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EFFECTS OF DEXAMETHASONE ON SOME REPRODUCTIVE HORMONES OF PREGNANT SHEEP

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Abstract

Dexamethasone is widely used in both veterinary and human medical practices. However, it seems to cause some deleterious effects on pregnancy probably by causing changes in the reproductive hormone levels. Twenty healthy adult sheep comprising 18 ewes and 2 rams were used for this study. Pregnancies were achieved by natural mating after estrus synchronization. Dexamethasone was administered at 0.25mg/kg body weight on days (1, 3, and 5) during first trimester; days (51, 53, and 55) during second trimester; and days (101, 103, and 105) during the third trimester. Blood samples were collected biweekly for hormonal assay. Results showed that dexamethasone significantly ($p < 0.05$) decreased progesterone concentrations and caused abortion in sheep but had no significant ($p < 0.05$) effect on estrogen. The abortion could probably be due to decreased progesterone concentrations as a consequence of the adverse effects on placenta.

***Keywords*:** Dexamethasone, estrogen, progesterone hormone, Pregnancy, Sheep.

Introduction

Dexamethasone is a fluorinated compound derived from corticosteroid and having 21-carbon steroid skeleton with hydroxyl (OH–) or methyl (CH_3–) group attached at C16. This compound has virtually no mineralocorticoid effect but remains potent anti-inflammatory and analgesic glucocorticoid with broad significant physiological and therapeutic uses (Pierre-Louis, 2010). Therefore, it is used to treat and manage several diseases and medical conditions in both animals and humans (Yahi *et al*., 2017). These include pregnancy related and metabolic diseases such as ketosis, pregnancy toxaemia and mastitis, prenatal foetal lung development and maturation, management of neonatal diseases (Yahi *et al*., 2017). However, since there is no single drug that produces just a single effect without being accompanied with other undesirable effects, dexamethasone is no exception. The drug has been reported and observed to cause abortions in some breeds/species of animals like cattle, sheep and dog (Greco and Davidson, 2017). The adverse effects range from glucose intolerance to more severe effects like decreased placental weights and efficiency, intrauterine growth restriction (IUGR), and altered hypothalamic-pituitary-adrenal axis (Yahi *et al.*, 2017). The mechanisms underlining some of these deleterious effects during pregnancy are not clear as different species do not always respond to medicines in the same way. However, changes in reproductive hormone levels and their receptor concentrations may be involved. Progesterone and estrogen are chemically classified as steroids and are regarded as the two main reproductive hormones in mammals, with progesterone playing a central role in the maintenance of pregnancy (Knight *et al*., 2016). During the course of normal pregnancy, progesterone concentration increases as the pregnancy progresses in order to maintain the integrity of pregnancy by the sustenance of uterine quiescence. However, decreasing progesterone concentration or its receptor (PR) expression and/or activity promotes parturition or abortion (Cost, 2013). During pregnancy, progesterone is mainly produced by corpora lutea (CL) and placenta and, to a lesser extent, adrenal cortex. Estrogen, on the other hand, is usually produced by the mammalian ovary, corpus luteum, or placenta and may be conjugated and has the widest range of physiological functions. In addition, estrogen is also known to be produced by both maternal and foetal adrenal glands during pregnancy (Wilsterman *et al.*, 2018)). Some of its actions during pregnancy include, but are not limited to, stimulation of the growth of mammary gland ducts and secretory activities of the oviduct and uterus to enhance foetal survival, regulation of gonadotropin secretion, relaxation of pelvic structures, softening of the pubic symphysis, and general enlargement of the perineal area (Hafez and Hafez, 2013). The actions of progesterone and estrogens are mediated by their respective nuclear receptors. The regulation of progesterone receptor (PR) genes in the uterine tissue is critical for the response of the organ to progesterone and thus the maintenance of uterine quiescence during gestation. Hence the mechanism regulating progesterone secretion and PR expressions are important in the understanding of uterine physiology during pregnancy. One conserved function of steroid hormone receptors is that they autoregulate the expression of their own genes (Borahay *et al*., 2017). In general, hormone receptors are regulated both by their own ligand (homologous regulation) and by other regulatory molecules (heterologous regulation). Endogenous glucocorticoids are known to be involved in the heterologous upregulation of several hormone receptors (Ahmadabad *et al*., 2016).). The synthetic glucocorticoid, dexamethasone, may have similar role during pregnancy (O'Connell *et al*., 2013). The objective of this study was to assess the influence of dexamethasone on progesterone and estrogen concentrations in pregnant sheep.

