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ANTI-MÜLLERIAN HORMONE SERUM CONCENTRATION IN PREPUBERTAL EWE LAMBS AS A MARKER OF THEIR FECUNDITY

By

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ABSTRACT

Anti-Müllerian Hormone (AMH) was considered to be one of the most endocrine markers that playing a major role in the prediction of the most future productive animals.

The objective of this study was to predict the fertility of young ewe lambs through a single estimation of AMH in their early breeding life. Twenty clinically healthy Barki ewe lambs, 8-10 months age. The ewes were shown to have normal cyclic activity before the beginning of the experiment. The body weight was ranged from 20-30 kg. The current work was conducted on Animal Reproduction Research Institute-Sheep farm. Inspected ewes were still under observation with collection of the required samples during the period of the study.

Trans rectal Ultrasonography was used for following up the progress of pregnancy and number of fetuses. Assay of AMH, progesterone and estradiol, in the conceived and control animals were determined till the parturition (end of the study). There was significant ($P<0.05$) increase in the serum AMH concentration in group conceived from first service. Meanwhile, a significant increased ($P<0.05$) of estradiol level in the group conceived from second service. In the same time, there was no significant variation in progesterone level between the two groups. In conclusion, serum AMH concentration is a good predictive biomarker for fertility in ewe lambs at the beginning of their breeding life.

Key words:

AMH, Progesterone, 17-β estradiol, Number of service, Pregnancy, Ewe lambs, Reproductive performance.

INTRODUCTION

As the previously described role of AMH as an indicator for successful ewe fertilization **(Lahoz *et al.,* 2012)**. AMH was found in fish, reptiles, birds, and mammals. In mammals, it was present in both males and females. Anti-Müllerian hormone could potentially had an

association with fertility characteristics such as puberty, pregnancy, conception rate, and fecundity. As the population of the world increased and the demand for food protein increases, the need for producers to increase production was extremely important.

Veiga-Lopez *et al.*, (2012) reported that granulosa cells of preantral and small antral follicles considered to be the main source of AMH in sheep. These stages of follicle development were gonadotropin-responsive. They added that small antral follicles expressing high levels of AMH was increased by FSH treatment. Moreover, **Kern *et al.*, (2010)** mentioned that ovine was born with a variable number of ovarian germ cells; moreover, there was an indirect relation between the number of these cells and age**.** Ability of the ewe for early successful lambing reflects high economic benefit on the producing farm reduce the generation interval and the culling risk. In addition, **Lahoz *et al*., (2012)** sated that AMH was present in prepubertal ewe lambs and can be used as a factor help the ewe producers in making accurate decision when selecting for replacement expected fertility at the first couple. AMH was estimated in plasma of prepubertal ewe lambs. Moreover, there was a direct relation between it is concentration and the occurrence of ovulation in ewe lambs after treatment with an ovarian stimulant **(Petrovic *et al*., 2012).** AMH expression begins in the granulosa cells of primary follicles and is highest in preantral and small antral follicles **(Estienne *et al.*, 2015).**

The aim of the present study was undertaken to determine the relationship between AMH serum concentration in Barki prepubertal ewe lambs and fertility.

MATERIAL AND METHODS

The current work was conducted on Animal Reproduction Research Institute-Sheep farm Giza Governorate. All ewes used for the study were housed under natural lighting condition as one flock. Twenty clinically healthy Barki ewe lambs, 8-10 months of agewere used.

The ewes were shown to have normal cyclic activity before the beginning of the experiment. The body weight of the experimental ewe lambs was ranged from 20-30kg. The animals were divided in to two groups each of ten. All ewes were kept under observation for two weeks prior to the experiment to be sure that they were free from any infectious disease and were given prophylactic treatment against internal and external parasites. Then synchronization of estrous cycles for all ewes were done using intra vaginal sponge saturated with medroxy progesterone acetate (Veramix, Upjohn L.t.d., Crawley, Sussex, and UK). Ewe lambs were treated by 250 IU I/M injection of pregnant mare serum gonadotropin (eCG) after 13 days

from synchronization **Swelum *et al.,* (2015).** After the ewe lambs had exhibited estrus, two intact mature rams were introduced to the flock to allow natural mating only for 30 min in each time. Pregnancy were diagnosed and fetal numbers were determined by mean of trans rectal sonar or Ultrasonography. Each lamb had weighed, and fertility data were collected throughout the study. According to the number of service per conception, experimental ewe lambs were classified into 2 groups.

Group I, ewe lambs were conceived from the first service and group II, ewe lambs were conceived after the second service and the rest of animals that not conceived after the second service were excluded from the experiment. During pregnancy, all the pregnant ewe lambs provided by a balanced ration, (requirements for maintenance and for pregnancy), and water was offered ad libitum according to **NRC (2001)**. After ten weeks of pregnancy, the animals allotted by age, weight, and number of foeti in the yards until the day of parturition.

