

Down-Regulation of Progesterone hormone in Recurrent pregnancy Loss

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Summary:

Background:

Progesterone hormone is important in the preparation for and in maintaining of pregnancy through different mechanisms but mainly by shifting the balance from Th1 to Th2 mediated immunity to avoid rejection of the fetus; in this study we tried to find out whether there is a relation between the level of the expression of progesterone and its stimulant HCG hormone and recurrent pregnancy loss (RPL).

Patients and Methods:

Immunohistochemistry technique was performed to detect and determine the expression of progesterone and HCG hormones using paraffin embedded sections of curate samples obtained from 40 women, who were divided into three groups: 24 women with RPL, 10 women with abortion for the first time, and 6 women with induced abortion.

Results:

The levels of the expression of both progesterone and HCG hormones were found to be significantly down-regulated in the first group as compared with the second group ($p=0.00$), and the third group ($p=0.00$), with a highly significant positive correlation between these two parameters ($r=0.866$, $p<0.01$).

Conclusion:

Low expression of progesterone in women with RPL could raise the possibility of underlying immuno-endocrine pathology responsible for this recurrent loss due to the important role of progesterone as an immunosuppressive hormone help in maintaining pregnancy.

Key words: Progesterone, HCG, RPL.

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Introduction:

Recurrent pregnancy loss (RPL) is defined as three or more consecutive spontaneous abortions (1). It affects about 1% of the child-bearing population (2). There have been numerous proposed causes of RPL, one of these causes can be identified in about 50% of patients, however; in the remainder the cause is unknown.

An immune-based etiology underlying unexplained RPL has been proposed; however the exact mechanism has not been elucidated (3).

The interactions between immune-endocrine and reproductive systems are heightened during pregnancy as an adaptive mechanism and are regulated by a complex array of hormones and cytokines that control the survival of a semiallogeneic conceptus (4).

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During early pregnancy progesterone and estrogen promote proliferation and differentiation of endometrial stromal and epithelial cells while human chorionic gonadotropin (HCG) maintains progesterone release from corpus luteum of the ovary (5). Progesterone is liberated by the corpus luteum, due to the actions of IL-4 and IL-6 from Th2 cells (6), after ovulation and acts on the endometrium to prepare it for implantation (7). Throughout pregnancy, progesterone produced by the placental cells inhibits T-cell mediated graft rejection in the uterus (8). This is achieved by the shift in balance from a Th1 to a Th2 cytokine environment (9) and the inhibition of lymphocyte activation and proliferation (10).

Lymphocytes of pregnant women have been shown to be particularly sensitive to the effects

Materials and Methods

Patients were divided into three groups; Group A: 24 pregnant ladies presented with incomplete first trimester abortion, all of whom gave a history of previous 3-6 consecutive first trimester abortions, with no medical diseases, family history of genetic diseases or uterine anatomical anomaly, also all of them were negative for acute infection with rubella, HCMV and toxoplasmosis. Group B: 10 pregnant ladies presented with incomplete first trimester abortion and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness, and Group C: 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indication under approved consent of two senior gynecologists and a physician. Curate samples of the materno-fetal interface were taken from

Results

Tables (1) and (2) show the percentages of progesterone and HCG expression respectively in terms of mean \pm SE, minimum and maximum values of the three groups. Tables (3) and (4) show the difference in the expression of progesterone and HCG among the three groups

of progesterone compared to those non-pregnant women (10). The state of pregnancy itself causes an increase in the number of progesterone receptors that are displayed on peripheral blood lymphocytes making them more susceptible to progesterone's inhibitory mechanisms (8). Progesterone also acts on syncytiotrophoblast cells driving them to produce cytokines that favour the Th2 environment necessary for successful pregnancy (9).

In this study, we attempt to explore the expression of progesterone and HCG hormones in women with RPL and compare it with that in normal pregnancy and women with pregnancy loss for the first time using monoclonal antibodies of progesterone and HCG hormones.

all these women at the end of evacuation curate operation.

