STEROID HORMONE CHANGES IN PREGNANT RATS

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Author’s note to the reader:

The work shown here was completed nearly 20 years ago. Some of the findings were presented at the 1983 FASEB Annual Meeting, and the associated abstract can be seen at: Lederman SA, Rosso P. Hormonal Changes in Undernourished Pregnant Rats. *Fed Proc* 1983;42(3):614, Abstr. 1885. Many things conspired to prevent us from preparing this manuscript for publication in a timely fashion. In the intervening years, the authors have moved on to other places and priorities. We long ago lost track of Mr. Frankl, who did the assays, and as a result cannot get more detail about the methodology, where we might have found it informative. We have also decided to leave the references as they were, rather than present more recent citations that had not determined our original interest in this study. We hope that those who are still studying rat pregnancy will find this report useful.

Sally Ann Lederman, Ph.D.  April, 2004
ABSTRACT

Plasma concentrations of estradiol, estrone, and progesterone were determined in pregnant rats fed ad libitum throughout gestation and in pregnant rats that were food restricted (45% of ad libitum intake), protein restricted (6% casein, ad libitum), or fasted from day 17 to day 19 after prior ad libitum feeding. Blood levels were determined on gestational day 19 in fasted rats and on days 5, 12, 19, and 21 in the other groups. The results show that all 3 hormone levels increase during normal gestation, but progesterone rises earlier than estrone or estradiol. In well fed rats, progesterone levels were greatest on day 19. In the 3 malnourished groups, plasma progesterone on day 19 tended to be lower than in the ad libitum fed group on day 19. In contrast, day 19 estradiol concentration was higher in the 3 malnourished groups than in the ad libitum fed group. Estrone concentrations was also higher than the ad libitum value on day 19 in both the food restricted and fasted rats.

Total circulating progesterone on day 19, determined from measurement of plasma volume and progesterone concentration, was markedly higher in the ad libitum fed group than in the malnourished groups. These results suggest that gestational hormone production may be affected by nutrition and may influence maternal physiologic adjustments to pregnancy.

Key words: pregnancy, steroids, estradiol, estrone, progesterone, malnutrition.
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Introduction

Undernutrition is known to affect plasma levels of many hormones. Several of the hormones that regulate metabolism, such as insulin, glucagon, growth hormone, and thyroid hormone, are known to undergo adaptive changes in nonpregnant individuals subjected to undernutrition (1-3). Furthermore, several studies have explored how undernutrition, especially fasting, alters these metabolic regulators in pregnant subjects. These studies have generally demonstrated that metabolic adaptation to starvation occurs more rapidly during pregnancy.

Pregnancy, however, is characterized by the elaboration of steroid hormones (estrogens and progesterone), which, though present in nonpregnant subjects, show marked increases during gestation. Many biological processes are influenced by these steroids and they may well play a fundamental role in maternal physiologic adaptations to pregnancy (4-6), adaptations that are essential for normal pregnancy outcome. Although physiological adaptations are dampened by undernutrition, it is not known whether hormonal changes mediate these effects, or whether the levels of these hormones are altered differentially by different types of maternal undernutrition. In humans, it has been found that women with high progesterone levels have a higher mean placental weight and a tendency for high fetal weight (5). Since the placenta is the main source of progesterone in humans, however, it is possible that improved growth is responsible for increased progesterone and not the converse. Since fetal growth and viability depend on appropriate maternal adjustments, it would be valuable to know if changes in gestational hormones, acting to affect maternal adjustments to pregnancy, contribute to the reduced fetal growth associated with undernutrition.

Although little is known about the effect of nutrition on gestational production of estrogens and progestins, several findings are suggestive. For example, in nonpregnant subjects, undernutrition is known to alter the levels of several of the hormones essential for sexual functions (7). Thus amenorrhea and infertility are the result of severe undernutrition, such as is observed in anorexia nervosa and in severe, primary starvation. Moreover, in rats fed a zero protein diet throughout gestation, spontaneous
resorption of fetuses could be largely prevented by the administration of estrone and progesterone (8), although it was not determined whether the diet had lowered blood levels of these hormones. Other workers studying early gestation (to day 13 or 15) in rats have shown that plasma progesterone is significantly reduced in rats fed zero protein diets (9) and is also somewhat lower in rats fed 6% protein (10). In rats restricted 75% during the first ten days of pregnancy, administration of a constant amount of progesterone daily, concurrent with the restriction, appears to alter the pattern of fetal growth (11). In humans, where infant weight has been correlated with plasma volume expansion (12), urinary estriol excretion in the first two trimesters showed a tendency to be reduced in women bearing low weight infants (<25th percentile) (12). In addition, it has been reported that undernourished, pregnant Indian women excrete a reduced amount of urinary estrogens (13). An increased estrogen excretion and an increase in infant birth weight were observed when these women were hospitalized and given better food and bed rest during late gestation. In order to be excreted in the urine, however, estrogen must be conjugated by the liver. It is conceivable that undernutrition reduces urinary levels by reducing conjugation, in which case blood levels could be increased rather than decreased by undernutrition. Furthermore, estriol, a major urinary estrogen, is produced by the fetal-placental unit (14). Thus, increased production could be the result, not a cause, of increased conceptus growth.

