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Estrogen and Progesterone Receptor Gene Expression During Different Stages of Uterine Involution in Sahel Goats

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ABSTRACT

The study investigated estrogen and progesterone receptor gene expression during different stages of uterine involution in sahel goats. Thirty Sahel does (30) and bucks (5) (2-3.5 years and 30-40 kg) were used for the study. Pregnancies were achieved by natural mating after synchronization. Repeated injection of cloprostenol at 250µg/kg intramuscularly were given at 11- day interval. The does were randomly separated into postpartum days 1,3,7,14, 21 and 28 and undergo ovariohysterectomy for uterine tissue sample collection through caesarean section. Collected tissues were fixed in 10% formalin and used for estrogen and progesterone ELISA assay using commercial kits. The results showed that, the concentration of progesterone receptor on day 1 was 5.15 ± 1.01 ng/ml, followed by progressive decrease on day 3 and 4 postpartum, and a fluctuating trend in increase between day 14, 21 and 28. The highest peak (26.6 ± 2.21 ng/L) concentration of estrogen receptor was observed on day 7 postpartum and significantly ($p < 0.05$) decrease to 17.4ng/L on day 28 postpartum. Conclusively, the concentration of progesterone and estrogen receptor protein at the various stages of uterine involution indicate that upregulation of these receptors were carried out by progesterone and estrogen.

Keywords: Estrogen, Progesterone, Receptor gene, Sahel goat, Uterine involution

INTRODUCTION

Progesterone and estrogen are the two primary reproductive steroidal hormones in mammals and are categorized as steroids chemically (Gruber *et al.,* 2004). Progesterone is essential for reproduction since it helps to keep a pregnancy going. The corpus luteum (C.L.) and placenta, as well as the adrenal cortex to a lesser extent, are the primary sources of progesterone during pregnancy (Enginler *et al.,* 2017). According to Fernandes *et al.* (2013), the corpus luteum, the placenta, and the mammalian ovary are the primary sources of estrogens, which can be conjugated. In addition to the corpus luteum and placenta, it is also known that during pregnancy, the adrenal glands of the mother and the fetus create estrogen. The primary source of placental estrogen precursors during fetal adrenal steroidogenesis is the foetal adrenal cortex (Abecia and González Bulnes, 2012; Badawi *et al.,* 2014). The adrenal glands of fetuses convert cholesterol to dehydroepiandrosterone (DHEAS). The placenta transforms the DHEAS into either estrogen or estradiol (Migale *et al.,* 2016). According to recent research, progesterone may have an indirect effect on uterine quiescence by preventing the uterus and cervix from expressing genes related to contraction and by preventing the creation of chemokines that aid in the chemotaxis of immune cells (Degefa *et al.,* 2006).

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Additionally, progesterone has been shown to reduce myometral contractility by inhibiting prostaglandin synthesis and activity in pregnant women (Gadkar-Sable *et al.,* 2005). Prostaglandin activity is blocked, prostaglandin production is reduced, and prostaglandin inactivation rate is increased, among other pathways that mediate this inhibition (Badawi *et al.,* 2014). Pregnancy-related progesterone declines are linked to higher prostaglandin synthetase activity and prostaglandin F2α synthesis, which increases the risk of abortion (Takayama *et al.,* 2010). Because progesterone's actions are mediated through its nuclear receptor (Takayama *et al.,* 2010), progesterone responsiveness in the uterus and, consequently, the preservation of uterine quiescence during pregnancy depend on the regulation of PR genes. According to Hamilton et al. (2017), progesterone and its receptor (PR) are therefore essential elements of uterine physiology throughout pregnancy. Autoregulates the expression of their own genes, which is one conserved function of steroid hormone receptors (Rajaram *et al.,* 2015). Hormone receptors are generally regulated by other regulatory molecules as well as by their own ligand (homologous regulation) (heterologous regulation). Numerous hormone receptors have been shown to be heterologously up-regulated when endogenous glucocorticoids are present (Alade *et al.,* 2024).

Although there are several reports on some aspects of the morphology, physiology, and pathology of the goat's reproductive organs (Zongo *et al*., 2014; Egbe-Nwiyi *et al*., 2015; Majama *et al*., 2023; Shaukat *et al*., 2024). There appears to be little information on endometrial estrogen and progesterone receptors gene expression in the uterus during the involution period in Sahel goats. Knowledge of these changes will improve our understanding of the reproductive performance of Sahel goats. Therefore, the goal of this work was to investigate the receptor gene expression during uterine involution in order to further our comprehension of potential gene factors governing uterine involution in the animal species under investigation.