The present study is carried out the aim to determine the effect of dexamethasone on the levels of serum progesterone and estrogen hormone in serum of sheep during gestation .

Materials and Methods

Animals and Management

Twenty healthy adult sheep comprising 18 ewes and 2 rams were used for this study. The animals were purchased from private farms in Babylon. The ages of the ewes ranged between 2.5 and 3.5 years, while the rams were 3 years. The ewes weighed between 35 and 40 kg and the rams weighed

40 kg. Before the commencement of the experiment, the health status of the animals was evaluated clinically and they were treated prophylactically with oxytetracycline LA (Introxin-200, Holland) at 20 mg/kg body weight and ivermectin (Pharma Swede, Egypt) at 200 µg/kg body weight and were allowed to acclimatize for four weeks. Their feed rations consisted of wheat offals, beans husks, and hay. The rams were kept separate from the ewes until the time of service. Throughout the period of the experiment, these animals were maintained under good conditions.

Estrus Synchronization

All the females were synchronized at the end of the acclimatization period using cloprostenol (Estrumate, Schering Trough Animal, Germany) given intramuscularly at 250 µg/kg, 11-day interval as reported previously (Akusu and Egbunike,1984). They were teased with apronned males daily and all the females that came into estrus after the second treatment were allowed to be served naturally by the males. Day of estrus was recorded and considered as day 0 of the gestation. Pregnancies were confirmed by failure to return to estrus and by ultrasonographic examination. After successful synchronization and fertile mating, the animals were then randomly separated into 2 groups of 9 each: dexamethasone treated pregnant sheep (DTS) as treatment group and nondexamethasone treated pregnant sheep (NDS) as control.

Treatments

Animals in the dexamethasone treated group were treated with dexamethasone (Dexaphan, Pharma Pharmaceuticals, Swede, Egypt) injection given intramuscularly at 0.25mg/kg body weight on days (1, 3, and 5) during the first trimester; days 951, 53, and 55) in the second trimester; and days (101, 103, and 105) in the third trimester. The animals were observed for possible clinical changes throughout the period of the gestation.

Blood Sample Collection and Analysis

Five ml of blood sample was collected with minimal excitement on day 0 and thereafter on biweekly basis from each animal through the jugular vein and transferred into sterile tube without anticoagulant. The blood was allowed to clot and centrifuged at 3000 rpm for 5 minutes.

ELISA Assay for Determination of estrogen and progesterone hormone Concentration:

ELISA assay was achieved according to the method described by the manufacturing company (Elabscience /USA).

Results and Discussion

The changes in serum estrogen and progesterone concentrations in pregnant sheep following the administration of dexamethasone are presented in Figures (1 and 2). While progesterone concentration increased progressively with advancing pregnancy in both the treated and control groups up to the second trimester, there were significant ($p < 0.05$) decreases at time-points in serum progesterone concentrations in dexamethasone treated sheep compared to the control (Figure 1). This decrease was observed at the beginning of the second trimester (day 56) and continued up to day 112 of gestation compared to the control for the same period. However, there was no significant ($p > 0.05$) variation in estrogen concentrations between dexamethasone treated group and the respective control group (Figure 2).

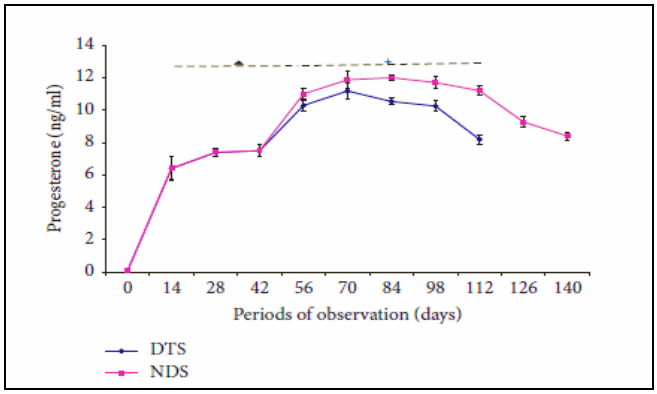

Fig. 1 : Effects of dexamethasone on progesterone concentration in dexamethasone treated pregnant sheep (DTS) and nondexamethasone treated pregnant sheep (NDS).

