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Evaluation of Serum Estrogen Progesterone Ratio in the Three Trimesters of Pregnancy in Wistar Rat

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ABSTRACT: Estrogen and Progesterone are two major hormones of pregnancy. As soon as a spermatozoon fertilizes an ovum, hormonal changes begin in preparation for implantation and subsequent maintenance of pregnancy. The aim of the study was to determine if there are changes in Estrogen/Progesterone ratios (E/P) during pregnancy in different trimesters of pregnancy in Wistar rats. Twenty female Wistar 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 5, 12 and 19corresponding to first, second and third trimesters of pregnancy respectively. Blood samples collected from the four groups were subjected to ELISA method for the assay of serum estrogen and progesterone levels. There were statistically significant ($p<0.05$) increases in progesterone in the second and third trimesters when compared with non-pregnant Wistar rats (5.70±1.35pg). There was a statistically significant increase in estrogen levels in the three trimesters of pregnancy. Also, there was a significant correlation between estrogen and progesterone in the second and third trimester of pregnancy but not in the control and first trimester. The E/P ratio in the control was significantly ($p<0.01$) higher than that of the second and third trimester but not for the first trimester. Conclusively, the E/P ratios are altered during pregnancy and knowledge of this is relevant in hormone therapy in assisted pregnancy technology.

Keywords: E/P ratio, Estrogen, Progesterone, Trimester, Pregnancy, Wistar rat.

Introduction

Pregnancy is the state of carrying a growing embryo or fetus inside the uterus of a woman from fertilization to birth. During pregnancy, there are changes in a woman's normal hormone patterns. Human pregnancy is somewhat arbitrarily divided into three trimester periods (Olatunji-Bello II *et al*., 2001) as a means to simplify reference to the different stages of prenatal development. During pregnancy, the placenta produces large quantities of estrogen and progesterone. 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. B-estradiol is considered to be the major estrogen, although the estrogenic effects of estrone are far from negligible (Guyton and Hall, 2000). They promote the development of female secondary sexual characteristics, and are also involved in regulating the menstrual cycle and prepare the uterus for pregnancy by enriching and thickening the endometrium (Ferenczy *et al*., 1979).

Estrogen also helps to protect the heart and bones, as well as maintaining the breasts, womb, vagina and bladder in their healthy state as well as less facial hair and smoother skin than men (Nelson and Bulun, 2001). Estrogen also plays a very important role in the development of the foetus by maintaining the endometrium during implantation.

Yunlong *et al*. (2001) reported that estrogen increases nitric oxide-dependent relaxation during pregnancy and that the estrogen receptor antagonist tamoxifen prevents a pregnancy–associated increase in nitric oxide synthase (NOS) activity in a variety of tissues. Diethylstilbestrol (DES) was for decades widely believed to prevent miscarriage and other undesirable outcomes (Bamigboye and Morris 2003). During the second trimester, the development of the foetus can be more easily monitored and diagnosed. The beginning of the third trimester often approximates the foetus to survive, with or without medical help, outside of the uterus (Richardson and Corfman, 2008).

Progesterone during pregnancy is primarily produced by the placenta. In women, progesterone levels during the preovulatory phase of the menstrual cycle is relatively low, rises after ovulation and are elevated during the luteal phase (Farage *et al*., 2009). Progesterone levels tend to be <2 ng/ml prior to ovulation and >5ng/ml after ovulation. If pregnancy occurs, human chorionic gonadotropin is released maintain ning the corpus luteum, allowing it to maintain levels of progesterone. At 8weeks, the placenta begins to produce progesterone in place of the corpus luteum. This process is called the luteal-placental shift (Chabbert *et al*., 2007). Progesterone function is to inhibit the smooth muscle in the uterus from contracting and decreasing prostaglandin formation, both of which allow the foetus to grow with the expanding uterus. It also serves as a precursor to most steroid hormones (Bowen, 2000).
Although both estrogen and progesterone are important in maintenance of pregnancy, the ratios of Estrogen/Progesterone might vary from one level of pregnancy to the other. The aim of this study is to investigate the normal ratios of these important pregnancy hormones in order to know the appropriate ratio to be used in hormone therapy during pregnancy especially with assisted fertility treatments.

Materials and Methods

Test Animals: Twenty female and six male Wistar rats, with initial weight of 165-180 g 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 Sciences, University of Benin, Benin City. They were acclimatized for seven days. A 12-hours photoperiodic light and dark cycle was maintained. They were fed with growers mash (produced from Ewu Flour Mills, in Edo State) and water ad-libitum until a weight between 210-290 g 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 overnight by introducing two (2) male rats to the separate cages containing five (5) female rats each. 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 days 5, 12 and 19 corresponding to first, second and third trimester periods respectively, the animals were anaesthetized and blood samples were collected using a 5ml syringe each. The blood sample was immediately transferred into a plain bottle for recovery of serum. The same procedure was undertaken for the control (non-pregnant) group. The collected sera were assayed for estrogen and progesterone respectively.
Calculation of estrogen and progesterone ratio was done by Estrogen/Progesterone.