MATERIAL AND METHODS

Ethical Approval

All applicable national and institutional guidelines for the care and use of animals were followed. All procedures performed in the studied animals were following the ethical standards of the Faculty of Veterinary Medicine Committee on Animal Use and Care where the study was conducted.

Study Location

The study was carried out in Maiduguri, the capital and largest urban city of Borno state in the Northeastern part of Nigeria. It is cosmopolitan in nature, situated at an elevation of 354 meters above sea level. The city is located between latitudes 11º and 14º N and longitudes 10 º and 14 º E within the conventional Sahel zone, and has a total landmass of 50,778 square kilometers (Egbe-Nwiyi *et al*., 2024). The temperature ranges from 35-40 º C for most parts of the year, with two distinct seasons, the rainy season with a mean annual rainfall of 647mm (from July to October) and a prolonged dry season for the rest of the year (Ishaku and Majid, 2010)

Study Animal

A total of thirty Sahel does and five bucks (2-3.5 years and 30-40 kg) were used. The does were purchased from the livestock market of Maiduguri and transported to the large animal clinic at the University of Maiduguri's Faculty of Veterinary Medicine. The does were suggested to routine physical examination to ascertain their health status as well as pregnancy status during 14 days' acclimatization period. Feed (wheat offals,

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bean husks, hay made from groundnut leaves, and salt licks) and water were provided throughout the experimental period (Bello *et al*., 2023).

Experimental Design

After the 14 days' acclimatization period, the does underwent an ultrasound scan in accordance with Medan and Abd El-Aty (2010) protocol to confirm they were not pregnant. Thereafter, the does were synchronized using cloprostenol (Estrumate®, Schering Trough Animal, Germany) at 250µg/kg intramuscularly at 11- day interval. The second dose was administered at 11th day, and the does came on heat (estrus) after the second treatment. Thereafter, the does were allowed to naturally mate with the bucks. At the end of the gestation period, all the does were checked for signs of parturition and were separated into groups by assigning them randomly in postpartum days 1,3,7,14, 21 and 28 to undergo ovariohysterectomy (OVH) according to Yahi *et al*. (2017)

Tissue Sample Collection and Processing

Uterine tissues were collected from the does on days 1, 3, 7, 14, 21 and 28 postpartum at ovariohysterectomy and all fixed in 10% formalin. For the estrogen and progesterone ELISA, each tissue was removed from the formalin and blotted on a clean tissue paper and rinsed thoroughly with ice-cold phosphate buffer saline (PBS 0.2mol/L) at a PH of 7.0 for several times so as to remove excess blood thoroughly. The tissues of 300-500 mg were then weighed using a sensitive weighing machine PW 254 (ae ADAM) and then minced into smaller pieces using scalpel blade for homogenization in 9 mls of PBS per 1gm of tissue with a glass homogenizer on ice IKA T25D digital ULTRA TURRAX rpm × 1000 (Germany). Three (3gm) gram of tissue was used in 27 mls of PBS and the tissue suspension was then subjected to freeze-thaw cycles to further break the cells. After that, the homogenate was further centrifuged using centrifuge machine 5430 EPPENDORF AG Germany for 5 minutes at 5000rpm and the supernatant collected into a separate tube for the ELISA (Collden *et al.,* 2023).

ELISA Assay

Goat Estrogen and Progesterone Receptor ELISA Kits

Immunoreactive PR and ER proteins were detected in uterine tissue sections using Goat Estrogen receptor (ER) ELISA Kit KTE50053 and Progesterone receptor (PGR) ELISA Kit KTE50057 obtained from Abbkine China. Analysis of the estrogen and progesterone receptors were done using the kits according to the manufacturer's instruction. The kits use enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to quantify Goat estrogen and progesterone receptor (Collden *et al.,* 2023).

Data Analysis

The data obtained were analysed using ANOVA and descriptive statistics (Graphs), Tukey Kramer as posthoc test with JMP version 11 software (SAS Institute Inc, Cary, NC) at $p < 0.05$.

RESULTS

Estrogen and Progesterone Receptor Gene Expression

The concentration of progesterone receptor observed on day 1 was 5.15 ± 1.01 ng/ml, this was followed by a progressive decreased on day 3 and 4 postpartum (4.77 ± 0.84 ng/ml and 5.42 ± 0.98 ng/ml; respectively), and a fluctuating trend in increase between day 14, 21 and 28 (4.42 ± 0.98 ng/ml; 5.17 ± 0.39 ng/ml; 5.79 ± 0.72 ng/ml, respectively) Figure 1.

