RESEARCH
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RELATIONSHIP OF HORMONAL AND IMMUNOLOGICAL FACTORS TO RECURRENT SPONTANEOUS MISCARRIAGE IN ERBIL CITY

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Abstract

The current study was conducted with the aim of evaluating some hormonal, immunological parameters and folic acid and their relationships with women with recurrent miscarriages. The samples of the current study were collected from Erbil women's hospital and some clinics for gynecological diseases. 64 women with repeated spontaneous miscarriage were included in the research and 41 women served as the comparison group each of whom had one or more healthy children, 32 were pregnant. During the period between January and December 2020, al abortions occurred during the first three months of pregnancy (1-12) weeks. The study recorded significant differences in the levels of the hormones estrogen, progesterone as well as prolactin between women with frequent spontaneous abortion and non-pregnant women, while significant differences were found in the levels of these hormones among women with frequent abortion compared with pregnant women and it was 24.61 for progesterone 110 mg/ml and 13.4 mg/ml for prolactin, the results showed that there were significant difference in folic acid levels, the highest in non-pregnant women was 14.24 mg/ml and the lowest in infected women, and the IL-2 immune factor, which was 120.23, which was the highest in women with recurrent miscarriages.

Keywords: Miscarriages, Progesterone, Estrogen, Prolactin.

INTRODUCTION

Repeated spontaneous miscarriage is one of the serious and important medical problems that has increased alarmingly in the world in general and Iraq in particular. Repeated spontaneous abortion is common in populations and is described as three or more pregnancies lost in a row with the same intimate partner within 28 weeks of pregnancy (Guerrero et al., 2020). The reasons that it stands behind it and the associated risk factors vary greatly between different societies. Pregnancy loss occurring before the twenty week of pregnancy is defined as RSA by the American Society for Reproductive Medicine (Homer, 2019). About 50% of RSAs and 1% to 2% of all partners attempting to procreate have RSA (Von Woon et al., 2022). The root reasons are still unclear. Maternal immunological variables are directly linked to 80% of inexplicable pregnancies, thrombophilia factors (including hereditary and acquired thrombosis), anatomical abnormalities of the uterus, as well as endocrine abnormalities are the 4 most common causes Importance (Coomarasamy et al., 2021).

Intermittent and repeated miscarriages are the two kinds. A loss occurs in about 15% of all clinically confirmed births (Ali et al., 2020). The exact cause of a particular miscarriage is rarely determined on an individual basis. The reason is frequently unknown despite intensive medical testing and novel therapies for many people (Brosens et al., 2022). Abnormalities associated with diethylstilbestrol or acquired (Hennessy et al., 2021), women who have experienced three or more straight losses may develop conditions like fetal adhesions or leiomyomas and undergo hysterosalpingography or hysterosalpingography (Ali et al., 2020).

The corpus luteum produces progesterone, which is required to keep the lining of the uterus in order to support and grow the embryo. A luteal phase deficiency occurs when progesterone production is inadequate after fertilization. A subpar or delayed menstruation is typically the cause of a luteal phase abnormality. Recurrent miscarriages have been associated with high levels of luteinizing hormone and polycystic ovarian syndrome (Peretyatko et al., 2021). In a research that contrasted women with abnormal ovaries and repeated loss with those with typical ovarian shape, the average Very comparable live delivery rates 58.5% and 60.9%, respectivelyare observed (Chakravarty, 2019), Furthermore, bromocriptine treatment for hyperprolactinemia resulted in an 85.7% success rate for pregnancies, compared to only 52.4% for the control group, despite the fact that hyperprolactinemia raises the risk of repeated loss (Min et al., 2020).

For expectant women, folic acid (Pteroylglutamic) is the most metabolized and stable type of folate (Matjila et al., 2017). There have been conflicting findings in published studies relating FA intake to abortion risk (Deng et al., 2022) that folic acid shortage, flaws in FA and homocysteine biosynthesis, and a higher chance of miscarriage. The mean IL-2 level was in patients hospitalized for termination of pregnancy due to early pregnancy failure who had first-trimester repeated inexplicable pregnancy loss, and the IL-2 level was lower in women. Low plasma folic acid levels were linked to an increased chance of miscarriage. First-trimester expectant women in good health who had at least one infant and had no prior history of having an unfavorable perinatal result (Maiter and Chanson, 2022), the research revealed a significant impact of raised IL-2 may be (also documented no substantial link between high IL-2 level and chance of spontaneous miscarriage, and Due to the low amount of losses, as well as the possibility that some of the patients were having their first abortions (Barapatre and Vaidya, 2017).