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

Results

The levels of estrogen, progesterone and E/P ratios in the stages of pregnancy and in non-pregnant rats (control) are shown in Table 1.

Table1: Levels of Estrogen, progesterone and E/P ratios in the stages of pregnancy and in non-pregnant rats (control). Values are represented as mean ± S. E. M

Stages of Pregnancy	Progesterone (pg/mL)	Estrogen (pg/mL)	Estrogen/Progesterone ratio
Control	5.70±1.35	9.74 ± 0.50	1.46±0.17
First Trimester	11.78 ± 2.83[b]	14.68 ± 1.36[a]	1.08±0.12[b]
Second Trimester	25.62± 3.74[a]	16.26 ± 1.22[a]	0.69±0.1[a]
Third trimester	36.36± 1.17[a]	23.60 ± 1.82[a]	0.65±0.06[a]

a – indicates that it was statistically significant when compared with control ($p< 0.05$)
b – indicates that it was not statistically significant when compared with control ($p> 0.05$)
n – 5 per group

There was a significant increase ($p<0.05$) in progesterone concentration as pregnancy advances, with the third trimester having the highest value (36.36±1.17 pg/mL), and the levels of progesterone during the three trimesters were significant higher ($p<0.05$) than the controls except in first trimester. There was also a significant increase ($p<0.05$) in estrogen concentration with the third trimester having the highest value (23.60±1.82 pg/mL), and the estrogen levels in each trimester of pregnancy was significantly higher than the control.

The control group had the highest estrogen/progesterone ratio compared to the first, second and third trimesters of pregnancy. There was a significant decrease ($p<0.05$) in estrogen/progesterone ratio in the pregnant group (second and third trimesters).

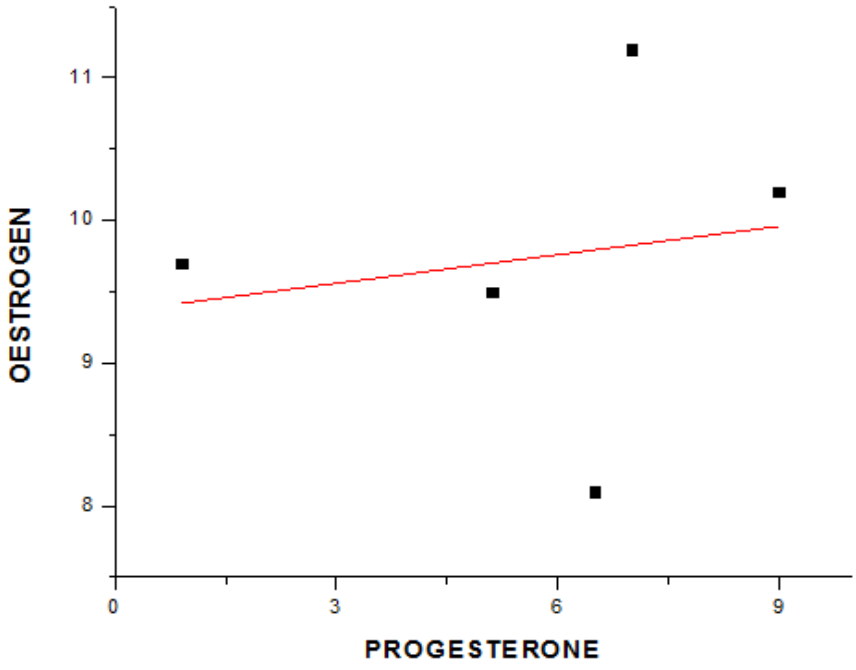

Figure 1: Correlation of estrogen and progesterone for control group ($p>0.05$)

There was no significant correlation between estrogen and progesterone values in the control group.

