

# Moderate anxiety in pregnant women does not compromise gestational immune-endocrine status and outcome, but renders mothers to be susceptible for diseased states development: A preliminary report

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## ABSTRACT

**High anxiety levels during pregnancy commonly lead to clinical complications that affect the mother/child's prenatal and perinatal health. Such complications are thought to result from combining deficiencies of the endocrine milieu and decreased immune tolerance that support pregnancy. It is yet unclear whether pregnant women subjected to moderate anxiety develop a similar state of endocrine deficits and compromised immune tolerance. Here, we evaluated this issue by monitoring endocrine and immune parameters during pregnancy in a sample of women suffering of moderate anxiety. The health of mothers and children, as well as the outcome of the pregnancies were also revised and recorded. Moderately anxious pregnant women present increments of state anxiety, serum cortisol and progesterone, but not of estradiol, as pregnancy progressed. No variations of leukocyte anti- and pro-inflammatory cytokine mRNA expression were found along pregnancy. However, these pregnant women did show an increased frequency of gestational and perinatal complications, conditions that had no major consequences for their health and that of their children at birth. Hence, moderate anxiety renders the mother to be susceptible to develop diseased states during or by the end of pregnancy, while keeping the endocrine milieu and immune tolerance reasonably in place.**

**Keywords:** Gestation; Pregnancy; Anxiety, Progesterone; Estradiol; Pro-Inflammatory Cytokines; Anti-Inflammatory Cytokines; Immune Tolerance; Endocrine Deficiency

## 1. INTRODUCTION

During the past three decades, we have witnessed an increment in the number cases of maternal and fetal/infant pre- and perinatal complications worldwide [1]. Although nutritional factors, infections of various sorts, stressful circumstances-whether social, biological or both-and a wealth of physiological (including behavioral) disorders alone or combined may predispose or trigger pathophysiological states during gestation and delivery, many intricacies of the mechanisms underlying them are still poorly understood.

Anxiety disorders are now recognized to have harmful effects on the course of gestation and on the mother/child prenatal and postnatal health status [2-6]. Such deleterious effects seem to result from immune-endocrine unbalances [7,8]. Most of the studies conducted up to this date, however, have documented high anxiety levels to be related with negative gestational health outcomes [5,9, 10]. To our knowledge, it is yet unclear whether pregnant women subjected to moderate anxiety develop a state of endocrine deficiency and compromised immunological tolerance as that described for pregnant women living under high anxiety levels. Here, we evaluated this issue by monitoring endocrine and immune parameters during pregnancy and the impact of moderate anxiety on the

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pregnancy outcome in a sample of pregnant women.

## 2. METHODS

### 2.1. Participants

This pilot study was conducted in a total of ten women (17 - 33 years old) having moderate anxiety. Volunteers were primiparous, mestizo, literate middle class pregnant women (n = 10; **Table 1**) attending to prenatal care. The physical exploration and routine laboratory analyses revealed no abnormalities or underlying pathologies. Family status, pregnancy outcome and pre/perinatal health were followed and/or supervised. All the participants signed the corresponding informed consent and the protocols used for sample withdrawing and handling were approved by the Ethics Committee at the Hospital Escuela, Universidad Veracruzana and concurred with the Norma Oficial Mexicana [11], section NOM-007-SSA2-1993.

### 2.2. Anxiety Evaluation

The magnitude of anxiety was scored by using the state-trait anxiety inventory (STAI) [12] <http://www.mhs.com/product.aspx?gr=cli&prod=stai&id=overview#>). This inventory has been used extensively in basic neuropsychological research, clinical settings and cross-cultural studies [10,13]. Recently it was adapted to the obstetric population to measure subjective feelings of tension, apprehension, nervousness and worries [14]. STAI measures both state and trait anxiety by assessing how subjects feel by the time of testing as well as how they felt at a particular time in the past. The inventory consists of 40 items presented as a Likert scale of almost-never to almost-always. In our case, women were asked to fill out the questionnaires with no time limit, working in undisturbed classroom within the hospital around 10 o'clock after breakfast. The questionnaire was applied when women first arrived to the hospital seeking for medical care then re-applied at 12 (first trimester) and 38 (third trimester) weeks of pregnancy. Anxiety scores were compared between the first and third trimesters taking the former as the reference value, since scores between the first (upon arrival to the hospital) and the second application (by the first trimester) did not differ. Statistical differences between first and third trimester anxiety scores were explored by using one way ANOVA tests ( $p < 0.05$ ).

