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STUDIES ON THE IMMUNOSUPPRESSIVE ROLE OF STEROID HORMONES DURING PREGNANCY

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ABSTRACT

It has been recognised that steroids can exert a profound influence over immunological reactivity. The present study analyzes the role of steroid hormones - estrogen, progesterone and cortisol - and their involvement in immunoregulation during pregnancy. As is known, the endogenous levels of all the three hormones increase during pregnancy. When the steroid levels in pregnancy serum were correlated with the lymphocyte response to mitogen, no correlation was observed. The suppressive effect of pregnancy serum was found to have no correlation with its steroid content. In general, steroids did not seem to affect the maternal immune system as evidenced by the present study.

INTRODUCTION

The mechanism by which the fetus, which is essentially an allograft, is able to survive against the immunologic defences of the mother is one of the less understood phenomena in present day immunology. It has been reported that there is a blocking of the maternal immune system by the regulatory factors present in the blood, particularly steroids, which induce changes in the immunological activity in the maternal host during pregnancy.

In general, steroid hormones - progesterone, estrogen and corticosteroids - inhibit normal T-cell proliferation by various agents such as PHA, Con A or PPD (1,2). Significant

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suppression by progesterone was observed on many occasions (3,4). But inhibition of mitogen, antigen and allogenic cell activation by estrogen was found only at high physiological levels (5,6). Marked reduction in PHA transformation of lymphocytes was shown by Tomoda et al., (7) when corticosteroids were added to the cultures.

On the contrary, it has also been reported that serum and placental levels of steroids are too low to have significant effect on lymphocytes (5,7). Poskitt et al., (8) found that progesterone and estrogens have little effect on the response of lymphocytes to PHA and PWM unless present in high concentrations not usually present in pregnancy blood. Although profound qualitative and quantitative changes occur during pregnancy, the effect of these changes on the maternal immune system is not well understood and requires further investigation.

MATERIALS AND METHODS

Blood samples: Blood samples from pregnant women were collected from the clinics of the Department of Obstetrics and Gynecology, A.I.I.M.S., New Delhi. Blood from voluntary donors served as control. The blood samples were collected in screw-capped tubes with heparin (Centron Laboratories, Bombay, India) for lymphocyte isolation and without heparin for serum separation.

Lymphocyte cultures: Lymphocytes were isolated by the Ficoll-Hypaque density gradient method. The cultures were done in 96 well microtitre plates (Nunc, Denmark). Cell suspension (50 µl) containing 0.2 to 0.3 million cells was taken for culture. A standardised optimum concentration of PHA and Con A was added to each well and the total volume of the cultures was adjusted to 0.25 ml with RPMI-1640 medium (Gibco Laboratories, USA). Wells without mitogen served as controls. The cultures were incubated at $37^{o}C$ with 5% CO_2. After 48 hours 0.5 µCi of tritiated thymidine (Bhabha Atomic Research Centre, Bombay, India) was added to each well and the cultures were terminated 24 hours after thymidine incorporation. The cells were harvested on a Whatman GF/C filter paper using a cell harvester (Nunc, Denmark). The filter paper was transferred to a scintillation vial and 8 ml of simple scintillation mixture (POPOP 100 mg and PPO 4g per litre sulfur-free toluene) was

added to each vial. The radioactivity present in the vials was counted in a LKB Wallac Minibeta liquid scintillation counter.

The proliferative capacity of the lymphocytes was analyzed as follows:

$$\text{Transformation index} = \frac{\text{Counts in cpm of cells with mitogen (T)}}{\text{Counts in cpm of cells without mitogen (C)}}$$

The percentage transformation of lymphocytes from pregnant patients was calculated by taking the transformation index of non-pregnant controls as 100%.

Effect of pregnancy serum on normal lymphocyte cultures:

Normal lymphocyte cultures were done as described earlier and 10% pregnancy serum was added to each well. Cultures without pregnancy serum served as controls. By taking the percent transformation in cultures without pregnancy serum as 0% suppression, the percent suppression in cultures with pregnancy serum was calculated.

Radioimmunoassay of hormones (RIA):

Estimation of steroid hormones 17-β estradiol, progesterone and cortisol was carried out according to the WHO method manual (9). Within and between assay coefficients of variation for estradiol, progesterone and cortisol were found to be 7.8% and 10.2%, 9.3% and 10.9% and 9.2% and 10.8%, respectively.

Analysis of the suppressive effect of serum after the extraction of hormones:

After the extraction of hormones for RIA from the serum, the residue containing serum proteins was once again analyzed for its suppressive effect on lymphocyte cultures. The residue was first dissolved in RPMI-1640 medium and added to normal lymphocyte cultures to study its effect.

