

# The Influence of Age, Gender and Weight on Sex Hormone Levels and Histological Development of Reproductive Organs in Arabi Sheep

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## ABSTRACT

This study was conducted in the College of Agriculture - University of Basra from 1/9/2020 to 15/1/2021 by collecting 120 blood samples from males and females of the Arabi sheep breed, as well as taking samples from reproductive organs (testes and ovaries) to evaluate the effect of age, weight and sex of animals on sexual hormone levels. Samples were divided into three age groups (5-8 months, 9-12 months and 1-1.5 years) and three body weight groups (15-25, 26-36, 37-47) kg. The results showed a significant ( $P < 0.05$ ) increase in the concentration of gonadotropin hormones (FSH, LH) and steroidal hormones (estrogen, progesterone and testosterone) in animals older than 9 months. The concentration of FSH and LH hormones were higher females compared to males. All levels of sex hormones significantly ( $P < 0.05$ ) increased when the body weight increased to more than 26 kg in both sexes. Reproductive organs in both sexes (testes and ovaries) developed when the animal age and weight increased.

**KEYWORDS:** Arabi sheep; sexual hormones; reproductive organs; age; weight.

## الخلاصة

أجريت هذه الدراسة في كلية الزراعة - جامعة البصرة في الفترة من 2020/9/1 إلى 2021/1/15 من خلال جمع 120 عينة دم من ذكور وإناث سلالة الاغنام العراقية ، وكذلك أخذ عينات من الأعضاء التناسلية (الخصيتين والمبيضين) لتقييم تأثير عمر الحيوانات ووزنها وجنسها في مستويات الهرمونات الجنسية. قسمت العينات إلى ثلاث فئات عمرية (5-8 أشهر ، 9-12 شهراً و1-1.5 سنة) وثلاث مجاميع لوزن الجسم (15-25 ، 26-36 ، 37-47) كغم. أظهرت النتائج زيادة معنوية ( $P > 0.05$ ) في تركيز هرمونات مغذيات الغدد التناسلية (FSH ، LH) والهرمونات الستيرويدية (الإستروجين والبروجستيرون والتستوستيرون) في الحيوانات الأكبر من 9 أشهر. كان تركيز هرمونات FSH و LH أعلى عند الإناث مقارنة بالذكور. زادت جميع مستويات الهرمونات الجنسية معنويًا ( $P > 0.05$ ) عندما زاد وزن الجسم إلى أكثر من 26 كجم في كلا الجنسين. تطورت الأعضاء التناسلية في كلا الجنسين (الخصيتين والمبايض) مع زيادة عمر الحيوان ووزنه.

## INTRODUCTION

The Arabi sheep is one of the three main local breeds in Iraq, it is located in the southern portion of the country, and it is smaller than the Al-Karadi and Al-Awassi breeds [1]. The age of sexual puberty in sheep differs between males and females, depending on the age, weight, season, critical body weight, development of the reproductive organs (testes, ovaries) and increase the effectiveness of the endocrine gland secretions [2,3]. Sex hormones play an efficient role in the completion of the sexual process in male and female sheep [4]. In addition, Biological and physiological processes in the animal body affect by many factors such as energy intake, season, sex, age, temperature, productive and hormonal

secretions [5,6]. [4] indicated in his study on Belclare ewes that the rate of ovulation increases high weight females This research group suggested that the animal weight plays an important role in improving the endocrine secretion of sex hormones and a positive reflection on the development of the reproductive activity in females. Most sex hormones, including testosterone hormone, affected by growth, age, sex, weight and physiological status in buffalo calves [7]. [8] reported that it is necessary to study the histological development of the animal reproductive organs to explain the relationship between the growth of these organs and the secretion of sex hormones. Therefore, the objective of the present study is to discover the effect of age, sex and weight on sex hormones and reproductive tissue development in

the Arabi sheep breed.