MATERIALS AND METHODS

Samples

64 individuals with a history of main recurrent spontaneous abortion (RSA), which is characterized by three or more sequential losses, were included in the study, were selected. Each patient was filled out a questionnaire form that included: name, age, weight, family history, and geographic origin. Any patients with from previous or current medical disorders related or unrelated to pregnancy from the study, the positive control group was 41 abnormal pregnant women in the first trimester of pregnancy. Blood samples were collected from these women who had had a previous miscarriage and healthy non-pregnant women: - Obtaining blood samples on the 21st day of the cycle. As for normal pregnant women, they were collected in the first trimester of pregnancy.

Hormonal assay

Hormonal concentrations were examined by the Vita Diagnostic Immunoassay Assay (VIDAS) using combinations of estradiol, progesterone and prolactin (Pandey et al., 2005).

Folic acid in bloodassay

The blood samples were diluted with sample buffer (1:100) before the test and folic acid was measured using the proper reagent and ELISA method. By mixing a 10 l sample with 40 l of the solvent, the recommended 100-fold reduction was accomplished. 15 mL of this fluid was added to 285 mL of solvent to finish the 100-fold reduction (Kaldygulova et al., 2023).

Measurement of interleukin-2

Huma Reader-HS was used, the serum was diluted to 10 microliters with Buffer 1000ml, after which it was washed and the steps were followed until the end of five minutes (Banerjee et al., 2013).

Statistical analysis

The t-test in the GrpaphPad Prism Software was served for comparing between values of the study groups. Differences between vales were considered significant at $P<0.05$ (Al-Eodawee et al., 2023).

Results and Discussion

Hormonal examination

Progesterone levels in blood were statistically different between women with RSA and healthy, reproductive women who were not pregnant in this study, but they were statistically different (P 0.05) between women with RSA and healthy expectant women (Figure 1)

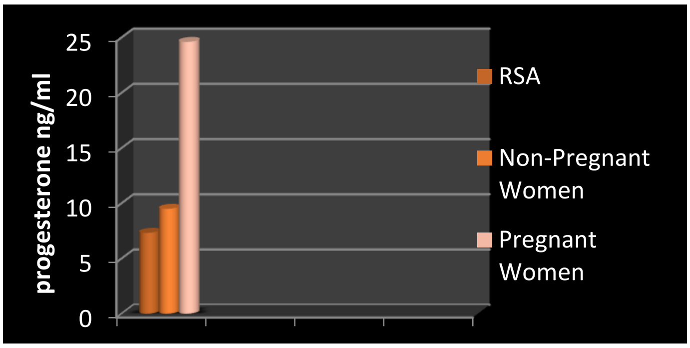

Figure (1): Serum progesterone ng/ml in women with RSA as compare to the normal control (LSD=0.397)

Serum progesterone concentrations were numerically lower in women with RSA at 7.32 ng /mL than in normal non-pregnant women at 9.49 ng/mL when compared to women without RSA; there was a significant drop (P 0.05). Females: 24.61 ng/mL Figure 1.Several hormonal processes that promote the embryo' healthy growth and development are necessary for the establishment and preservation of pregnancy. Endocrine disorders are thought to be the cause of 8 to 12% of all RPL instances. The progesterone synthesis-related corpus luteum deficiency, placental abruption, and maternal progesterone malfunction can be differentiated from endocrine diseases linked to sporadic and repeated pregnancies.

It was also found in the results a significant decrease in progesterone levels in blood serum in all aborted women at different stages of pregnancy compared with other treatments, which indicates an important role for progesterone in spontaneous abortion for women during the first and second trimesters of pregnancy (Raghupathy and Szekeres-Bartho, 2022).

Progesterone and estrogen levels rise during ovarian stimulation and ovulation induction, and they fall during a normal pregnancy, but they rise again during the typical transition phase (weeks 7–11) and may be higher than they were during the first trimester, and the high rate of miscarriage in cases with syndrome Overstimulation, where progesterone and estradiol drop from a very high level supports this concept (Thiele et al., 2019).

