



## Influence of Maternal and Lamb's Factors on the Level of Estrogen During Three Physiological Statuses of Ewes

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### ABSTRACT

The physiological status of the reproductive life of females is controlled by several hormones which are affected by numerous factors that can be classified into intrinsic and extrinsic ones. This study aimed to detect the level of estrogen hormone, the impact of parity number and kind and sex of lambs on its level in three physiological statuses (third trimester of pregnancy, parturition, and postpartum period), which was achieved by estimation of plasma estrogen level of 24 pregnant ewes through ELISA technique. A significant difference between primiparous (50.60) and multiparous (64.78) ewes on the level of estrogen hormone on week 18 of gestation was observed. Estrogen levels on the day of parturition were 51.48 and 70.50 for the primiparous and multiparous groups, respectively. Furthermore, the sex of lambs also varied significantly between the two parity groups under study. Additionally, the type of birth (single or twins) had a significant effect on the level of estrogen in multiparous ewes only. It was concluded that parity number has a significant effect on the level of estrogen at the end of the gestation period and during parturition. The sex of lambs in both parity groups affected the level of estrogen significantly. Nevertheless, the type of birth significantly affected the level of estrogen in multiparous ewes.

**Key words:** Estrogen, Parity, Pregnancy, Parturition, Postpartum.

### INTRODUCTION

One of the most crucial phases of every female's life for sustaining species is a pregnancy which mammalian species engage in a highly synchronized process that requires hormones and reproductive system. The structure of the endometrium and the uterine immune system are significantly affected by endocrine alterations, which include variations in the profile of estrogen and progesterone (Abu Nasar 2006).

A dominance of estrogen during a period of parturition is started by the secretion of cortisol from the fetal adrenal gland (Nagel et al. 2019) to stimulate placental 17 $\alpha$ -hydroxylase to convert progesterone to estrogen that is related directly to the size of the fetus and uterine fluid (Amin et al. 2010; Probo et al. 2011).

Estrogen cooperates with relaxin to relax the birth canal which aids in the easier delivery of the fetus (Probo et al. 2011).

Postpartum is the period from parturition to the first estrus which is characterized by endometrium regeneration, uterine involution, and ovarian cyclicality resumption that take place under hormonal interaction of

the pituitary gland and hypothalamus (Medan and El-Daek 2015). Postpartum is influenced by breed, diet, season, lactation, suckling, and intensity of suckling (Hernández et al. 2021). There are significant differences in the interval between parturition and the first ovulation (Ascari et al. 2016).

According to Wise et al. (1986), a drop in the number of estradiol receptors in the anterior pituitary gland may be the cause of estradiol negative feedback effect after parturition. The number and location of estradiol receptors have a direct impact on the pituitary's reactivity to GnRH. For the sheep industry, the length of the postpartum period is crucial. Animal farming practices contribute to the welfare and health of people (Salmon et al. 2020). Nevertheless, there are challenges to produce agricultural products due to the increasing market demand for animal products. Furthermore, societal pressure exists to encourage environmentally responsible production to avoid the deterioration of the environment (Kusch-Brandt 2020).

As a result, the present issues of the global community of animal production scientists are the intensification of production and the application of creative techniques to maintain our living environment (Mayberry et al. 2020).

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In the case of sheep farming, system intensification necessitates more than one lambing each year (Miguel-Cruz et al. 2019). The ability of the ewes is not optimally utilized since current management practices prohibit two lamb births per year. According to the studies, sheep have a biological maximum limit of six months between lambing and five lambs during each pregnancy. The sheep producers are unable to accomplish this due to the prolonged postpartum period as well as the seasonal breeding behaviors of sheep. Numerous questions about the postpartum period have been addressed by thorough investigations while numerous important questions are yet unsolved.

## MATERIALS AND METHODS

This study followed international and national guidelines for the humanitarian treatment of animals and their welfare in compliance with relevant legislation.

The study was carried out in the sheep and goat rearing station in Baghdad which is located in Abu-Ghraib (a district 20km to the west). It is 29 meters above sea level. Weather in Abu Ghraib is influenced by Subtropical Dry Arid (Desert) climate. The average temperature of this district in the summer months, mainly in July and August reaches 48-50°C. To achieve the study goals, 24 pregnant ewes from a flock of 35 ewes (Turkish Awassi) in the last month of pregnancy were detected by ultrasonography. In addition, ewes-related data was recorded. Their ages ranged from 2-4 years, while parity was either primiparous or multiparous. All ewes were isolated from males and sheltered in semi-open fields under veterinarian observation.