From the result of this study, the lowest concentration of estrogen receptor was 16.2 ±

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4.01 ng/L observed on day 1 postpartum, the was followed by a progressive increased, reaching the highest peak (26.6 ± 2.21 ng/L) concentration on day 7 postpartum. The peak concentration begins to decrease progressively ($p < 0.05$) to a concentration of 17.4ng/L on day 28 postpartum (Figure 2). Therefore, the concentration of Estrogen receptor was significantly ($p<0.05$) higher throughout the days of uterine involution (1, 3, 7, 14, 21, 28) compared to progesterone receptor (Figure 3).

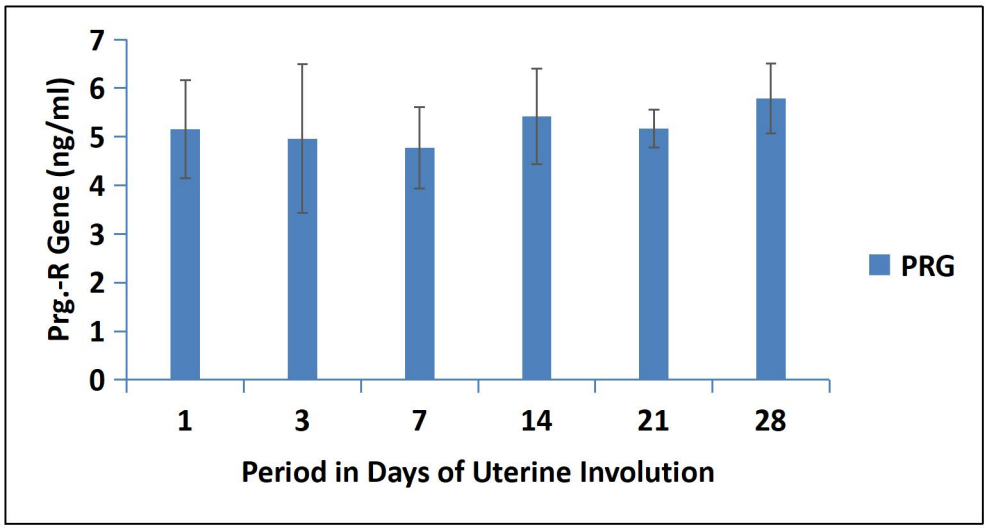

Figure 1: Concentration of Progesterone (P4) receptor gene during days 1, 3, 7, 14, 21 & 28 postpartum in Sahel goats.

Keys: PRG- Progesterone, R- receptor, ng/ml-nanogram per millilitre

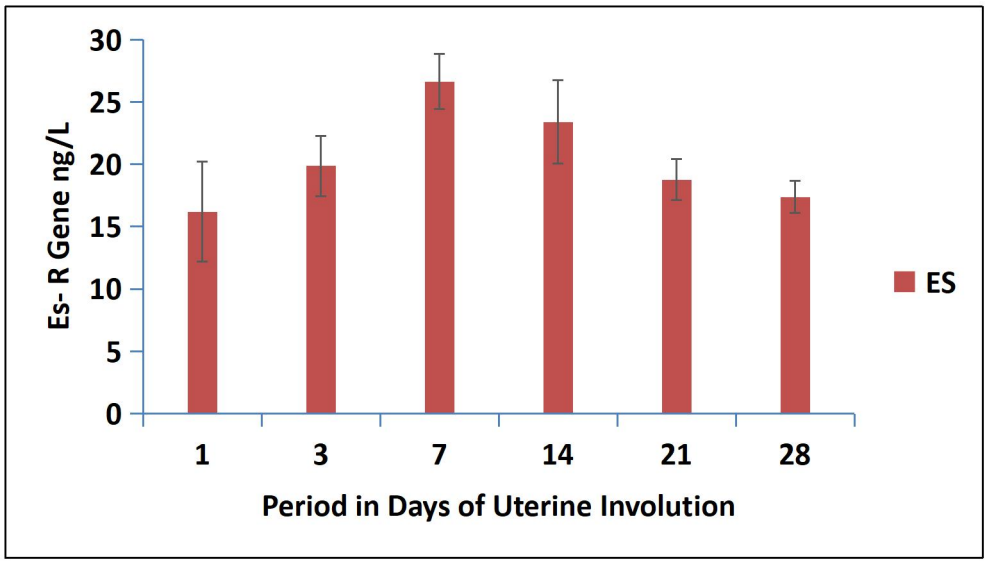

Figure 2: Concentration of Estrogen (ES) receptor gene during days 1, 3, 7, 14, 21 & 28 postpartum in Sahel goats.