### 2.3. Hormonal Profiling

A progressive increase of cortisol, estradiol and progesterone serum availability is required for maintaining pregnancy [2-6,15,16]. During gestation, however, ab-

**Table 1.** Main description of 10 female participants.

	Pregnant (n = 10)
Age, years (Mean $\pm$ SD)	22.5 $\pm$ 4.6
Marital and Family Status (%)	
Single	20
Married/living as married	70
Divorced	10
Husband support	90
Family support	70
Financial burden	20
Dysfunctional family relationships	30
Anxiety factors	
Fear to delivery	40
Health child concerns	100
Undesired pregnancy	20
Schooling, years (%)	
$\leq 12$ years	80
$\geq 13$	20

SD-Standard deviation.

normal rises of cortisol serum levels may down regulate estradiol and progesterone thus increasing the risk of abortion or premature delivery [17,18]. High anxiety is frequently associated with increased cortisol serum levels [18]. However, the relationship between progesterone levels and moderate anxiety in pregnant women has not been assessed before. We then determined cortisol, estrogen and progesterone serum levels (blood samples were withdrawn between 8:00 and 9:00 a.m.) in pregnant women living under moderate anxiety at 12, 27 - 29 and 38 - 40 weeks of pregnancy by clinical conventional means and standard protocols. The serum obtained was used to determine hormonal concentrations (ng/ml) by ELISA following the protocol provided by the supplier (Sensitivity 0.11  $\mu\text{g/dl}$  and intra-assay variability coefficient  $< 3$  percent; VITROS Kit; Ortho-Clinical Diagnostics; Johnson & Johnson). Temporal differences of hormonal concentrations were assessed by using repeated measures one-way ANOVA tests ( $p < 0.05$ ).

### 2.4. Cytokine Profiling

Experimental evidence suggests that sources different from the placenta, likely maternal leukocytes, release cytokines that mediate inflammatory states during high risk pregnancies [19], including those subjected to high

anxiety levels [7]. Since no previous work has studied the immunological profile of pregnant women living under moderate anxiety, we evaluated levels of mRNA expression of pro-(INF- $\gamma$ , TNF- $\alpha$ , IL-12) and anti-inflammatory (TGF- $\beta$ , IL-4, IL10) cytokines in peripheral leukocytes. Lymphocyte mRNA was extracted by using the RNeasy Mini Kit following the protocol recommended by the supplier (QIAGEN). The integrity, purity and concentration of mRNA was estimated following conventional protocols having as an internal control actin mRNA [20]. Then, cDNA was obtained [21] and used to amplify cytokine genes by multiple PCRs (37 cycles, aligning temperature 59°C) following the protocol provided by the manufacturer (Maximbio). Densitometric, semi-quantitative analyses (LabWorks 4.5) of the amplified products relative to GADPH expression were then carried out using digitized photographs (Bio Doc It; UVP Co., USA) of ethidium bromide stained polyacrylamide gels. We then pulled together, summed up and averaged intensity values of all cytokines per cytokine group analyzed for each trimester. Although we recognized that this manipulation masks cytokine individual variations along pregnancy, it provides a global, easy to compare and interpret, picture on the behavior of the pro- and anti-inflammatory immunological profiles throughout pregnancy. The average values referred above were also used to estimate the anti-/pro-inflammatory cytokine ratio (*i.e.*, Th2/Th1 response balance; for a similar approach see [17]). Statistical differences of both parameters over time were tested by using repeated measures one-way ANOVA tests ( $p < 0.05$ ).