Samples were embedded in paraffin and subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark). Refer to the immunohistochemistry procedure and signal evaluation in references (11, 12), dilutions of the monoclonal antibodies were: (progesterone 1:30, HCG 1:50) (BioGenex-USA). Negative controls were obtained by omitting the monoclonal antibody and using antibody diluent alone to verify the signal specificity.

Statistics: ANOVA test was used to determine the difference in the expression of progesterone and HCG among the three groups, and the relationship between these two parameters was measured using the correlation coefficient (r). Values of $p < 0.05$ were considered as statistically significant. and within the groups respectively using ANOVA analysis.

The study demonstrates a highly significant positive correlation between the expression of progesterone and HCG in the RPI group ($r=0.866, p < 0.01$).

Table 1. The expression of progesterone among the studied groups

Progesterone	n	Mean \pm S.E. ^ψ	Min. Value	Max. Value
Group A	4 ²	43.8 \pm 2.4	30	70
Group B	0 ¹	67.2 \pm 3.9	40	82
Group C	6	66.5 \pm 3.6	55	77

ψ Standard error
Total mean 53 \pm 2.5 %

Table 2. The expression of HCG among the studied groups

HCG	N	Mean \pm S.E. ^ψ	Min. Value	Max. Value
Group A	4 ²	60.4 \pm 1.9	45	80
Group B	0 ¹	76.8 \pm 1.9	66	88
Group C	6	78.3 \pm 4.6	60	95

ψ Standard error
Total mean 67.2 \pm 1.9 %

Table 3. The significance of difference in the expression of progesterone in between the groups

PROGESTERONE	P Value
Among the groups	0.000
Between group A and B	0.000
Between group A and C	0.000
Between group B and C	0.907

Table 4. The significance of difference in the expression of HCG in between the groups

HCG	P Value
Among the groups	0.000
Between group A and B	0.000
Between group A and C	0.000
Between group B and C	0.744

