±42.03
AA	6	2.45±13.88	3.12±18.26	4.85±67.84	1.76±25.75	4.10±36.17	3.22±38.06
Sig.		N.S	N.S	N.S	N.S	N.S	N.S

Relationship of age groups on laboratory maturing of Arabi sheep:

The results of Table (2) showed that there were no significant differences between the age groups for the maturation of the Oocytes of Arabi sheep in the laboratory in each of the damaged Oocytes, immature Oocytes and mature Oocytes in the presence and absence of bacterial aggregates. The results of the first, second and third age groups were as follows. In the presence of bacterial aggregates, 16.17, 11.73 and 12.89 for damaged Oocytes. 20.50, 16.03 and 16.52 for immature Oocytes. 63.32, 72.20 and 70.51 for mature Oocytes. In the absence of bacterial aggregates 26.48, 26.55 and 27.25 for damaged Oocytes. 32.37, 36.28 and 29.16 for immature Oocytes. 41.12, 37.15 and 43.57 for mature oocytes, respectively.

Table (2) The relationship between age groups and in vitro maturation of Oocytes in the case of presence and absence of bacterial aggregates of Arabi sheep.

Age group	Animal No.	Presence of germinal cumulus cells			Absence of germinal cumulus cells		
		damaged Oocytes	immature Oocytes	mature Oocytes	damaged Oocytes	immature Oocytes	mature Oocytes
First	27	1.96±12.89	2.07±16.52	3.67±70.51	2.95±27.25	2.03±29.16	3.69±43.57
Second	25	1.54±11.73	3.20±16.03	4.07±72.20	2.48±26.55	3.55±36.28	3.06±37.15
Third	24	2.13±16.17	2.25±20.50	3.59±63.32	2.03±26.48	3.58±32.37	3.99±41.12
Sig.		N.S	N.S	N.S	N.S	N.S	N.S

The interaction between GDF9 gene polymorphism and age groups on the in vitro maturation of Arabi sheep Oocytes:

The study showed that there were significant differences ($0.05 \geq P$) in the percentage of damaged Oocytes and mature Oocytes in the presence of germinal cumulus cells between the genotypes GG, GA, AA resulting from the mutation at position 205 of the studied segment of the GDF9 gene. Animals with genotype GA for the third age group of damaged Oocytes in the presence of germinal cumulus cells outperformed animals with genotype AA GG and genotype GA for the first and second age groups. No significant differences were observed between genotype GG for the first and second age group, genotype GA for the first age group, and genotype AA for the second and third age group. Also, no significant differences were observed between the genotype GA for the first and third age groups, and between the genotype GA for the third age group and GG for the first and second age groups, and the genotype AA for the second and third age groups. The percentage of damaged Oocytes was in the presence of germinal cumulus cells, for genotypes AA, GA, and GG for the three categories 15.50, 10.58, 11.42, 24.16, 14.66, 10.83, 11.37, 12.50, and 18.12, respectively.

There were significant differences in the percentage of mature Oocytes for in vitro maturation in the presence of germinal cumulus cells ($P \leq 0.05$). The

genotype AA of the second age group was superior to the ewes carrying the genotype GG. No significant differences were observed between AA category II and (AA category I and III, GG, GA category I and II). GA class III genotypes (AA class I and III, GG, GA class I and II). The results were 67.78, 72.71, 74.87, 51.66, 60.66, 71.66 and 64.25, 78.00, 58.75 for the genotypes AA, GA, GG for the first, second and third age group, respectively. The study also showed that there were no significant differences for immature Oocytes on genotypes AA, GA, GG and age groups. The results were 24.16, 24.66, 17.50, 16.71, 16.70, 13.60, and 24.37, 9.50, 23.12, respectively.

Also, the percentage of in vitro maturation in the absence of germinal cumulus cells of genotype GG and age groups, first 44.25, 28.00 and 27.75. The second was 39.72, 32.04 and 28.22. Third 38.57, 32.85 and 28.57, for damaged oocytes, immature oocytes, and mature oocytes, respectively. As for the GA genotype and age groups, The first was 44.43, 31.93 and 23.60. The second was 30.83, 40.00 and 29.16. Third 50.83, 30.00 and 19.16, for damaged oocytes, immature oocytes, and mature oocytes, respectively. The AA genotype concentrations for the age groups were, first 41.25 , 30.00 and 28.75. The second 35.30, 43.40 and 21.30. The third was 38.32, 33.32 and 28.32, respectively.

Table (3) The interaction between GDF9 gene polymorphism and age groups on the in vitro maturation of Arabi sheep Oocytes.

