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Quantitative Evaluation of Serum Oestrogen Levels in the Three Trimesters of Pregnancy in Albino Rat.

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Abstract
An experimental investigation using DRG Estradiol ELISA Kit to determine the levels of oestrogen in the serum of the first, second and third trimesters of pregnant and non-pregnant albino rats were undertaken.
Twenty albino rats were categorized into four different groups of five rats each. Group one (non-pregnant rats) served as the control. Groups two, three and four were pregnant rats in days 7, 14 and 21 of first, second and third trimesters respectively. Blood samples collected from the experimental controls were subjected to ELISA method for the assay of serum oestrogen levels. Results from this study revealed progressive increase in the mean oestrogen levels from 14.68±1.36pg/ml, 16.26±1.22pg/ml and 23.60±1.82pg/ml in the first, second and third trimesters respectively that significantly differed ($p< 0.001$) from control (9.74±0.50pg/ml). This report furthers supports the relevance of oestrogen in pregnancy.

Key words: Oestrogen, trimester, pregnancy , albino rat.

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Introduction

Pregnancy is the state of carrying a growing embryo or fetus inside the uterus of a woman or female animal carrying unborn offspring inside her womb from fertilization to birth. It starts when a male's sperm fertilizes ovum and implants into the lining of the uterus. In pregnancy, there are changes in a woman's normal hormone patterns.

In many societies, medical or legal definition, human pregnancy is somewhat arbitrarily divided into three trimester periods[1], as a means to simplify reference to the different stages of prenatal development. During pregnancy, the placenta produces large quantities of oestrogens, up to 100 times the amount secreted by the ovaries during the normal monthly cycle. Estrogen and progesterone stimulate the endometrium to support fertilized egg if pregnancy occurs.

In the normal non-pregnant female, estrogens are secreted in significant quantities mainly by the ovaries, although minute amount are also secreted by the adrenal cortices. β-estradiol is considered to be the major oestrogen, although the estrogenic effects of estrone are far from negligible[2].

These hormones promote the development of female secondary sexual characteristics such as the growth and development of the breast, regulation of the menstrual cycle and preparation of the uterus for pregnancy by enriching and thickening the endometrium [3]. Such endometrium under the influence of oestrogen is essential for implantation and consequent development of the fetus.

Furthermore, Oestrogen helps to protect the heart and bones, maintains the breasts, womb, vagina and bladder in their healthy state and causes less facial hair and smoother skin in women than men[4]. Oestrogen had been reported[5] to increases nitric oxide-dependent relaxation during pregnancy which results in increased blood supply to the endometrium. This role is opposed by oestrogen receptor antagonist tamoxifen which was said to prevent a pregnancy - associated increase in nitric oxide synthase (NOS) activity in a variety of tissues. The indispensable role of oestrogen or its derivative and its derivative Diethylstilbestrol (DES), is supported by a report[6] which claimed that Diethylstilbestrol (DES) for decades was widely used as it is believed to prevent miscarriage and other undesirable outcomes. During the second trimester, the development of the fetus can be more easily monitored and diagnosed.

The aim of this study is the determination of estrogen levels in first, second and third trimesters of pregnant albino rats.

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Materials and Methods

Test subjects

Twenty female albino rats and six male albino rats, with initial weight of 165-180g were purchased from the animal house of the Department of Animal and Environmental Biology, University of Benin, Benin city, Edo state, Nigeria. The twenty female rats were housed in wooden cages in groups of five (5) rats per group while the 6 male rats were kept in a separate cage in the Animal House facility of the Department of Animal and Environmental Biology (AEB), Faculty of Life Science, University of Benin, Benin City. They were acclimatized for seven days. A 12-hours photoperiodic light:dark cycle was maintained. They were fed with growers mash (produced from Ewu flour mill, in Edo state) and water *ad libitum* until a weight between 210-290g was achieved.

All procedures adopted complied with the International Guidelines for the Care and Use of Laboratory Animals as stated by the Ethical Committee, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

The female rats in estrous in three of the cages were mated by introducing two (2) male rats to the separate cages containing five (5) female rats each overnight. The following morning, the female rats were checked for signs of pregnancy by confirming presence of sperm in vagina smears or cervical plug.

The female rats were divided into four groups; the non pregnant group serving as control; the pregnant first trimester, second trimester and third trimester groups.

At day 5, 12 and 19 corresponding to first, second and third trimester periods respectively, the animals were anaesthetized and blood samples were collected from the abdominal aorta using a 5ml syringe each. The blood sample was immediately transferred into a non EDTA bottle for recovery of serum. The same procedure was undertaken for the control (non pregnant) group.

Statistical analysis was performed using the computer program GRAPHPAD INSTAT. All data were expressed as Mean + SEM. All data were analyzed by a simgle factor design ANOVA. Statistical significance was accepted at ($p < 0.05$).