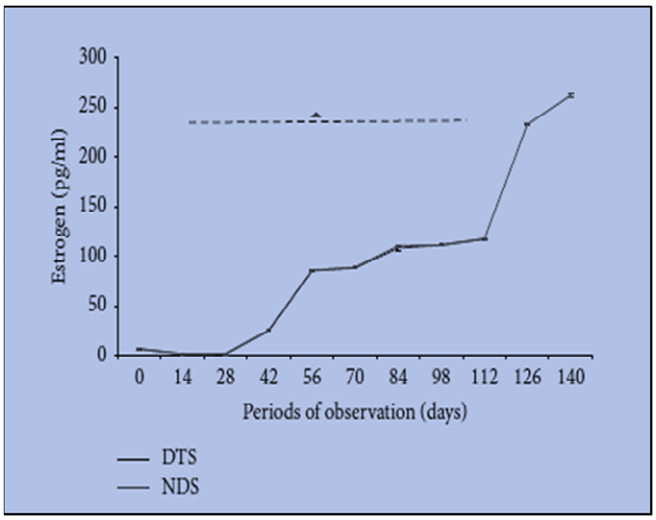

Fig. 2 : Effects of dexamethasone on estrogen concentration in dexamethasone treated pregnant sheep (DTS) and nondexamethasone treated pregnant sheep (NDS).

In this study, dexamethasone treatment caused abortion in sheep during advanced pregnancy which could be associated with decreasing progesterone concentration in systemic circulation. This results are similar to result by (McDonald *et al*., 2003). However, from our study, the significant decrease in progesterone concentration in the dexamethasone treated sheep may be in part, due to the adverse effects of dexamethasone on placenta. Dexamethasone has previously been reported to have adverse effects on placenta like decreased placental weights and efficiency ((Pierre-Louis, 2010). Progesterone is mainly produced by corpora lutea (CL) and placenta during pregnancy (Luconi *et al*., 2012). However, in sheep progesterone is mainly produced by placenta with little contribution from the corpus luteum especially during advanced pregnancy (Moore *et al*., 2014). Therefore the maintenance of normal progesterone concentration and pregnancy in sheep is placenta-dependent. (Bearden and John, 2015) found that placental weight was decreased in the dexamethasone treated sheep which could have compromised placental efficiency and led to lowered progesterone production and secretion. During pregnancy uterine quiescence is maintained by elevated levels of circulating

progesterone acting through its receptor (PR), whereas decreasing progesterone concentration or activity promotes parturition or abortion (Virgo and Bellward, 2014). The low progesterone concentration could be the possible cause of abortion in dexamethasone treated sheep in this study. Progesterone inhibits prostaglandin synthesis and activity in pregnant subjects and consequently decreases myometrial contractility. This inhibition is mediated by a number of pathways that include blocking prostaglandin action, decreasing prostaglandin synthesis, and increasing its rate of inactivation (Perrot-Applanat *et al.*, 2011). A fall in progesterone concentration during pregnancy is associated with increased prostaglandin synthetase activity and prostaglandin F2α production that can predispose to abortion (Perrot-Applanat *et al*., 2011). The decreased progesterone concentration observed in this study is similar to that reported by (Ahmadabad *et al*., 2016) in pregnant mice treated with dexamethasone, but (Gale, 2008) who worked with dexamethasone to induce foetal lung maturation in human did not observe any alteration in the plasma concentrations of progesterone. In the present study, dexamethasone treatment did not alter circulating estrogen level during pregnancy in the sheep although (Ohrlander *et al*., 2013) previously reported suppression of estrogen production in pregnant women by dexamethasone. These differences may be due to differences in source progesterone secretion during pregnancy. The primary source of progesterone during pregnancy in human is the corpus luteum (CL). The placenta does not contribute substantially to progesterone production during pregnancy until mid-gestation. Therefore the negative influence of dexamethasone on placenta as reported in literature may not have significant effects on progesterone and estrogen production. In humans and other primates in particular, maintenance of the corpus luteum itself is favored by the hormone, Human Chorionic Gonadotropin (HCG). This hormone has luteinizing hormone- (LH-) like activity that protects the corpus luteum from regression and stimulates its production of progesterone. If conception occurs, the corpus luteum is maintained and grows and secretes increasing amounts of progesterone. Hence in humans, the corpus luteum continues to produce progesterone until around midgestation when placenta begins substantial contribution to progesterone production and release (Ylikorkala *et al*., 2012).

Conclusions

From this study, it was found that, Dexamethasone decreased progesterone concentrations and caused abortion in sheep but no aberrant effect on estrogen concentrations was observed.

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