Abnormalities of the luteal phase in advanced stage RPL have been historically reported in women with RPL (Csabai et al., 2020). However, there is currently no agreement on the optimal technique for identification, which may include measuring blood progesterone amounts or performing uterine sample. Extremely high serum progesterone levels during the midst of the luteal period, greater than 10 ng/mL, are linked to luteal abnormalities. Progesterone levels can vary during pregnancy, and those below 12% of average have been linked to a higher chance of abortion. Measurements Due to the pulsatile nature of release (Shah et al., 2019) determining progesterone levels can be a constant challenge. If progesterone levels are low, the fetus may not be able to be sustained. Low progesterone levels may come from a lack of human chorionic gonadotropin (hCG) and thus from a miscarriage.

In this study, statistically significant differences were found in serum estradiol in women infected with RSA with an amount of 110 mg / ml compared to normal non-pregnant women 100 mg / ml, while there was a significant ($P < 0.05$) difference between women infected with RSA and pregnant women. Normal (245 mg/mL) results show the frequency of serum estradiol concentrations in RSA women compared to pregnant and non-pregnant women (Figure 2).

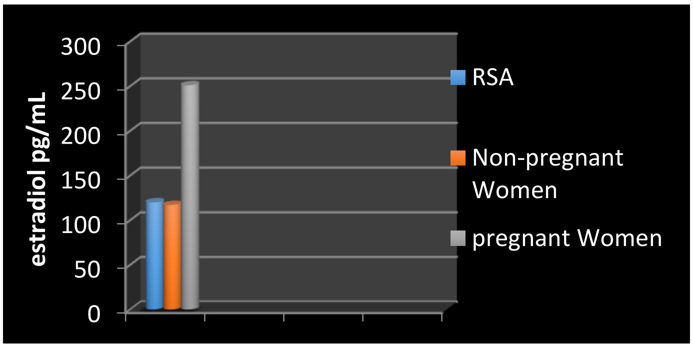

Figure (2): Comparison of serum estrogen amounts (pg/mL) between women with RSA and healthy controls (LSD=9.120).

The concentration of estradiol was significantly lower in the aborted women and there was a significant difference between the aborted women and the control groups but there was no difference between the first and second trimesters of the aborted women group. In other studies, it was also found that the concentration of estradiol was significantly lower in women who had a miscarriage than in women who had a live birth (Li et al., 2023). In contrast, some studies reported that the concentration of estradiol was much higher in women who had an abortion than those who had a live birth. Estradiol is essential for normal pregnancy development (Abdul-Hussain et al., 2020). Oligomenorrhea and a solitary estrogen deficit during the luteal phase of the menstruation period were associated with an increased chance of abortion in women. Estradiol in early pregnancy comes from the placenta and the corpus luteum. Therefore, any deficiency in the contents of the corpus luteum estradiol or a delay in the biosynthesis of estradiol by the placenta may cause miscarriage (La et al., 2021)

Women with RSA had blood prolactin concentrations of 14.3 mg/mL, while viable non-pregnant women had concentrations of 7.4 mg/mL. However, there were statistically significant variations between women with RSA and typical expectant women, who had concentrations of 13.4 mg/mL (Figure 3).

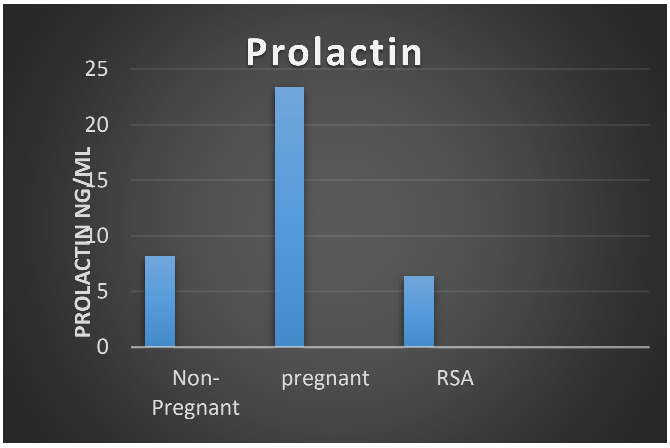

Figure 3: Frequency of serum prolactin concentrations in RSA women and healthy controls (LSD 5%=1.76).

