Evaluation of Ovarian Reserve by Using AMH Test before Collection the Oocytes for ICSI Technique

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Abstract

This study was conducted on goats with the purpose to evaluate the ovarian reserve before females submit to the ova retrieval for intracytoplasmic sperm injection (ICSI). The study was conducted at the college of veterinary medicine – the University of Baghdad and Middle East Labs - Baghdad (2019-2020). The study included thirty female goats (does) within the age of puberty and sexual maturity. The animals were divided into two groups: group I (< 4 years old), and group II (> 4 years old). A blood sample (6 ml) was collected from each animal through the jugular vein (before slaughter) in tubes containing anticoagulants and then sent to the laboratory for estimation of antimullerian hormone (AMH). The study showed significant increases in the level of AMH (4.02 ± 0.17) of the group I (< 4 years old) comparative with (2.22 ± 0.54) to the group II (> 4 years old) at the (<0.01), and this result relation with the number of oocytes obtained from ovarian samples of animals after slaughtering, it was found that the number and quality of oocytes increased in the group I (29 oocytes from a 15ovarian samples , compared with group II (16 oocytes from 15ovarian samples. This result explains the decrease in the ovarian reservoir of follicles with an increase in the age in the doe as a result of the decrease in the level of AMH in the serum, which is considered an indicator for knowing the ovarian reservoir from the follicles and determining the productive age of the ovaries. According to the results of this study, the decrease in the level of AMH in the serum is an indication of a decrease in the number of follicles on the surface of the ovaries, which negatively affects the fertility and productivity of the goats or may affect the fertilization rate and the number of fertilized embryos. The AMH test is considered one of the important tests that must be taken before oocyte collection to reduce the effort and costs involved in conducting any assisted reproduction technique that requires collecting the oocytes from the donor.

Keywords*: Reproductive, ovarian reserve, AMH, Oocytes, goat*

Introduction

Ovarian development of and primordial follicles occurs during the fetal period in several mammalian species including ruminants and humans, and in turn, each female is born with a fixed number of primordial follicles, which is considered as the ovarian reserve [1]. Given that there is no development of primordial follicles after birth, the size of ovarian reserve depletes as continuous reproductive cycles occur as a female age until a limited number of primordial follicles remains, at which time ovarian activity becomes irregular causing ovarian failure [2]. Furthermore, the size of the ovarian reserve is associated with oocyte quality [3], embryonic competence [4], and fertility [5].

Anti-Mullerian hormone (AMH), also known as Mullerian-inhibiting hormone (MIH), is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor-beta superfamily, whose key roles are in growth differentiation and folliculogenesis [6]. AMH is activated by SOX9 in the Sertoli cells of the male fetus [7]. Its expression inhibits the development of the female reproductive tract, or Mullerian ducts (paramesonephric ducts), in the male embryo, thereby arresting the development of fallopian tubes, uterus, and upper vagina [8]. AMH is also a product of granulosa cells of the preantral and small antral follicles in a female. As such, AMH is only present in the ovary until menopause in women [9].

Production of AMH regulates folliculogenesis by inhibiting the recruitment of follicles from the resting pool to select for the dominant follicle, after which the production of AMH diminishes [10]. As a product of the granulosa cells, which envelop each ovum and provide them energy, AMH can also serve as a molecular biomarker for the relative size of the ovarian reserve [11]. In bovine, AMH can be used for the selection of females in multi-ovulatory embryo transfer programs by predicting the number of antral follicles developed to ovulation [12]. AMH is expressed by granulosa cells of the ovary during the reproductive years and limits the formation of primary follicles by inhibiting excessive follicular recruitment by FSH [13].

AMH expression is greatest in the recruitment stage of folliculogenesis, in the preantral and small antral follicles. This expression diminishes as follicles develop and enter the selection stage, upon which FSH expression increases [14]. AMH is a predictor for ovarian response in vitro fertilization (IVF) and estimates a female's remaining ova supply [15]. This study should be confirmed in female goat (doe) aimed to determine the reproductive age and evaluation of ovarian reserve by estimating the blood level of AMH before the ICSI technique perform.