RESULTS

Steroid hormone profile during pregnancy:

Endogenous levels of estrogen, progesterone and cortisol were estimated by RIA in pregnancy sera from different trimesters of pregnancy and the results are summarized in Table I.

TABLE I

Steroid Hormone Levels (mean ± SD) During Normal Pregnancy

HORMONE	TRIMESTER		
	FIRST	SECOND	THIRD
ESTRADIOL (pmol/l)	3154 ± 1183 (25)	6558 ± 1768 (19)	11003 ± 3886 (22)
PROGESTERONE (nmol/l)	20.84 ± 5.72 (16)	33.42 ± 7.39 (18)	48.87 ± 4.25 (18)
CORTISOL (nmol/l)	411.4 ± 146.44 (20)	616 ± 189.51 (17)	769 ± 266.52 (20)

Figures in parentheses represent the number of samples studied.

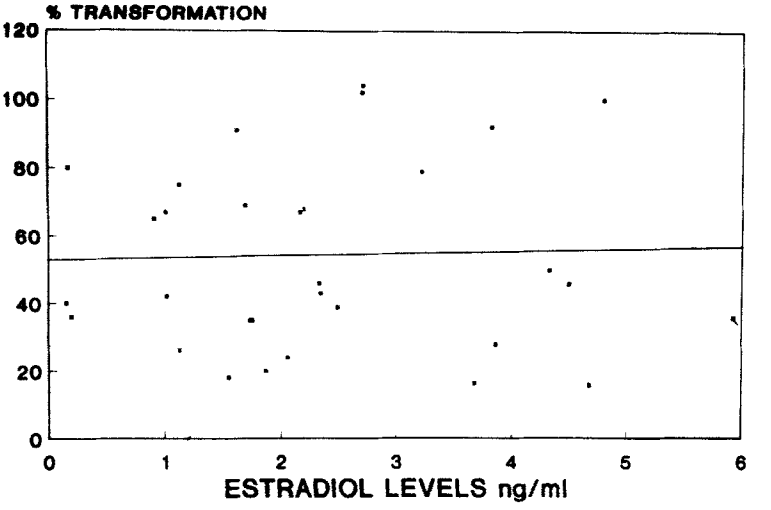

FIGURE 1

Correlation between Estradiol level and PHA induced lymphocyte transformation.

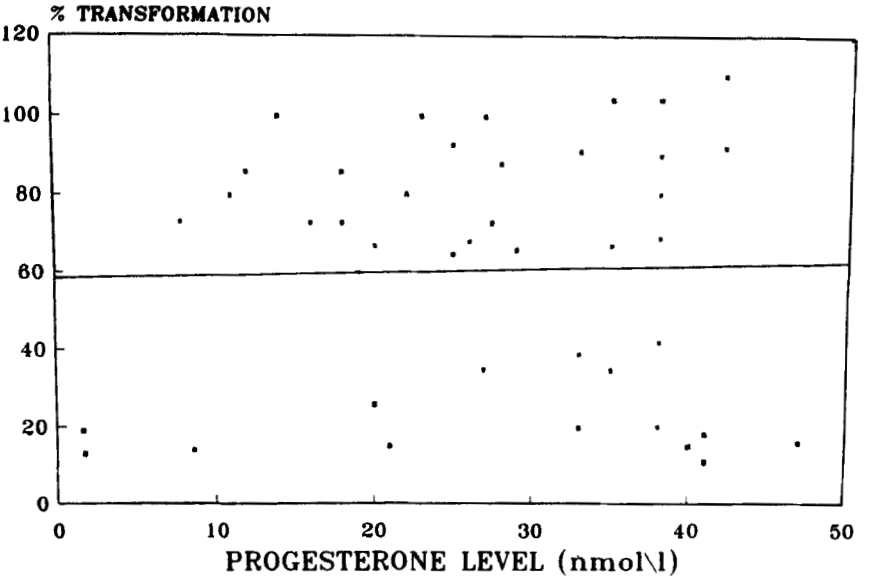

FIGURE 2

Correlation between Progesterone level and PHA induced lymphocyte transformation.