## MATERIALS AND METHODS

This study was conducted in Basra from 1/9/2020 to 15/1/2021 by collecting tissue samples from slaughterhouses and one hundred twenty blood samples from males and female Arabi sheep. Blood samples were classified into the following age (5-8 months, 9-12 months, 1-1.5 years) and weight (15-25, 26-36, 37-47) kg. Blood samples (8 ml) were taken from the jugular vein using sterile medical syringes and placed into sterile plastic test tubes without anticoagulant and left for 30 mins to clot. Tubes were centrifuged at 3000 rpm for 5 mins to separate the serum. The serum was kept at (-20 °C) until the time of analysis.

Levels of follicular stimulation hormone (FSH), luteinizing hormone (LH), estrogen, progesterone and testosterone hormones were determined using a procedure associated with MonobindInc - USA Company kit.

Twelve testicle and ovarian samples were collected from animals slaughtered in slaughterhouses in the province of Basra. The specimens were in the weight and age groups studied. Three samples were taken from each individual organ studied by cutting them with a special medical scalpel. Samples were placed in 50 ml plastic containers including 10% formalin, kept for 72 hrs and transported to the pathology laboratory of Veterinary Medicine College / University of Basra to study the histological development of the reproductive organs. Tissue cutting steps were carried out according to [9] method using Microtome /china. YIDI-1195.

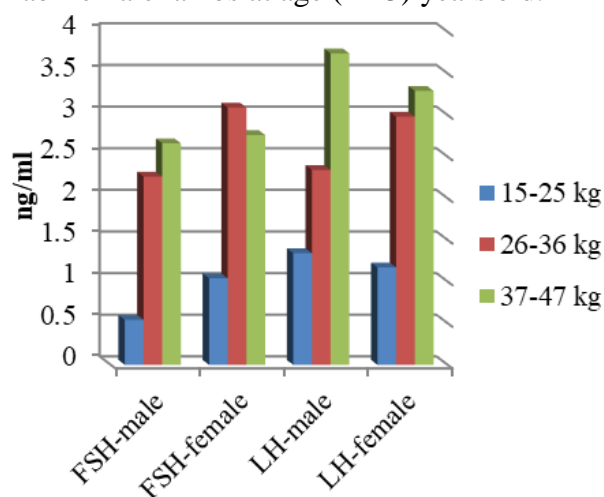
Data were analyzed using the Statistical Readiness Program [10] with the effect of age and weight in sex. Statistically significant differences were determined by significance levels ( $P < 0.05$ ) using the following general statistical model (1):

$$Y_{ij} = \mu + T_i + S_j: T_i + e_{ij} \quad (1)$$

## RESULTS AND DISCUSSION

**Gonadotropin-stimulating hormones (FSH, LH)**  
Significant differences ( $P < 0.05$ ) were observed between males and females Arabi sheep, because there was a significant rise in the FSH and LH hormones concentration when the animal age increased in both sexes (Figure 1). The concentrations of the two hormones in females were (1.02, 3.29, 2.76) and (1.18, 3.16, 3.09) ng/ml

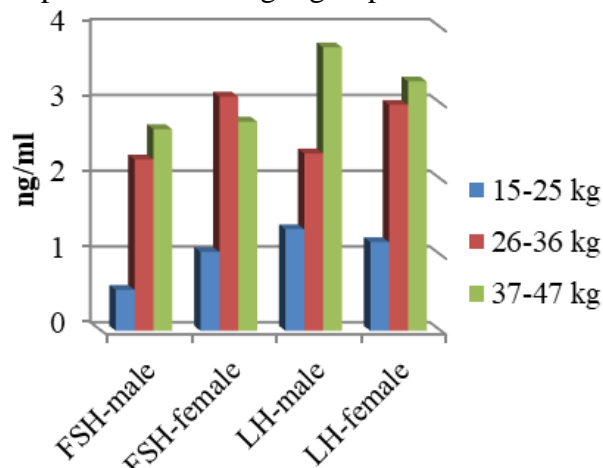
for the three age groups (5-8 months, 9-12 months, 1-1.5 years), respectively. For males, the concentration means of the two hormones were (0.55, 2.36, 1.68) and (0.35, 1.43, 2.61) ng/ml for all three age groups, respectively. These hormones were significantly higher in females than in males of all age groups. The rise in LH and FSH concentrations with the increased animal age is due to the complete growth and development of endocrine glands and gonadotropin axis (hypothalamus - pituitary - ovaries/ testes). This was reflected on an increased efficiency of their activity in the final result [11]. The parallel increase in hormones concentration and animal age might be also due to the high metabolism in the animal body at early ages, which contributes to the gradual increase of the body ability to send nerve impulses to the endocrine nervous system in later ages (more than 9 months). Furthermore, to stimulate the start of hormones secretion (GnRH) from the hypothalamus gland which leads it to an increase in secretion of the two hormones from the anterior lobe of the pituitary gland [12]. This result agreed with [13] who recorded a significant increase in the concentration of two hormones (LH, estrogen) in Arabi female lambs at age (1 - 3) years old.