All pregnant ewes were kept under observation throughout the gestation period to record any clinical manifestation occurred during the experiment. Follow up groups of sheep on the reproductive performance, hormonal changes were done from fertilization till successful lambing. Blood samples were collected in sterilized plane tubes from jugular vein from each animal in October month once before the study. Then Ultrasound was used for detecting pregnancy and number of fetuses on day 40 post mating.

Blood samples were collected from jugular vein from all animals before the study, then at time of introducing ram. Samples were immediately placed on ice and later centrifuged at 3000 rpm for 15 min. for plasma separation and samples were kept frozen at -20 °C until determination of Anti-Müllerian Hormone (AMH), progesterone and 17- β estradiol levels.

Measured traits throughout the experimental period:

Fertility was monitored **(Ozyurtlz *et al.,* 2011)**.

Pregnancy rate = number of pregnant ewes / numbers of mated ewes x 100.

Twinning rate = number of pregnant ewes bearing twins/number of mated ewes x 100.

Lambing rate = number of lambing ewes / numbers of mated ewes x 100.

Conception rate = number of conceived ewes / numbers of mated ewes x 100.

Prolificacy= number of lambs born (alive) / number of ewes mated x 100.

Fecundity rate =number of lambs born (alive) / number of ewes conceived x 100.

Hormonal assays:

Serum AMH, progesterone and 17-beta estradiol were assayed by ELISA technique using commercial diagnostic kits (Nova Tec. Immudiagnostica GmbH, Waldstrafle 23 A6, D-63128 Dietzenbach, Germany).

Statistical analysis:

All data were subjected to the statistical analysis according to **Sendecor and Cochran (1989).** Student "t" test and least significant differences were carried out to test differences between treatments using a computer program "COSTAT".

RESULTS

Primary investigation of the level of AMH in sera of inspected animals at zero day of the study aged (8-10 months) ranged between 32.0±0.06–38.07±0.05 (pg/ml). These data proved that there was no significant difference between the level of AMH in different body weight and age.

In the first group nine ewe lambs were conceived and eight ewe lambs were conceiving from the second by using Ultrasonography on day 40 post mating.

In the same time, there was highly significant increased in the concentration of AMH in the 1st group of ewe lambs which conceived and became pregnant from the first insemination (135±1.02 pg/ml) than those in the second group (49±1.05 pg/ml) as illustrated in (Table 1). There was no significant difference in the level of progesterone hormone between the two experimental groups.Meanwhile, estradiol 17-β hormone showed a highly significant increase in the serum level (52.70 ±1.28 pg/ml) of group II than group I (21.49 ±0.68 pg/ml) (Table 2).

Table (1): Serum concentrations of anti-Müllerian hormone (AMH pg/ml), progesterone (ng/ml) and 17-β estradiol (pg/ml) in ewe lambs in 1st insemination (Group I) and 2nd insemination (Group II) (mean ± SE).

Parameters	Group I (n=9)	Group II (n=8)
AMH(pg/ml)	135±1.02[a]	49±1.05[b]
Progesterone (ng/ml)	14.91±1.63[a]	19.72±0.94 [a]
17-β Estradiol (pg/ml)	21.49 ±0.68 [a]	52.70 ±1.28[b]

Means with different superscripts differ significantly: pa, b at P<0.05 in the same raws.

The fertility and lambing rate were significant increase in group I than those in groups II (90.00% vs 80.00%) as mentioned in (Table 2). The single lambing rate was significantly higher in group II than that in the group I (87.50% vs 33. 33%) The twinning rate was significantly higher in group I then that in the group II (66.66% vs 12.50 %). Also the prolificacy rate was significantly higher in group I than that in the group II (150.00% vs 90. 00%) and the fecundity was high significant in group I than that in the group II (166.66 % vs 112%).

Table (2): Reproductive efficiency in ewe lambs (mean ± SE).

Parameters	Group I (n=9)	Group II (n=8)
Fertility (%)	**9/10 (90.00%)[a]**	**8/10 (80.00%)[b]**
Lambing rate	**9/10 (90 %)[a]**	**8/10 (80%)[b]**
Total no of lambs born	**15[a]**	**9 [b]**
Single lambing	**3/9 (33.33%)[a]**	**7/8 (87.50%)[b]**
twin lambing	**6/9 (66.66%)[a]**	**1/8 (12.50%) [b]**
Prolificacy	**15/10 (150.00%) [a]**	**9/10 (90.00%)[b]**
Fecundity	**15/9 (166.66%) [a]**	**9/8 (112%) [b]**

Means with different superscripts differ significantly: a, b at P <0.05 in the same raws.