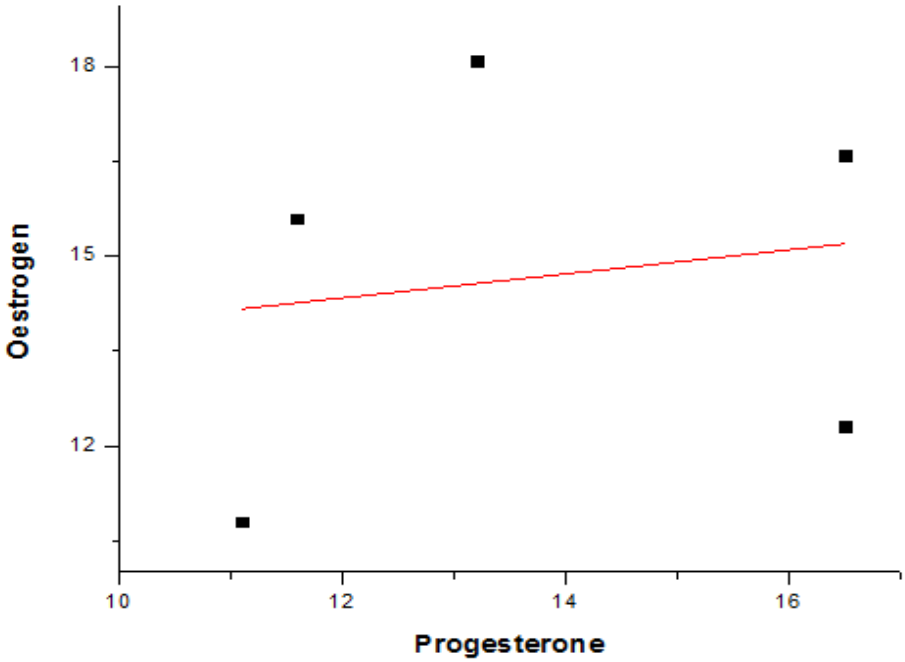

Figure 2: Correlation of estrogen and progesterone for first trimester ($p>0.05$)

There was no significant correlation between estrogen and progesterone values in the first trimester of pregnancy.

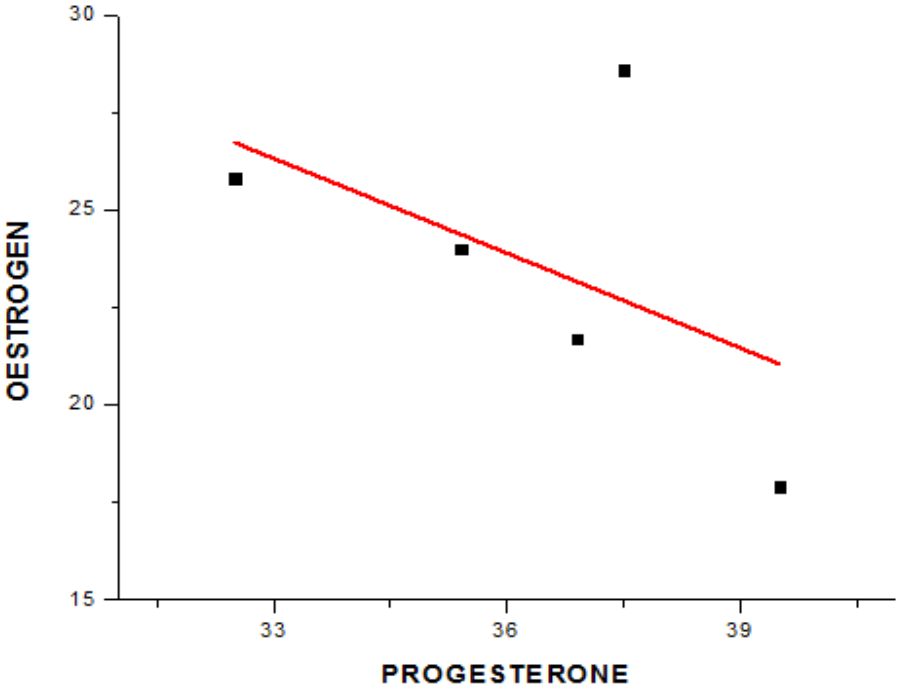

Figure 3: Correlation of estrogen and progesterone in second trimester ($p<0.05$)

There was a significant correlation between estrogen and progesterone values in the second trimester of pregnancy