**Figure 1.** The effect of age and sex on FSH and LH hormone levels in male and female Arabi sheep.

Heavy animals (males and females) showed a significant increase ( $P < 0.05$ ) in LH and FSH hormone levels compared to light animals (Figure 2). No significant differences were observed in the concentration of LH and FSH among males and females for all weight groups. [14] reported that the body weight increase leads to an increase in the weight and activity of gonadotropins glands, which positively has an influence on the rise of sex hormones secretion. This result was similar to that

found by [15, 16, 17] in their studies on goats and sheep at different weight groups.



**Figure 2.** The effect of weight and sex on FSH and LH hormone levels in male and female Arabi sheep.

### Steroid hormones (Estrogen, Progesterone, Testosterone)

All levels of steroid hormones in both males and females significantly increased with age development Table 1. The concentration of testosterone hormone elevated from 1.04 to 2.32

ng/ml when the animal's age increased from 5 months to a year and a half, this result agreed with [18] who indicated that the level of testosterone hormone in bulls is affected by age, breed, environmental factors and season. Furthermore, when the age of the animal increased from 5 months to a year and half, the concentration of progesterone and estrogen increased from 0.34 to 2.85 ng/ml and from 34.6 to 49.01 pg/ml, respectively. The reason for the rise of progesterone and estrogen levels with the advancing age in females may be due to the development and growth of ovaries, formation of mature follicles on a continuous basis and the increase of the corpus luteum activity in sexually mature animals [19]. These findings are consistent with [20, 21, 22].

Table 2 shows the effect of weight on the concentration of steroidal sex hormones, it was observed that all of these hormones significantly ( $P < 0.05$ ) increased with the increase of animal weight. In males, the concentration of testosterone increased from 0.99 to 2.41 ng/ml.

**Table 1.** The effect of age on steroidal hormones in male and female Arabi sheep.

Age group Hormones	5-8 months	9-12 months	1-1.5 years	Sig. level
Testosterone (ng / ml)	1.04 ± 0.3b	2.81 ± 0.7a	2.32 ± 0.4a	*
Progesterone (ng / ml)	0.34 ± 0.2c	1.40 ± 0.3b	2.85 ± 0.5a	*
Estrogen (Pg / ml)	34.6 ± 0.8b	50.5 ± 0.5a	49.01 ± 0.3a	*

Small letters in the row indicate the significant differences ( $P < 0.05$ ) between age groups. (Mean ±S.E.).

While the concentration of progesterone and estrogen increased from 0.66 to 2.67 ng /ml and from 34.1 to 49.6 pg/ml in females when the animal weight ranged from 15 to 47 kg, respectively. These results were in agreement with [23] which reported a significant increase in testosterone level

in the blood of heavy buffalo males compared to the lightest animals. Moreover, [17] suggested that the increase in sex hormones including estrogen, simultaneously improves with the improvement of body condition, which mainly depends on the female weight.

**Table 2.** The effect of weight on steroid hormones in male and female Arabi sheep.

Weight group(kg) Hormones	15-25	26-36	37-47	Sig. level
Testosterone (ng / ml)	0.99 ± 0.3 b	2.71 ± 0.7a	2.41 ± 0.4a	*
Progesterone (ng / ml)	0.66 ± 0.2b	1.04 ± 0.3b	2.67 ± 0.6a	*
Estrogen (Pg / ml)	34.1 ± 0.8b	49.5 ± 0.5a	49.6 ± 0.4a	*

Small letters in the row indicate the significant differences ( $P < 0.05$ ) between age groups. (Mean ±S.E.).