Genotype	Age groups	Anim. No.	Presence of germinal cumulus cells			Absence of germinal cumulus cells		
			damaged Oocytes	immature Oocytes	mature Oocytes	damaged Oocytes	immature Oocytes	mature Oocytes
GG	First	26	1.69±11.42 b	1.67±13.60	2.76±74.87 ab	4.63±27.75	2.62±28.00	5.84±44.25
	Second	22	2.08±10.58 b	4.76±16.70	6.14±72.71 ab	3.92±28.22	4.11±32.04	3.58±39.72
	Third	14	3.11±15.50 ab	2.29±16.71	4.90±67.78 ab	3.03±28.57	5.43±32.85	5.53±38.57
GA	First	2	0.83±10.83 b	6.29±17.50	6.00±71.66 ab	6.04±23.60	3.67±31.93	5.56±44.43
	Second	3	5.17±14.66 ab	8.95±24.66	10.66±60.66 ab	5.46±29.16	8.66±40.00	9.61±30.83
	Third	3	3.63±24.16 a	3.63±24.16	7.26±51.66 b	3.34±19.16	12.66±30.00	±50.83 13.97
AA	First	2	7.31±18.12 ab	5.89±23.12	12.97±58.75 ab	3.75±28.75	6.77±30.00	5.90±41.25
	Second	2	2.61±12.50 b	2.14±9.50	3.72±78.00 a	1.64±21.30	8.72±43.40	7.21±35.30
	Third	2	2.10±11.37 b	5.65±24.37	6.30±64.25 ab	2.88±28.32	3.11±33.32	2.64±38.32
Sig.			0.05	N.S	0.05	N.S	N.S	N.S

References

Abd El-Aziz, A.H., Mahrous, U.E., Kamel, S.Z. and Sabek, A.A., (2016). Factors influencing in vitro production of bovine embryos: a review. Asian J of Animal and Veterinary Advances, 11(12) 737-756.

Abd-Allah; S.M. 2010. Effects of storage conditions of dromedary camel ovaries on the morphology, viability and development of antral follicular oocytes. Anim. Reprod. 7(2):PP:65-69.

Abdelnour, M.F. 2011. The most important sheep breeds in the Arab world and their types, physiological and reproductive characteristics. Dar Al-Hikmafor. printing, publishing and distribution, Second edition, Cairo, pp. 481.

Al-Jubouri, A.A.H. 2019. The use of different concentrations of soy milk in laboratory maturation of sheep Oocytes and freezing them using vitrification. Master's thesis, College of Agriculture - Al-Muthanna University.

Al-Saadoon, A.A. (2014). Effect of cryopreservation technique on oocytes and early stage embryos in sheep. Ph.D, Dissertation. Collage of Agriculture, University of Baghdad, Iraq.

Camargo, L.S.D.A., Viana, J.H.M., Sá, W.F.D., Ferreira, A.D.M., Ramos, A.D.A. and Vale Filho, V.R., (2018). Factors influencing in vitro embryo production. Animal Reproduction (AR), 3(1) 19-28.

Gracia, C. and Woodruff, T.K.(2012). Clinical Cases in Oncofertility. In Oncofertility Medical Practice, pp:203-223. Springer, New York, NY.

Herdmann, A., Nuernberg, K., Martin, J., Nuernberg, G. and Doran, O. (2010) . Effect of dietary fatty acids on expression of lipogenic enzymes and fatty acid profile in tissues of bulls. Animal, 4(5): 755-762.

Ibrahim, W.I., Hassooni, H.A., Alkhazraji, W.J. 2019. Association of β-lactoglobulin gene polymorphism with milk production and composition in local Awassi sheep. Plant Archives, 2019, 19: 284-288.

Krisher, R.L., (2013). In vivo and in vitro environmental effects on mammalian oocyte quality. Annu Rev Anim Biosci, 1(1), 393-417.

Mardenli, O., Mohammad, M.S., Hassooni, H.A. 2020. Efficiency of dimethyl sulphoxide and ethylene glycol on subsequent development of vitrified Awassi sheep embryos. Jurnal Ilmu Ternak dan Veterinerthis, 25(2): 60-67.

Mardenli, O., Saleh Mohammad, M., Hassooni, H.A., Aryan, A.-B.H. 2019. Effects of season, ewe age and their interactions on in vitro embryo production of Syrian Awassi sheep. Plant Archives, 2019, 19(1), pp. 1292-1298.

Mohammed AA, Al-Suwalegh S, Al-Shaheen T. (2019). Effects of follicular fluid components on oocyte maturation and embryo development in vivo and in vitro.

Adv Anim Vet. Sci.7 (5): 346-355.

Nogueira, D.; Romero, S.; Vanhoute, L. 2009. Oocyte in vitro maturation In: Gardner DK, Weissman A, Howles CM and Shoham Z. Editors. Textbook of Assisted Reproductive Technologies. Laboratory and Clinical Perspective. 3rd ed.. Informa healthcare UK:111-151.

Nosratollah, T., Mahnaz, S., Farhad, M., (2012). Evaluation certain environment factors on wool characteristics of Arabi sheep breeds Open J. Anim. Sci., 2(1): 11-14.

Saini S, Sharma V, Ansari S, Kumar A, Thakur A, Malik H, Kumar S, Malakar D (2022). Folate supplementation during oocyte maturation positively impacts the folate- methionine metabolism in pre-implantation embryos.Theriogenol. 182: 63-70.

Smitz, J.E., Thompson, J.G. and Gilchrist, R.B .2011.The promise of in vitro maturation in assisted reproduction and fertility preservation. In Seminars in reproductive medicine. 29, (1):024-037). Thieme Medical Publishers.