DISCUSSION

A positive association between AMH and fertility in ewe lambs were recorded by **Lahoz *et al.*, (2012) and Jimmenez- krassel *et al.*, (2015)**. The current results showed that, the age (8-10 months) and weight (20-30 kg) of ewe lambs did not affect the AMH concentration. These finding was agreed with that recorded in ewe lambs **(Joshua Austin 2015; Vissser *et al.*, 2006) and Bartlewski *et al.*, (2002).** The results showed that prepubertal serum AMH concentration was significantly higher in ewe lambs in group I than those in group II which confirmed that AMH was correlated more intensely with early antral follicular counts.

Serum AMH concentration used as a predictor of the fertility at first mating before puberty. Farmers can perform a precocious selection of replacement ewe lambs with the highest expected fertility at first mating by this test **(Lahoz *et al.*, 2012)**. The current results agreed with **(Visser *et al.*, 2012)**. AMH concentrations are maintained relatively constant by the production of preantral and small antral follicles which may have paracrine actions in

preovulatory follicles. In addition, cumulus cells of preovulatory follicles still express AMH **(Monniaux *et al.*, 2013).** The ovaries respond to gonadotropins with both functional and structural changes, when exposed to gonadotropic stimulation, certain follicles increase in size and secrete sufficient amounts of estradiol and increasing estradiol feeds back to the brain triggering estrus. Estradiol concentrations signal the brain that, the mature follicles are present. Mature follicles are 0.4 to 0.7 cm in size when estradiol reaches the peak in sheep reproduction. At peak estradiol production,Gonadotropin-releasing hormone (GnRH) is released causing a massive release of LH (LH surge) and initiates the release of mature ova **(Riberio *et al.*, 2014; Inskeep, 2002)**. The ovarian reserve is the size of the ovarian follicle pool and the number of oocytes with-in those follicles. The ovarian reserve declines with age and results in the decrease of a female's reproductive function **(Visser *et al.*, 2006)**. **Lahoz *et al.*, (2012)** recorded that correlation between AMH and fertility later in life when measured AMH in sheep during the prepubertal period.

Ovulatory response to gonadotropin hormone and plasma AMH concentrations could be explained by the truth that they likely both mirror to the presence of antral gonadotropin-responsive follicles in the ovaries of premature ewes. **(Rico *et al.*, 2009)**.

Successful pregnancy was affected by age, body weight, nutritional status, season and endocrine status or uterine conditions **(Visser *et al.*, 2012)**. Healthy ewe lambs were reared under the same conditions to minimize the impact of such factors, although possible unknown individual disorders cannot be discarded. The results illustrated that ewes with higher prepubertal plasma AMH concentrations displayed a higher chance of becoming pregnant in group I than those with lower plasma AMH concentrations in group II. As far as we know, this is the first time that a relationship between AMH and productive performance has been established in a domestic species. The present study reinforces the prognostic value of AMH, even when the physiological status is not fully known. The phase of the ovarian cycle was almost independent on AMH levels, explaining why a single AMH measurement is usually sufficient **(Visser *et al.*, 2012)**. The AMH values would be a mirror of the beginning of follicular growth showing an ovarian activity different between animals of the same age in premature animals, it was probable that ewe lambs in our study with the highest ovarian activity at 8-10 months were also the earliest puberty, resulting in the better fertility observed when testing reproductive performance of different ewes at the same age. The lambs with the precocious follicular development would be fertile at an early age. An increase serum AMH

concentrations were supposed to have high number of healthy follicles in premature ewe lambs. AMH could be used as a screening test of fertility in the premature ewes at first mating. The establishment and maintenance of pregnancy depend upon several genetic and environmental factors. A good predictor of the ovarian response in the human is increased of AMH levels before IVF treatments, it has not confirmed to be as expect the successful pregnancy. **(Grynnerup *et al.,* 2012)**. It is stay to be assessed at which age and weight the diagnostic test based on the measurement of AMH concentrations is the most efficient for accurate prognostication. The reproductive performance of premature ewes at first and second service were correlated wAith their AMH concentrations in the prepubertal phase, increasing the premating weight and age of ewes could increase the pregnancy rate and multiple births. Confirmed with that **(Aliyari *et al.,* 2012; Akta¸s *et al.,* 2015)**. Highly prolific sheep herds are a very important subject in Lamb outlive **(Kott, 2014).** The survival rate (SR) of the lambs is affecting with LW, age of ewes was reported by **(Casellas *et al.,* (2007) and Kott, (2014)**. Increasing the premating weight of ewes through improved feeding strategies performed by a better reproductive performance and high profitability,mainly important in first lambing ewes that are produced under miserable nutritional conditions **(Akta¸s *et al.,* 2015).**

CONCLUSIONS

AMH concentration had a positive correlation with high fecundity and ovary function after synchronized estrus at first mating premature ewes. Furthermore, predict high reproductive performance of ewes.

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