Keys: ES- estrogen, R-gene, ng/L- nanogram per Litre

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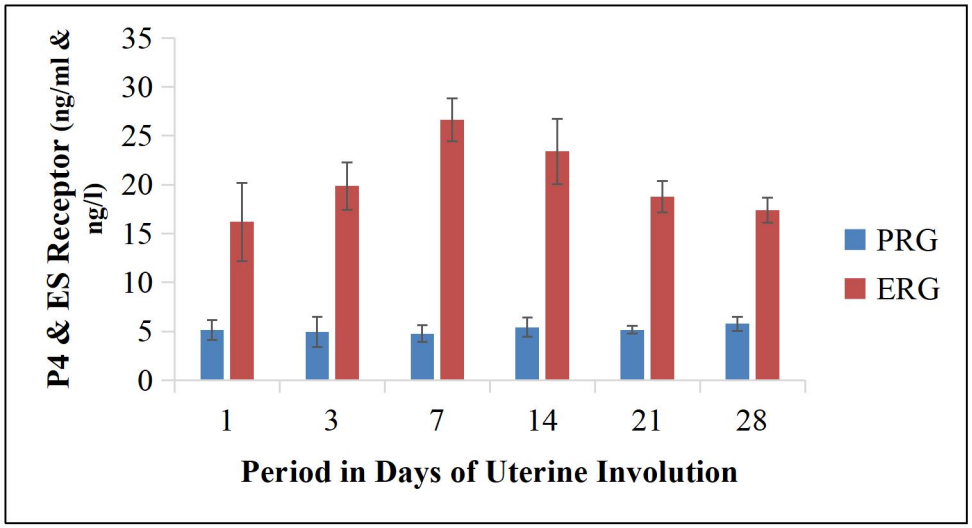

Figure 3: Comparison of Concentration of Progesterone (P4) and Estrogen (ES) receptor genes during days 1, 3, 7, 14, 21 & 28 postpartum in Sahel goats.

DISCUSSION

The mechanisms involved during postpartum uterine involution in goats are not completely understood. A crucial component of this process are the steroid receptors, which are particular proteins that are only found in the nuclei of endometrial epithelial and stromal cells as well as the endothelial cells of the stromal capillaries. Progesterone and estradiol are the two substances for which they have a strong affinity (Madak-Erdogan *et al*., 2016). According to Medin and El-Daek (2015), appropriate uterine function requires both progesterone receptor (PR) and estrogen receptor alpha (ERα) expression, although normal uterine growth requires only one of them.

In the present study, the concentration of the progesterone receptor protein was at the basal level up to day 1-14 postpartum. This finding was ascribed to upregulation of this receptor by estrogen and progesterone withdrawal and the upregulation of progesterone receptors in the endometrium. This is in accordance with the report of Patel *et al.,* (2015); Arnal *et al.,* (2017); Wu and DeMayo (2017)*;* Palaniappan *et al* (2019). However, these researchers did not consider the period of the involution as reported in this study. From day 21-28 postpartum, there was downregulation of progesterone receptor (PR) in the endometrium as observed in the present study. Bhurke *et al*. (2016) and Grimm *et al*. (2016) observed similar findings of downregulation of progesterone receptor expression immunohistochemically in the luminal epithelium by day 13 postpartum and in glandular epithelium by day 15 postpartum. This could be as a result of the increase in progesterone hormone in the endometrium towards the end of uterine involution which decreased the number of PR in the Sahel goat. The result also corresponds to the finding of Kandiel *et al.* (2012) who reported progesterone receptors (PR) to be significantly higher during late lactation in postpartum goats. Therefore, progesterone induced progesterone receptor gene.

Also, in the present study, concentration of estrogen receptor protein in the endometrium was high throughout the period of uterine involution with slight decrease on day 3 postpartum indicating that upregulation of these receptors was carried out by estrogen.

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This finding is in accordance with the report of Rajbhandari *et al.* (2012); Care *et al*. (2014); Palaniappan *et al.* (2019) that reported highest concentration of estrogen receptors during the follicular phase when concentration of progesterone hormone was low. In another study, Levin and Hammes *et al*. (2016) showed higher concentrations of estradiol receptors expressed in the epithelial cells than in stromal cells and glandular. The present result also showed a fall in the concentration of estrogen receptor proteins towards the end of uterine involution in the endometrium. This could probably be as a result of high level of progesterone concentration. Change in estradiol and lack of progesterone can be correlated with the increase in estrogen receptor (ER) protein. One conserved function of steroid hormone receptors is that, they autoregulate the expression of their own genes. In general, hormone receptors are regulated both by their own ligand (homologous regulation) and by other regulatory molecules (heterologous).

CONCLUSION

Finding from present study showed that, the concentration of the progesterone receptor protein was at the basal level up to day 1-14 postpartum, also, the concentration of estrogen receptor protein in the endometrium was high throughout the period of uterine involution with slight decrease on day 3 postpartum indicating that upregulation of these receptors was carried out by estrogen.

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