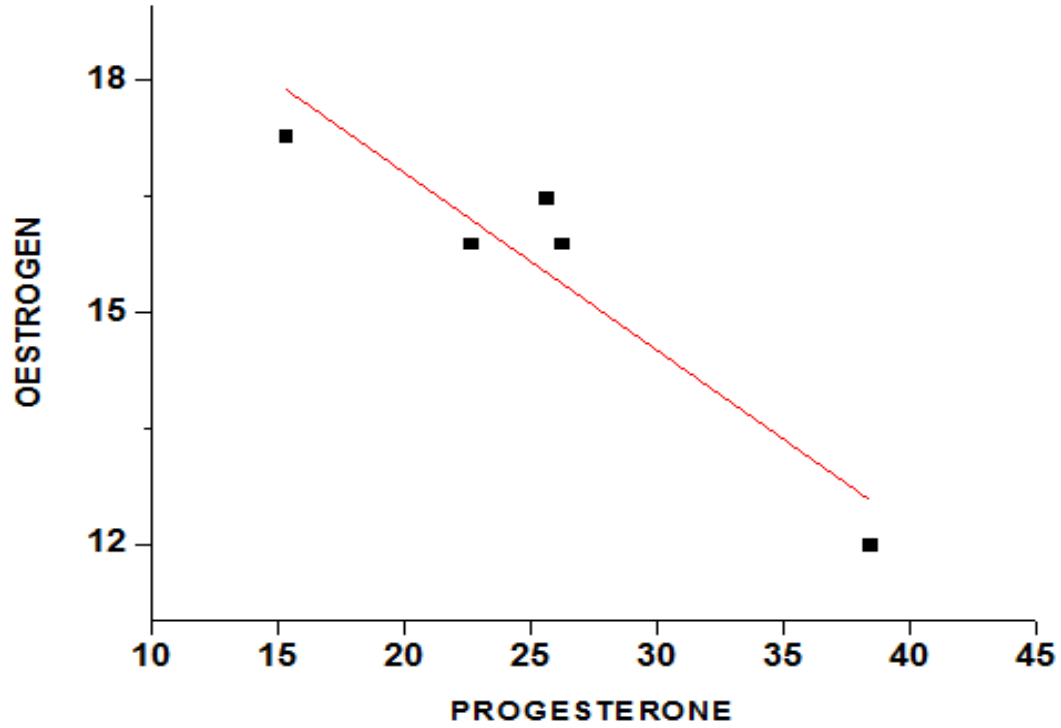

Figure 4: Correlation of estrogen and progesterone in third trimester ($p<0.05$)

There was a significant correlation between estrogen and progesterone values in the third trimester of pregnancy.

Discussion

Estrogen and Progesterone are sex hormones found in greater quantities in females and are the dominant hormones for the maintenance of pregnancy. These two hormones have been reported to increase remarkably with progress of pregnancy as reported by Agoreyo and Okeke (2014) and Agoreyo and Onwegbu (2015). There was increase in progesterone levels in the trimesters of pregnancy (Agoreyo and Onwegbu 2015). This was similar to the observed statistically significant increases in the levels of estrogen in pregnant female wistar rats (Agoreyo and Okeke 2014).

The increase in progesterone level at the different stages of pregnancy is as a result of conversion of uterine endometrium to its secretory stage to prepare the uterus for implantation (Guyton and Hall, 2006). During implantation and gestation, it decreases the maternal immune response to allow for the acceptance of the pregnancy and decreases contractility of the uterine smooth muscle (Bowen, 2000). Therefore, the progressive increase in the level of progesterone during the stages of pregnancy shows that progesterone plays a major role in the development of the foetus thus it is sometimes called the "hormone of pregnancy" (Bowen, 2000).

There was no significant correlation between estrogen and progesterone in the control group and first trimester but a significant correlation was seen in the second and third trimesters of pregnancy. This further indicates that as estrogen increases, progesteronealso increase up till the delivery is near when progesterone starts decreasing and estrogen continues to increase to aid in the delivery process.

The Estrogen/Progesterone ratio in the control was significantly ($p<0.01$) higher than that of the second and third trimester but not for the first trimester. The estrogen/progesterone ratio of the first trimester was higher than that of the second and third trimester although this was not statistically significant ($p>0.05$) which was due to greater increases in progesterone concentration.

Both progesterone and estradiol are essential for the endometrial preparation in order to be able to harbor the growing blastocyst. Controlled ovarian hyperstimulation used during intracytoplasmic sperm injection(ICSI) cycles

leads to abnormally high serum levels of estradiol (E2) and progesterone (P) secondary to excessive follicular growth. Such an imbalance can adversely affect the luteal phase and the implantation rates in ICSI cycles. According to Gidley-Baird *et al.* (1986), the comparison of steroid levels between women who became pregnant and those who did not revealed that women who failed to become pregnant had significantly higher E2 levels and a lower ratio of P/E2 than women who became pregnant, also they found out that the P/E2 ratio was a better predictor of implantation failure than was the absolute level of either progesterone or estradiol (Gidley-Baird *et al*., 1986). A higher E/P ratio was seen in the control group, this may be an indication for a period of effective implantation of the fertilized ovum. The ratios in the three trimesters of pregnancy can be used as a tool to access the functionality of these hormones of pregnancy.

Conclusion

In our study, it was concluded that Estrogen/Progesterone ratio is altered during pregnancy. Knowledge of this is relevant in hormone therapy in assisted pregnancy technology.

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