All ewes were offered 14% of protein and roughage as concentrated feed. Water and minerals were accessible the whole day. Every ewe was allowed to move in an open area and get some sunshine to provide a sufficient quantity of vitamins. To satisfy essential dietary requirements, each lamb was continuously left with its dam to acquire appropriate colostrum and for optimal suckling.

Blood from the jugular vein was taken in an anticoagulant-free vacutainer tube in the early hours (7.00-8.45 am) preceding feeding each 14 days. The first samples were taken on the 16<sup>th</sup> and 18<sup>th</sup> week of pregnancy. The parturition day and successive samples were taken every 14<sup>th</sup> day (weeks 2, 4, 6, and 8 postpartum). Cold transportation chain was maintained while sampling transportation. In the laboratory, serum was separated by centrifugation at 3000 rpm for 20 minutes. The serum was stored at -18°C until hormonal analysis by ELISA technique.

### Statistical Analysis

Results were presented as mean±SD using Statistical Package for The Social Sciences Version 18 (SPSS v. 18). To measure the differences between groups t-test was applied while the level of analysis of the data was 5-1%.

## RESULTS AND DISCUSSION

There were increased levels of estrogen hormone in the 16<sup>th</sup> and 18<sup>th</sup> week of pregnancy in the primiparous (46.48, 50.60) and multiparous (53.75, 64.78) ewes (Table 1). Due to the conversion of  $\alpha 17$  - hydroxyprogesterone to estrogen from the placenta which is associated with increasing fetus size and uterine fluid to decrease the effect of negative feedback of progesterone hormone (Amin et al. 2010).

While another study reported an increase in estrogen in this period to prepare the uterus for the delivery process. This result agrees with Amin et al. (2010) in goats and AL-Khafaji and AL-Akkam (2017) in ewes whereas the significant increase in this hormone in the multiparous ewes explains the effect of age and size of fetus in the large parity compared to primiparous ewes as reported by a previous study that showed increased parity order increases fetus weight which has an important role in the cellular mechanism of the uterus to support and produce nutrients (Amin et al. 2010).

An elevation of estrogen levels in the primiparous (51.48) and multiparous ewes (70.50) on the day of parturition (Table 1) was explained by the study of Probo et al. (2011) who reported that the status of placental estrogens around parturition is extremely complicated and the information on its definite effect has differed among animal species. In ewes, the previous studies demonstrate that domination of estrogen around parturition is ordinarily rated as a significant agent, that precedes parturition to soften the birth passageway and promotes excitation of the myometrium and liberates prostaglandins. However, Nagel et al. (2019) recorded that in sheep, lambing causes a severe liberation of cortisol from the fetal adrenal glands, which promotes estrogen and reduces progesterone concentration. Consequently, by stimulating the release of PGF2 $\alpha$ , the concentration of oxytocin increases, leading to an increase in myometrium contraction. In the current study, the significant difference between the two groups (Table 1) confirms the claim that increased uterus status coincides with the age of the mother and the size of the fetus (Takayama et al. 2010; Mirzaei et al. 2011).

Regarding postpartum, estrogen appears in the levels around parturition in the two parity groups which is inconsistent with the study of Bekeova et al. (1991) who reported that the level of estradiol decreases sharply from two weeks before lambing to 4 days post-lambing. This difference in the results may be because of the long duration of sampling of the current study whereas the same references assert slight increases between days 17 and 42 postpartum.

The present study reveals that there is no statistical difference between the two parity groups in the level of estrogen which reflects the effect of lactation which inhibits fertility in almost mammalian species (Mcneilly 2001). Due to a lowering in thereproduction function of the hypothalamus, through some neural changes according to Dobek et al. (2013) which is hard to detect precisely. Among the recognized suppressors of the GnRH/LH system, endogenous opioid peptides have a crucial role in the alteration of the reproductive process (Dobek et al. 2013). Oliveira et al. (2013) explained that lactation affects reproductive activity associated with prolactin and oxytocin production. Furthermore, Gaafar et al. (2005) assert the impact of the suckling process on ovarian resumption.

Takayama et al. (2010) emphasized suckling is a critical factor that interferes with ovarian resumption after parturition whereas Hernandez et al. (2009) indicated suckling delays ovulatory resumption after parturition thus weaning time is an essential agent in the interval of postpartum anestrus (Ronquillo et al. 2008). Another study by Gonzalez-Stagnaro et al. (2002) rejects this finding. Takayama et al. (2010) found that there is no ovulation in nursing does.