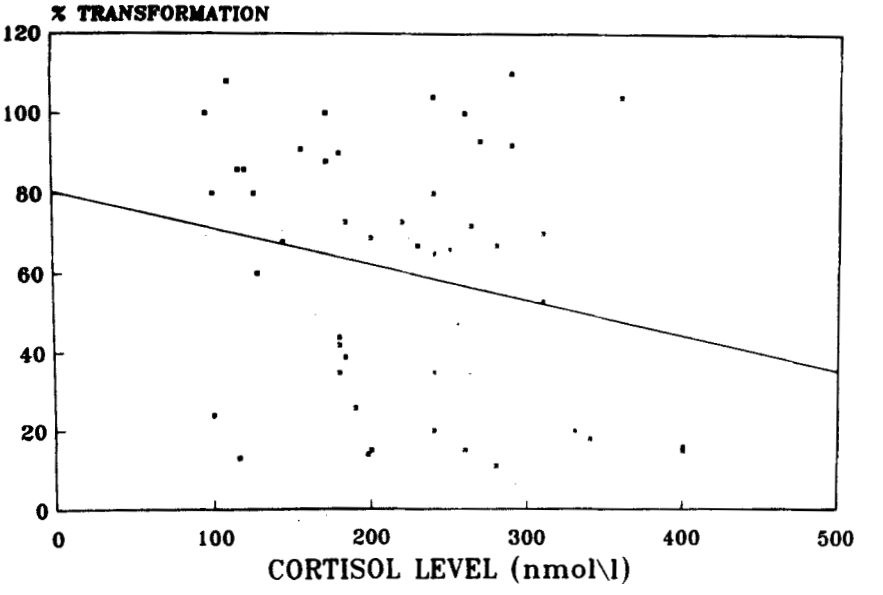

FIGURE 3

Correlation between Cortisol level and PHA induced lymphocyte transformation.

Correlation of steroid levels with lymphocyte transformation:

The levels of steroid hormones, estradiol, progesterone and cortisol, were compared with the proliferative capacity of lymphocytes from the same subject to find whether there was any correlation between the hormone levels and the lymphocyte transformation. Figures. 1,2 and 3 show the relation between the hormone levels and the PHA induced transformation of lymphocytes from pregnant women. No correlation was found between the levels of steroid hormones and the pattern of lymphocyte transformation. The same observation was made in Con A induced cultures.

Suppressive effect of pregnancy serum in relation to steroid levels:

After estimating the hormone levels in pregnancy sera the samples were analyzed for their effect on normal lymphocyte cultures. The inhibitory effect of these pregnancy sera was once again compared with the serum levels of steroid hormones. The results indicate a lack of correlation between steroid levels and inhibition of PHA induced normal lymphocyte blast transformation by the pregnancy serum (Fig 4,5 and 6). The same observations were also made in Con A induced cultures.

Effect of precipitated serum proteins after hormone extraction on normal lymphocyte culture:

After the extraction of hormones for RIA, the pellet containing the residual serum proteins was analyzed for its effect on lymphocyte cultures to ascertain whether the serum, depleted of steroid hormones, still has any inhibitory activity on normal lymphocyte culture. No significant reduction in the suppressive activity of the steroid depleted serum was observed.

DISCUSSION

The immunosuppressive role of steroid hormones, particularly estrogen, progesterone and cortisol, in general as well as during pregnancy has been reported to be conflicting. The serum levels of progesterone, estrogen and cortisol increase considerably during pregnancy. It was interesting to find out whether increased levels of steroids had any relation with lymphocyte transformation rate or the suppress-

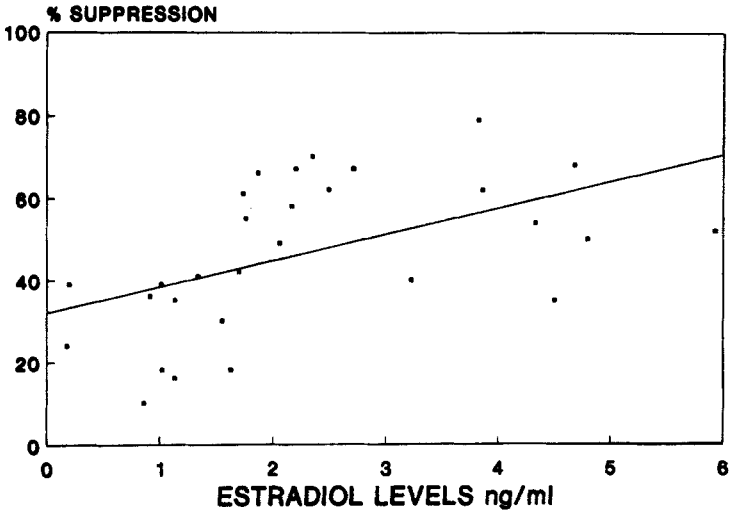

FIGURE 4

Correlation between Estradiol level and % suppression of PHA induced normal lymphocyte transformation.