The frequency of serum prolactin concentrations in RSA women and healthy controls was detected in the current study (Figure 3). This finding is related to another study of women with RPL, in which only three had numerically elevated levels of prolactin and one had a significantly high level. It was reported that a clinical trial of a group of women Successful pregnancies were more common in patients whose hyperprolactinemia was managed with bromocriptine. Women who experienced losses had noticeably greater prolactin levels. While the live delivery percentage for the untreated group was 52.4%, it was significantly higher for the treatment group. They administered bromocriptine. Serum prolactin levels (%) were stabilized before fertilization and remained so until the end of the ninth week of pregnancy in this cohort of patients.

Lactotroph cells in the pituitary gland and elsewhere (including the breast gland, placenta, uterus, and T lymphocytes), release and make prolactin. Many studies point to prolactin's importance in achieving conception. Women with RPL often have their prolactin levels checked because high amounts of this hormone are linked to a lack of ovulation. Endocrine diseases like diabetes, polycystic ovarian syndrome, hyperprolactinemia, and luteal phase abnormalities should always be checked (Hudić *et al.,* 2021).

-Some hematological and immunological parameters

In the current study, the folic acid level was measured as 5.31 ng/mL in women with RSA with a significant difference (P≤0.05) compared to fertilized women either pregnant 14.24 ng/mL or non-pregnant 12.93 ng/mL Table 1

Table 1: Folic acid concentrations and IL _2 in women with RSA and controls

Groups	Folic acid ng/ml	IL _2 pg/m
RSA	5.31	120.23
Non-pregnant	12.93	55.81
Pregnant	14.24	40.24
LSD	1.70	1.230

There was no correlation found between taking folic acid before conception and an increased risk of abortion. In early pregnancy, women who consumed 400 milligrams (mg) of folic acid alone had a reduced loss incidence than those who did not (Cheng et al., 2022). Women who naturally lost a baby between 6 and 12 weeks of gestation were used as cases, and women whose pregnancies were of a similar length were used as controls, in a research examining the link between folic acid levels and the occurrence of abortion. Miscarriages were more common in women with lower plasma folic acid concentrations than in those with greater concentrations, but the risk of miscarriage did not rise in women with higher plasma folic acid concentrations (Husebye et al., 2022), In patients with inexplicable repeated pregnancy loss in the first trimester referred for termination of pregnancy due to early pregnancy failure, the mean folic acid was 8.7 ng/mL, whereas in healthy first-trimester expectant women, the mean folic acid was 10.8 ng/mL have at least one healthy childbirth and no history of maternal complications. There is a major dissimilarity between the two categories (Löb et al., 2021).

Table 1 showed a rise in interleukin 2 in aborted women, reaching 120.23 Pg/m, while it was 55.82 Pg/m and 40.24 Pg/m for non-pregnant and pregnant women, respectively, and that Fetal development and pregnancy maintenance are aided by a lack of inflammatory responses to cytokines, or vice versa. Because interleukin-2 and tumor necrosis factor are part of the inflammatory cytokines, it's safe to say that these cytokines work to quell inflammation, and their action in the cell is a response that works to delay hypersensitivity reactions and high concentrations, including increased abortions due to increased uterine muscle contraction by the action of interleukin- 2, which stimulates the production of cytokines by increasing more. It leads to miscarriage and fetal death (Gatti et al., 2020). Repeated abortions are used to stimulate apoptosis of widow cells, that cytokines participate in the immune interaction of the mother and fetus and have a role in determining the success of pregnancy, and that immune factors have a role in abortions through cytokines in rejection in the work of immune rejection of the fetus and lack of development the fetus and placenta and thus the pregnancy fail (Peng et al., 2021).

CONCLUSION

There have been many changes and differences in hormone levels in women exposed to recurrent miscarriages compared to pregnant women who are not exposed to abortion and non-pregnant women, in addition to changes in the levels of immune response in general and in the levels of interleukin-2 in particular, since interleukin-2 and tumor necrosis factor are part of inflammatory cytokines, it is safe to say that these cytokines calm inflammation, and their action in the cell is a response that delays hypersensitivity reactions and high concentrations, including increased abortions due to increased contraction of the muscles of the uterus by the action of interleukin-2, which stimulates the production of cytokines by increasing further leads to miscarriage and fetal death.

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