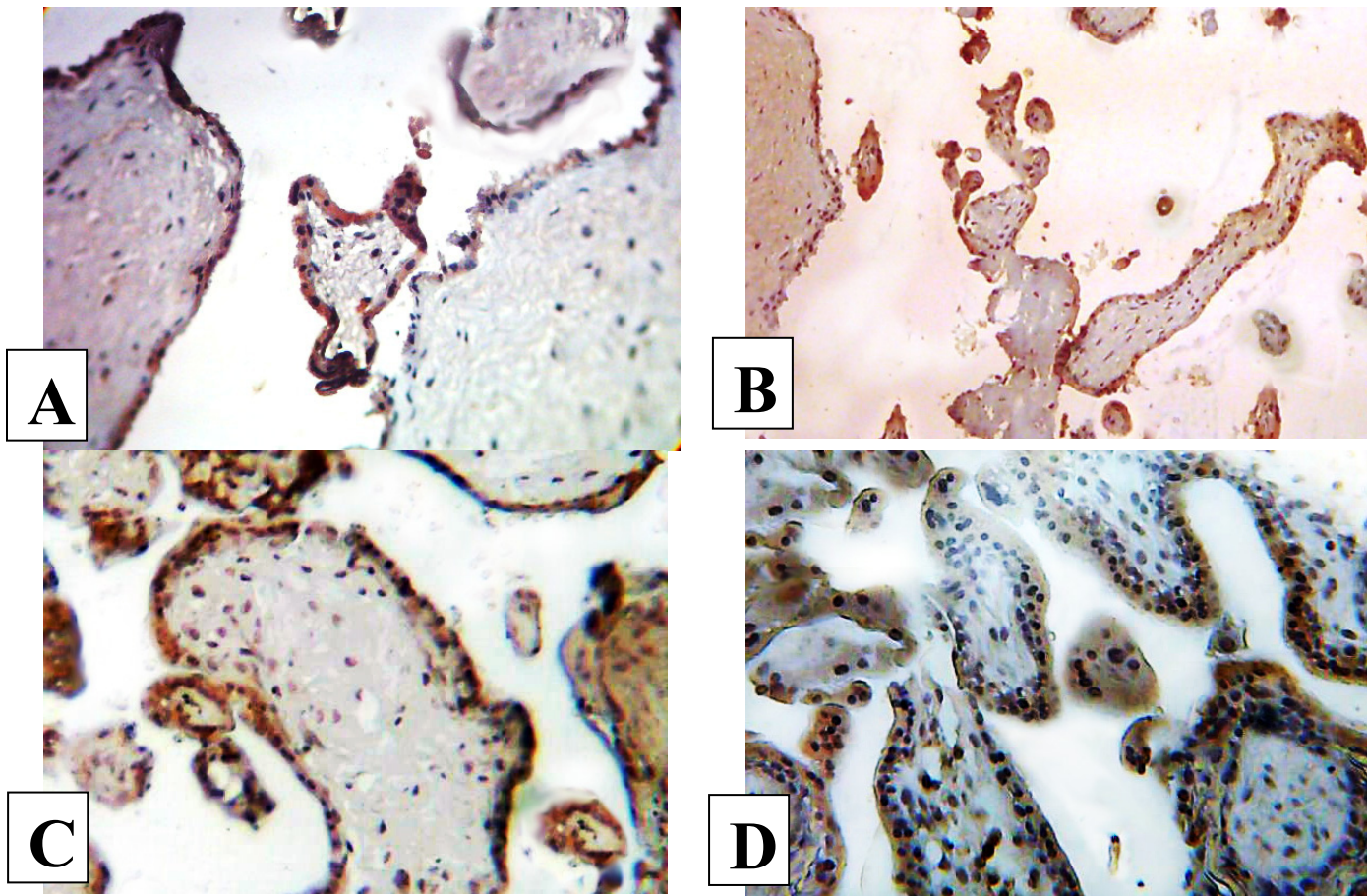


Figure: Detection of Progesterone and HCG by immunohistochemistry in women with pregnancy loss. (A & B) Expression of progesterone in women with RPL and normal pregnancy respectively. (C & D) Expression of HCG in women with RPL and normal pregnancy respectively. Magnification power of A-D (X400).

Discussion

In this study we observed a significantly lower expression of progesterone in the tissue of women with RPL as compared with the controls which is in agreement with a study showed lower expression of progesterone and progesterone receptor in the RPL group using immunohistochemistry analysis on endometrial tissue (but not during pregnancy) (13). In addition, progesterone deficiency is suggested in 10-60% of couples experiencing RPL (14). Recently, a study showed an inappropriate immune response to sex hormones especially estrogen and progesterone in these patients as compared with the control group (15).

On the contrary, a study compared the serum level of progesterone and estradiol between a

group of non-pregnant women with history of RPL during the follicular phase, and nulligravid females with tubal or male-factor infertility without miscarriage, showed comparable results in both groups with very few cases showing higher estrogen and lower progesterone levels in the study group (16). But our data come from the local expression of the hormone at the materno-fetal interface which means that we tried to study the actual hormonal environment during pregnancy. Also apart from systemic changes in the maternal immune system, local immune-modulation at the materno-fetal interface via wide array of hormones and cytokines and immune effector cells also play a very critical role in maintaining the balance of a desirable immune response (17, 18).

It is known that progesterone can directly affect T cell differentiation *in vitro*, suppressing the development of Th1 pathway and enhancing the differentiation along the Th2 pathway (19). Furthermore, in response to progesterone, $\gamma\delta$ T cells produce progesterone-induced blocking factor (PIBF) (10), which has been shown in mice to inhibit NK cell activity and have anti-abortion effects (20). All these studies goes with our previous studies on these cases that showed a significant increase in the expression of the Th1 cytokine (IFN- γ) in women with RPL as compared with the control groups (21).

In addition, low expression of HCG in the study group supports our results because, firstly;

HCG stimulates and acts on maintaining the release of progesterone from the corpus luteum (5), and secondly; it was found that progesterone secretion from the trophoblasts cells can be doubled *in vitro* in the presence of pure HCG (22).

In conclusion, down-regulation of progesterone could be suggested to have an effect in pregnancy loss in those who are suspected to have an underlying immune mechanism in the pathology of RPL due to the important role of progesterone in maintaining pregnancy through facilitation of immune tolerance (5).

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