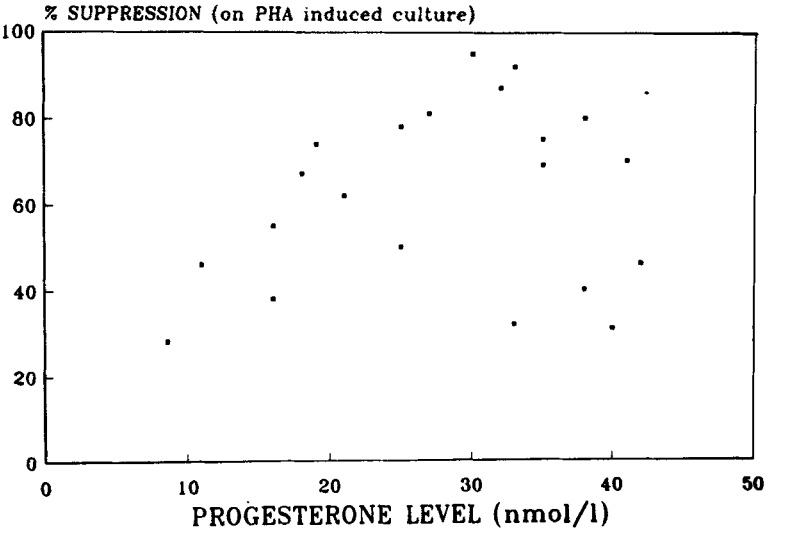

FIGURE 5

Correlation between Progesterone level and % suppression of PHA induced normal lymphocyte transformation.

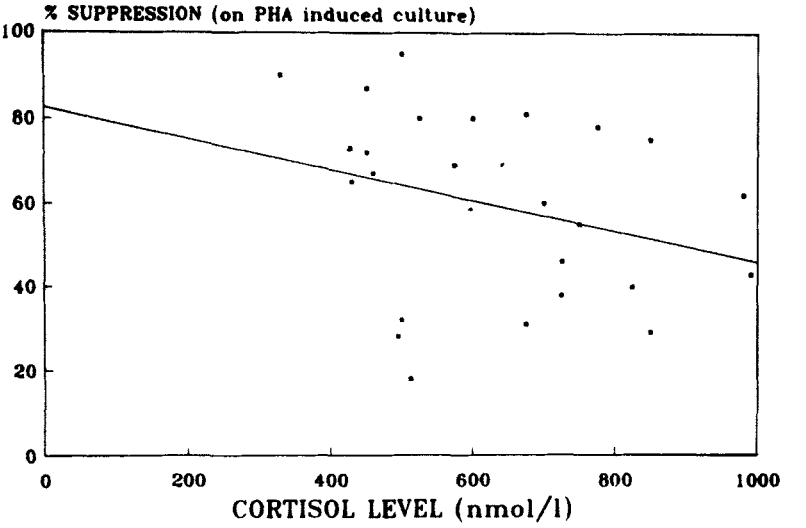

FIGURE 6

Correlation between Cortisol level and % suppression of PHA induced normal lymphocyte transformation.

ive effect on pregnancy serum. The present study failed to observe any correlation between steroid concentration in pregnancy serum with either lymphocyte responsiveness or immunosuppressive effect of serum during pregnancy. This is in agreement with the earlier report of Yoshida et al., (10) who have shown that the amount of steroids present in pregnancy serum did not inhibit T-cell proliferation. Poskitt et al., (8) found that progesterone and estrogens have little effect on the response of lymphocytes to PHA or PWM unless present in high concentrations, not usually present during pregnancy. The suppressive effect of estrogen on immune cell function was found only at high physiological concentrations (5,6) while no suppression was observed by Szekeres et al., (4). The work of Kasakura (11) also showed a lack of correlation between the cortisol levels in pregnancy plasma and the inhibition of MLC during pregnancy.

Another approach was followed to study the suppressive effect of steroid hormones. The pellet, which remained after the extraction of steroids from the serum, was analyzed for

its inhibitory activity. The results did not show any difference in the inhibitory activity after the extraction of the steroids. This reaffirms that serum steroid hormones are not responsible for the observed inhibitory activity of pregnancy serum.

The present study strongly suggests that the steroid hormones - estradiol, progesterone and cortisol - do not play an immunosuppressive role. Although a number of studies have indicated that steroids are immunosuppressive in vitro (5,6), these studies utilized much higher concentration of steroids than the normal physiological levels. Therefore, it has been suggested that the hormones might be responsible for local immunosuppression around the placenta which contains enormous amount of these steroids. Even this possibility does not seem to be true as Yoshida et al., (10) have found that the amounts of hormones present in the retroplacental serum did not inhibit T-cell proliferation. However, Stimson and Hunter (12) proposed that steroids may affect the immune response by inducing the thymus to produce immunoregulatory factors or immunoregulatory cells. The evidence presented indicates that the increasing amount of steroid hormones during pregnancy play a physiological supportive role rather than an immunological role.

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