**Table 1:** Descriptive statistics of estrogen (pg/m)/primiparous and multiparous ewes with the t-test of estrogen/primiparous and estrogen/multiparous

Days of blood calculation	Min.	Max.	Mean	SD
Week 16/ pregnancy	43.2	53.2	46.48	3.94
Week 18/ pregnancy	45.5	54.3	50.60	3.72
Post Parturition Week	42.3	57.8	51.48	5.12
2	45.9	81.0	57.42	12.32
4	43.5	60.1	53.85	6.05
6	45.8	61.7	55.75	5.85
8	50.1	64.9	57.17	4.83
Descriptive Statistics of Estrogen /multiparous ewes				
Week 16/pregnancy	42.4	83.5	53.75	15.13
Week 18/pregnancy	43.3	80.5	64.78	12.68
Parturition day	50.2	89.1	70.50	14.66
Postpartum Week				
2	42.9	59.8	53.42	6.47
4	53.60	64.70	58.80	3.67
6	55.00	84.10	62.88	10.84
8	44.5	87.4	61.13	15.10
t- Test of Estrogen/primiparous and Estrogen/multiparous				
Days of blood calculation	T	P-Value	C.S	
Week 16/primi/multiparous	1.6091	0.1218	P>0.05 (NS)	
Week 18/Primi/multiparous	3.7319	0.0012	P<0.01 (HS)	
Parturition primi/multiparous	4.2406	0.0003	P<0.01 (HS)	
Week 2/Primi/ multiparous	0.552	0.605	P>0.05 (NS)	
Week 4/Primi/multiparous	1.338	0.239	P>0.05 (NS)	
Week 6/Primi/multiparous	1.536	0.185	P>0.05 (NS)	
Week 8/primi/multiparous	0.573	0.592	P>0.05 (NS)	

The current study agrees with the study of Zdunczyk et al. (2004) who reported parity order does not significantly affect uterine involution in comparison to primiparous and multiparous ewe as well as the finding of Hayder and Ali (2008) disagrees with Webb et al. (2007) results in the period of postpartum anestrus in primiparous ewes due to growing process and the first-time nursing of ewe.

Regarding the influence of birth type on the level of estrogen, the current study reveals no significant differences between single and twins in the primiparous (Table 2) which disagrees with the finding of Khan and Ludri (2002a) in goat. However, Takayama et al. (2010) and Mirzaei et al. (2011) confirmed the effect of the type of lambing on the size of the uterus prepartum and postpartum which affects the uterus involution. This is in agreement with the current study finding in the multiparous ewes. Neffel et al. (2021) found a significant effect of the number of fetuses on the level of cortisol at parturition in contrast with other study reported no significant differences in serum cortisol in the prepartum period between single and twin-bearing ewes (Medan et al. 2015). While, in the multiparous ewes, there is a significant increase in estrogen levels (Table 2) in the twin compared with the single in the current study, which agrees with Sumaryadi and Manalu (1999) who obtained the same result regarding significant differences in estrogen in the twin than single lambs. Khan and Ludri (2002a) detected a significant level of estrogen in the crossbred goats and it is also in conformity with the findings of Douglas et al. (2015) and Gamit et al. (2019) in goats.

Regarding the effect of sex of birth on estrogen levels, the present study identified significant differences between males and females in the primiparous ewes and multiparous ewes that agree with Khan and Ludri (2002b), Fazio et al. (2013) and Luigi et al. (2021) in goat, due to stimulation of

the placenta to resynthesize estrogen from progesterone causing an increased level of estrogen in the male-bearing goat as the report of Ford et al. (1998).

**Table 2:** t-test of Estrogen in primiparous and multiparous ewes with the type of parturition and sex

Parameters	Mean±SD	t	P-value
Primiparous Ewes			
Type of Parturition			
Single	30.10±1.34	0.613	0.559NS
Twin	31.75±4.49		
Sex lamb			
Female	28.88±2.54	3.042	0.019*
Male	34.09±2.55		
Multiparous Ewes			
Type of Parturition			
Single	48.07±5.24	2.528	0.050*
Twin	57.99±0.00		
Sex lamb			
Female	50.59±6.50	3.042	0.019*
Male	51.68±8.91		

NS=non-significant. \*Significant difference at P<0.05.

## Conclusion

Indicating the results of the current study, it is concluded that the level of estrogen hormone increases when the parity order increases at the end period of pregnancy and near parturition. It is also elevated in the male-bearing ewes in both parties, whereas the type of birth affects only multiparous ewes. The role of hormones in reproductive statuses is complicated because of interactions of intrinsic and extrinsic factors. The postpartum period represents the most important period that is affected by the overlapping factors mentioned above. Thus, novel studies about estrogen and its connection with ovarian resumption in the postpartum period are needed to suggest new strategies aimed to shorten the postpartum intervals.

### Author's Contribution

All these study steps are the work of the author Amel Mustafa Kamil including the theory of study, field watching, laboratory testing along with statistical analysis. and its writing.

### Conflict of interest

The author declares there is no conflict of interest.

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