Results

The results of serum level of oestrogen in pregnancy are presented below in figures I showing significant increases in estrogen levels in first, second and third trimesters of pregnancy in albino rats.

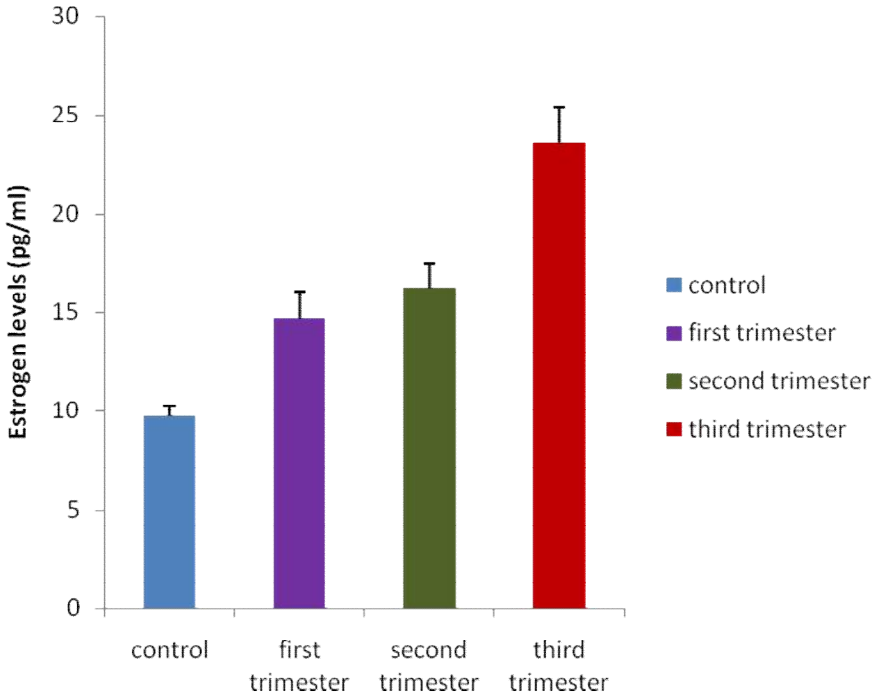

Figure I: A bar chart showing Estrogen levels (pg/ml) in first, second and third trimesters of pregnancy in albino rats.

The value in the first trimester (14.68±1.36pg/ml), second trimester (16.26±1.22pg/ml) and third trimester (23.60±1.82/mlpg) were compared to control ((9.74±0.50pg/ml). This reveals statistically significant increases ($p < 0.001$)

Discussion

The present data showed the quantitative difference between the oestrogen levels in pregnant and non-pregnant albino rats. Results from this study showed that there was an increase in oestrogen levels during pregnancy. The growth-promoting and vascularizing effect of oestrogen on the uterus is in harmony with the fact that increasing amounts of it are present in the body fluids as pregnancy progresses**.** Analysis of variance of the control, first, second and third trimesters showed that there was a significant increase in the amount of oestrogen levels in the three trimesters of pregnancy. The mean value of oestrogen levels recorded in the non-pregnant was significantly lower ($p<0.001$) when compared with the pregnant groups. The concentration of estrogen increased steadily with the duration of pregnancy. The increases observed were significantly higher when compared with the non pregnant rats. This is in line with the work done by Nelson and Bulun[4].

During the first trimester, estrogen production was mostly made by the placenta, and this continues until near the end of the pregnancy when the amount circulating in the body is a thousand times the amount when not pregnant[5]. This rapid increase in estrogen level during pregnancy could be as a result of a massive production of estrone and estriol by the placenta[4]. The increase in estrogen during pregnancy gets to its peak just before parturition. Absence of estrogen just before parturition has been known to affect parturition negatively as reported by Csapo, et al.[7] who showed that rats ovariectomized two days before term maintained life litters but failed to deliver them; but estrogen replacement therapy on the expected day of parturition resulted in normal delivery. Waynforth, et al.[8] concluded that the greatly increased ovarian estradiol-17β secretion in the last 1 to 2 days of gestation, when progesterone secretion has fallen to low values, provides physiologically the high estrogen levels, required for normal delivery. Oestrogen is a hormone involved in the ripening of the neck of the womb (cervix) and preparing it for the birth of the baby. It is possible that oestrogen increases the release of other local hormones (prostaglandins) which help ripen the cervix[9].

This study has shown that oestrogen levels increases during pregnancy in Albino rats compared to when not pregnant. This could be as a result of massive production of oestrogen by the placenta during pregnancy. This increase in the level of estrogen in pregnancy supports previous reports thus validating the indispensable role of this hormone in sustaining normal gestation and parturition.

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