## 2.5. Additional Statistical Analyses

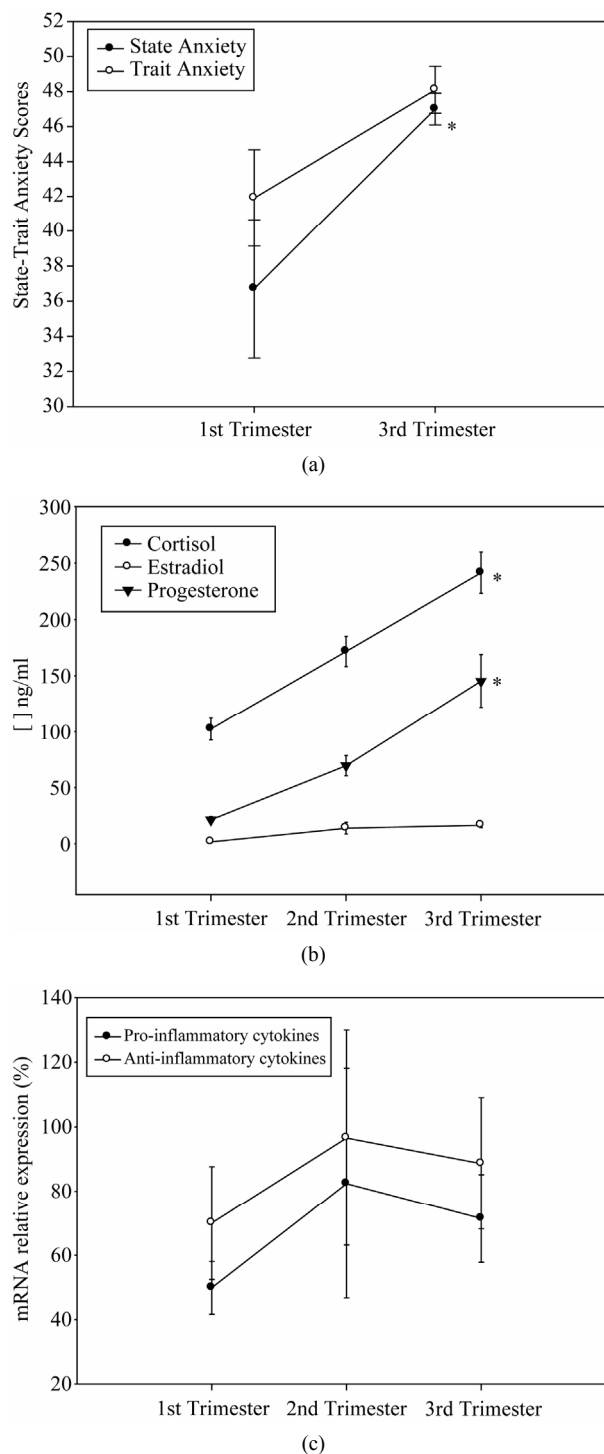
To investigate possible temporal correlations among all parameters measured, we used Pearson's correlative analyses ( $p < 0.05$ ).

## 3. RESULTS

As shown in **Figure 1(a)**, pregnant women with moderate anxiety increased their state anxiety as pregnancy progressed. In spite of these change, cortisol and progesterone serum concentrations increased in the course of pregnancy (**Figure 1(b)**). In contrast, estradiol serum levels remained relatively constant during gestation (**Figure 1(b)**). Moderate anxiety did not modify anti- and pro-inflammatory cytokine mRNA profile expression during pregnancy (**Figure 1(c)**). With the exception of gestational anti- and pro-inflammatory cytokine profile patterns (Pearson's correlation coefficient 0.79 at  $p < 0.05$ ), no interaction among anxiety, endocrine and immune variables were found.

**Table 2** shows pregnancy complications and outcomes developed by the mothers. Seven out of ten of these

women developed complications throughout pregnancy or labor. Four out of these seven women acquired urinary tract infections; two had hypertensive states, two presented premature labor and one an upper respiratory tract



**Figure 1.** Anxiety scores (a), cortisol, progesterone and estradiol (b) serum levels and anti-/pro-inflammatory mRNA cytokine expression (c) in pregnant women showing moderate anxiety during pregnancy.

**Table 2.** Pregnancy and birth outcome details of ten the pregnant women.

Caesarean birth/Normal vaginal birth (%)	50/50
Gestation at delivery, weeks (Mean $\pm$ SD)	40.6 $\pm$ 1.6
Pregnancy complications (%)	70
Urinary tract infection	40
Hypertension	20
Preterm labor	20
Upper respiratory infection	10
Delivery complications (%)	50
Hypertension	20
Pregnancy beyond 41 weeks	10
Lack of labor progression	10
Fetal distress	10
Birth weight, g (Mean $\pm$ SD)	3084.5 $\pm$ 387.9
Birth length, cm (Mean $\pm$ SD)	49.5 $\pm$ 2.5

SD-Standard deviation. The **Table 2** displays the diseased states developed by the mothers during pregnancy or by the time of birth. This table also shows body weight and length of children. Notice that the last parameters reach values within the reported normal range. Repeated measures one way ANOVA: \* $p < 0.05$ , \*\* $p < 0.001$ .

infection. Interestingly, the women with urinary tract infection also presented hypertensive conditions and premature labor. Also, half of the participants-all of them developed pregnancy complications-had the highest anxiety scores (not shown) and were all subjected to cesarean sections. In spite of these pathophysiological findings, body weight and length of the children delivered by this group of women were normal (**Table 2**).