## HISTOLOGICAL STUDY

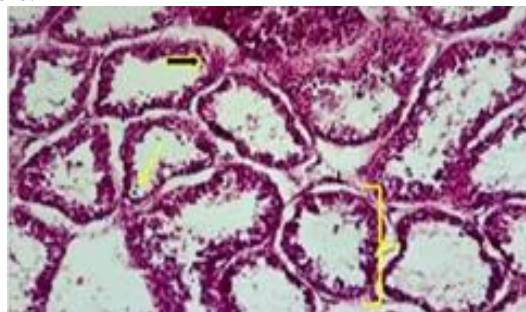
### Testis

Picture 1 indicated the histological section of testicles at age (7) months and weight (18) kg. The thick fibrous lobule surrounded by thick fibrous of epididymal tissue, the epididymal tubules were small, without sperm, undeveloped and a very weak proliferation of spermatogonia cells occurred. Furthermore, the testicular interstitial tissue, Leydig cells and testicular cords were small. The histologic section of male lamb testicles at the age of 10 months and the weight of 29 kg was shown in Picture 2. There was a simple development in spermatogenesis and some spermatogonial cells, which appeared to be attached to the membrane of seminiferous tube. The number of primary and secondary spermatocytes in the interstitial tissue was small, whereas there were many Leydig cells. Picture 3 indicated the histological section of a mature ram testis which was 14 months of age at weight of 40 kg. It was observed that the seminal tube was more effective during the process of sperm formation, the number of primary and secondary spermatogonial cells was high a lot, sperms accumulated inside the spermatic tubule and Sertoli cells appeared more clearly. The reason for the growth and development of testicles with animal age and weight was due to the increase of sex hormones production (testosterone) and its direct impact on the function of testicles [24]. [25] Al-Tarabany *et al.*, (2015) reported another reason in their study on Egyptian sheep. As They found that the increase in body weight associated with an increased secretion of metabolic hormones, such as the growth hormone, which has a direct role in the growth of body organs, including testes. [26] reported a positive relationship between testicular growth and body weight.

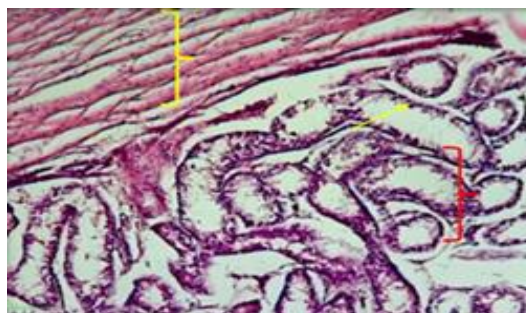
### The ovary

A histological section of the ovary of an 8-month-old female lamb with a weight of 19 kg was shown in Picture 4. The germinal epithelium, the tunica albuginea layer, follicles and fibroblast cells in the cortex tissue seemed completely immature. Furthermore, the ovum in the ovary cortex was small and undeveloped. Picture 5 shows the histological section of a 10-month-old female with a weight of 28 kg. The presence of the mucous membrane of the ovary, a large number of ova in the ovary cortex, and pre-basic germ cells were noted. Finally, Picture 6 shows the histological section of the ovary of a 13-month-old mature ewe weighing 40 kg. It

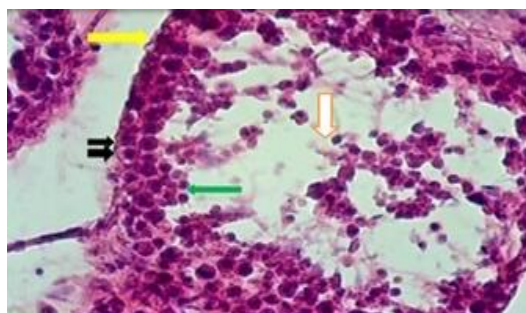
was observed that there was a primary follicle surrounded by theca interna and theca externa cells with a mature graafian follicles. The ovum surrounded by corona radiates and contained the follicular fluid and the corpus luteum in the cortex area. [27, 28] suggested that the development of the ovary structure correlated with the age of sheep. [29] reported that the reach to the complete stage of ovary development and the growth of its tissues clearly depends on the age increase of female camels.