Materials and Methods

This study was conducted on goats with the purpose to evaluate the ovarian reserve before females submit to the ova retrieval for intracytoplasmic sperm injection (ICSI) in the goat. The study was conducted at the college of veterinary medicine – the University of Baghdad and Middle East Labs - Baghdad (2019-2020). The study included thirty female goats (does) within the age of puberty and sexual maturity. The animals were divided into two groups: group I (< 4 years old), and group II (> 4 years old). A blood sample (6 ml) was colleted from each animal through the jugular vein (before slaughter) in tubes containing anticoagulant and then sent to the laboratory for estimation of antimullerian hormone (AMH).

Blood sample and AMH assay

Blood samples were collected from the does before slaughter using venipuncture from the jugular vein. 30 blood samples were collected. Send to the lab. Blood samples were centrifuged for 10 min at 2000 × g within 2 h after collection. Serum was stored at 20 °C until hormonal assay.

Statistical Analysis

Data were collected, revised, coded, and entered into the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as numbers and percentages while quantitative data were presented as mean, standard deviations, and ranges when their distribution was found parametric. The comparison between two independent groups with quantitative data and parametric distribution was done by using an ***independent t-test.*** The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: [P> 0.05 = non-significant (NS), P < 0.05 = significant (S), P < 0.001 = highly significant (HS)] [16].

Results and Discussion

The study showed significant increases in the level of AMH (4.02 ± 0.17) of group I (< 4 years old) comparative with (2.22 ± 0.54) to the group II (> 4 years old) at the (<0.01), table (1), and this result relation with the number of oocytes obtained from ovarian samples of animals after slaughtering, it was found that the number and quality of oocytes increased in the group I (29 oocytes from 15 ovarian samples , compared with group II (16 oocytes from15ovarian samples , table (2). This result explains the decrease in the ovarian reservoir of follicles with increase the age in the doe as a result of the decrease in the level of AMH in the serum, which is considered an indicator for knowing the ovarian reservoir from the follicles and determining the productive age of the ovaries related to the association of AMH with age or parity seems contradictory in cattle [18] and compatible with [17] reported there were greater conception rates in primiparous than multiparous heifers and similar fertility. While with the study of [19] there was not any significant correlation between AMH and parity, in other studies concentrations of AMH in second and third lactation cattle were greater than in cows with their first and fourth or more lactations [5]. Nevertheless, AMH concentration has been reported to decrease with age in

humans [20] and mice [21]. According to the results of this study, the decrease in the level of AMH in the serum is an indication of a decrease in the number of follicles on the surface of the ovaries, which negatively affects the fertility and productivity of the goats or may affect the fertilization rate and the number of fertilized embryos. The AMH test is considered one of the important tests that must be taken before oocyte collection to reduce the effort and costs involved in conducting any assisted reproduction technique that requires collecting the oocytes from the donor. The AMH concentration is closely correlated with the size of the ovarian primordial follicle reserves [3] and [18].

Table 1: Comparison between Group I and Group II regarding antimullerian hormone (AMH)

AMH	Group I	Group II	Test value	P-value	Sig.
	No= 15	No= 15			
Mean ± SE	4.02 ± 0.17	2.22 ± 0.54	3.156	0.004	HS
Range	3.1 – 5.5	0.02 – 6.5			

A highly significant (HS) difference between the two groups for the AMH at P-value <0.01

Table 2: number and quality of collected oocytes from different ages of the donor does

Age of doe	Number of samples of reproductive systems	Number of collected oocytes			Total Number of oocytes
		G A	G B	G C	
(< 4 years old)	15	11	8	10	29
(> 4 years old)	15	5	3	8	16

Conclusion

According to the results of this study, it is concluded that the application of the protocols of any to assisted reproductive technologies in mammals must take into interpretation the type of animal used, age, and reproductive status. Therefore, the AMH test is one of the most important tests that indicate the ovarian reservoir of follicles and its effectiveness, which saves time and materials in the process of recovery the ova in quantity and quality, which will positively affect the success of the technique used to assist reproduction. Obtaining a larger number of ova increases the chance of fertilization, and thus increases the rates of pregnancy and reproduction.

Conflict of Interest: None

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Ethical Clearance: Not required

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