#### 4. DISCUSSION

High anxiety during the course of gestation leads to a variety of clinical complications by the time of birth. Such deleterious effects have been attributed to endocrine deficiencies and reduced immune tolerance. However, another psychological condition that could affect pregnant women is moderate anxiety. To our knowledge, it is yet unclear whether pregnant women subjected to moderate anxiety develop a state of endocrine deficiency and compromised immune tolerance comparable to those observed in pregnant women with high anxiety levels. In this work we approached this issue by carrying out a preliminary study in a cohort of pregnant women having moderate anxiety. Our results show that even when moderately anxious women increased their anxiety scores as pregnancy progressed, overall endocrine and immune parameters behave as reported for women ongoing a

normal pregnancy [22-26]. In our study moderate anxiety did not associated with a compromised gestational immune-endocrine status in contrast to that reported in pregnant women with high anxiety levels. It is interesting, however, that pregnant women in our study developed gestational and perinatal complications by the end of pregnancy, suggesting that moderate anxiety renders women to be susceptible to developed diseased states. Although the mechanism is currently uncertain, it may be that the lack of the gestational rising of estradiol serum levels observed. Indeed, the women studied increased their anxiety levels and developed hypertensive conditions, infectious diseases, preterm or delayed labor or lack of labor progression by the end of gestation. Estradiol is known to reduce anxiety [27,28], is a potent progestational hormone [29], an immune modulator [30] and a regulator of uterine and blood vessel contraction [7]. Hence, future studies must address this relationship.

Another interesting observation was that moderate anxiety did not associate with child's low body weight or short length at birth. What makes the embryos/fetus somehow resistant to the effects of moderate anxiety? Once again, the answer is yet unclear. Nevertheless, it may be that the increments of cortisol and progesterone serum levels and the maintenance of an adequate balance of pro/anti-inflammatory cytokines during pregnancy may suffice to procure a pro-gestational state in moderately anxious women. In addition, since estradiol can also stimulate pro-inflammatory processes under certain circumstances [7,17], a reduction of estradiol serum levels in pregnant women suffering of moderate anxiety could also be protective. Future experiments must address this important issue.

A surprising result was that maternal leukocyte anti- and pro-inflammatory cytokine mRNA expression did not show variations along pregnancy in spite of the fact that anxiety levels and progesterone and cortisol serum concentrations increased significantly. Commonly, during implantation and labor the placenta releases pro-inflammatory cytokines that are counteracted by increments in anti-inflammatory cytokines secreted by maternal leukocytes and the placenta itself as pregnancy proceeds. This last response appears to depend upon increments of cortisol, progesterone and estradiol [7,8,17]. As the date of birth approaches cortisol increases further, pro-inflammatory cytokines also increase whereas progesterone and estradiol drop, a combination that precipitates labor [7,14,17,18]. So, it is puzzling that women included in our study did not modify their leukocyte cytokine expression during the course of their pregnancy. One possibility to explain this finding is that the code needed to trigger shifts of anti-/pro-inflammatory cytokine expression in maternal leukocytes heavily depends upon estrogen increments, a condition that was not pre-

sent in pregnant women suffering of moderate anxiety levels. A second possibility is that the participation of cytokines released by maternal leukocytes is minor during the response of the immune system to moderate anxiety levels. Thirdly, moderate anxiety levels might decouple immune-endocrine relationships thus protecting the embryo/fetus, but certainly not the mother. Decoupling, however, might also be a sign that foresees worse things to come if anxiety levels are kept moderate or high for a longer time. Upcoming studies must sort out the merits of each of these possibilities.

## 5. CONCLUSIONS

Moderate anxiety in pregnant women associated with increments of anxiety levels, serum cortisol and progesterone, but no increase of estradiol, as pregnancy progressed. In addition, no variations of leukocyte anti- and pro-inflammatory cytokine mRNA expression were found along pregnancy. However, these pregnant women presented an increased frequency of gestational and perinatal complications, conditions that had no major consequences for their health and that of their children at birth.

Consequently, pregnant women with moderate anxiety are more susceptible to develop complications during or by the end of pregnancy, while keeping the endocrine milieu and immune tolerance reasonably in place.

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