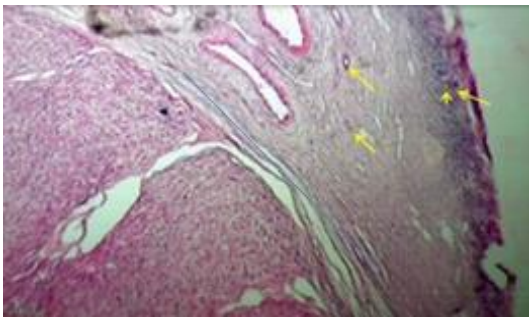
**Picture 1.** Anatomical section of a male testis at the age of (7) months and the weight of (18) kg. Thick fibrous lobula surrounded by thick fibrous tissue of epididymis (yellow arc), epididymis tubules (red bow), undeveloped spermatogonia cells (yellow arrow). Testicular interstitial tissue (blue arrow). The testicular cortex (black arrow), (H & E, 100x.).



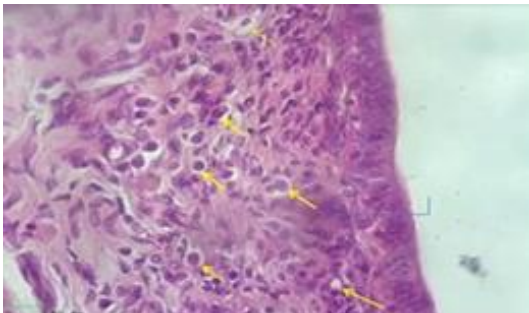
**Picture 2.** Anatomical section of a male testis at 10 months of age and a weight of 29 kg. Spermatogenesis and some spermatogonia (yellow arrow). Seminiferous tubule (yellow bow). The primary and secondary spermatocytes (black arrow). Leydig cells (blue arrow), (H & E, 100x.).



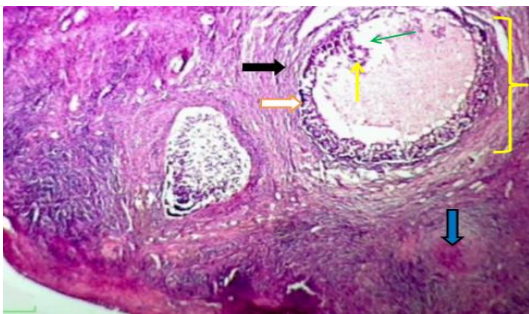
**Picture 3.** Anatomical section of a mature ram testis at 14 months old and 40 kg weight. Primary and secondary spermatogonia (black arrow). The spermatic tubule (white arrow). Sertoli cells (blue arrow), (H&E, 100x.).



**Picture 4.** Anatomic section of a female lamb ovary at the age of 8 months and the weight of 19 k. The follicles (yellow bow). Fibroblast cells (blue bow). Tunica albuginea (black arrow), (H & E, 100x.).



**Picture 5.** Anatomic section of a female ovary at the age of 10 months and the weight of 28 kg. The ovary (blue bow). The ovum (yellow arrow). Primordial germ cells (green arrow), (H & E, 100x.).



**Picture 6.** Anatomic section of a mature ewe ovary at the age of 13 month and the weight of 40 kg. Theca interna (white arrow). Theca externa (black arrow). Graafian follicles (yellow row). The ovum (yellow arrow) surrounded by corona radiata (green arrow). The corpus luteum (blue arrow), (H & E, 100x.).

## CONNCLUSION

This study concluded that the age and weight of the Arabi sheep breed clearly affect the function of gonadotropin and endocrine glands and raise the level of sex hormones. These concentrations are higher in females than in males. According to the study histological development, the growth of reproductive organs also correlates with the animal age and weight.

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**Author Contributions:** All authors contributed equally in writing original draft preparation, all authors have read and agreed to the published version of the manuscript.

**Informed Consent:** All patients gave their written informed consents before inclusion.

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