We have previously proposed that inadequate maternal plasma volume expansion and the associated reduction in uterine blood flow resulting from undernutrition during pregnancy may be a major determinant of the associated fetal growth retardation (15, 16). We have documented that low protein feeding, food restriction (16, 17) or fasting (18) can prevent the normal expansion of plasma volume during pregnancy in the rat. These studies have shown that the decrement originally observed in maternal plasma volume at term (16), may be observed by day 19 of pregnancy as well. Since estrogen and progesterone increase total body water and reduce vascular tone, it is conceivable that the marked increase in plasma volume occurring during gestation is partly mediated by these hormones. If so, undernutrition would be expected to alter the levels of these hormones, since undernutrition largely prevents this plasma volume expansion.
The present study was designed to determine if the levels of progesterone or estrogens were altered by undernutrition during pregnancy. In addition, the relationship of these hormones to maternal plasma volume expansion was also explored. Because we had shown that food restriction, low protein feeding, and fasting all induced alterations in maternal plasma volume as well as affecting fetal growth, all three models were studied for their effects on maternal hormone levels.

**Materials And Methods**

**Animals**

Most of the animals used in this study were involved in a study of plasma volume expansion reported previously, (17). Briefly, Sprague Dawley rats (Holtzman Co., Madison, WI) weighing 250 ± 1.5 (X ± SEM) on day 0, the sperm positive day, and bearing 13 ± 4 fetuses were used in the final analyses. All animals were fed ad libitum till day 5 of the experimental period. A small blood sample was drawn (about 1 ml) from the leg vein and then plasma volume was determined as previously reported. Studies were done on representative pregnant rats on day 5. The remaining rats were then divided among 3 groups, ad libitum fed controls (25% casein diet), food restricted (fed 45% of the average intake of the controls), and low protein fed (6% casein diet, ad libitum). Representative rats from each group were similarly studied on days 12, 19, and 21. A subgroup of ad libitum fed rats was fasted from day 17 to day 19, and studied on day 19. Not all samples drawn were sufficient for the hormone analyses, so initial group sizes were larger than the number of hormone analyses finally reported.

Body weight and food intake were recorded about three times each week. Between 9 AM and noon of the morning that an animal was killed, it was anesthetized (24 mg/kg Ketamine + 45 mg/kg pentobarbital sodium) and plasma volume was measured, as previously reported (17, 19). The animal was killed by aortic exsanguination. Blood samples were kept on ice, then centrifuged cold at 25000 rpm for 15-20 minutes, and the plasma separated and frozen (-20o C) until analyzed.
Estradiol assay

Estradiol was measured by radioimmunoassay (Pantex kit, Santa Monica, CA) after extraction of estrogens with 3:2 ethyl acetate: hexane. A second antiserum was used to precipitate the estradiol-antibody complex. Both protein-bound and free, unconjugated estradiol are measured in this assay.

Estrone assay

Estrone was determined by radioimmunoassay (Estrone Test Set, Wien Laboratories, Succasunna, N.J.) after extraction with purified methylene chloride. Dextran-coated charcoal was used to remove the estrone that did not bind to the antibody.

Progesterone assay

Progesterone was measured by radioimmunoassay (Progesterone RIA Kit, Nuclear Medical Systems, Inc., Newport Beach, CA) using a second antibody to precipitate the progesterone-antibody complex.

Estriol assay

Estriol was measured by radioimmunoassay (Estriol RIA kit, Nuclear Medical Systems, Inc., Newport Beach, CA). Assay of 12 samples, including at least one sample from each group and time point, showed all values to be less than 0.5 mg/ml plasma. No additional estriol assays were performed and the results are not discussed further.

Plasma volume

Plasma volume measurements used here to estimate total circulating hormone levels were determined as reported previously (17, 19), by injecting 0.3-0.4 g of a 5 mg/ml Evans blue dye solution into the exposed femoral vein, and removing aortic blood 3 to 10 minutes later. The concentration of the dye in the plasma was determined spectrophotometrically after four-fold dilution with physiologic saline. Turbidity corrections were done as previously reported (19).

Results

The numerical data are shown in Table 1. The figures help to illustrate the trends.
Estradiol

As shown in Figure 1, estradiol increased throughout gestation in ad libitum fed rats. Values for food restricted, low protein fed rats and fasted rats also increased. However, at day 19 the mean values for all three of the undernourished groups were above the control group's value. The marked rise in plasma estradiol observed in the ad libitum fed group from day 19 to 21 was not observed in the undernourished groups.

Estrone

As Figure 2 shows, plasma estrone rose almost linearly between days 5 and 21. A similar pattern was observed in food restricted rats. In both fasted and low protein fed rats plasma estrone, like plasma estradiol, was higher at day 19 than it was in the control group. At day 21 plasma estrone values were similar for the low protein fed, food restricted, and control groups.

Progesterone

Plasma progesterone increased about 65% between day 5 and day 19 in well fed rats and then declined to day 21 (Figure 3). On day 12 plasma progesterone was nearly identical in the low protein fed, food restricted and ad libitum fed groups. In contrast to current findings for estrogens, progesterone tended to be lower on day 19 in all undernourished groups, whether food restricted, low protein fed, or fasted. Nevertheless, in the undernourished groups, as in the well fed group, a pre-term drop in plasma progesterone was observed.

Total Circulating Progesterone

The data for total circulating hormones are shown in Table 1. Figure 4 shows the plotted values for total progesterone. The data illustrated suggest that the undernourished animals do not increase net progesterone production during the course of pregnancy. Between days 5 and 19, when there is a marked increase in total progesterone in the ad libitum fed rats, there is virtually no change in total progesterone in the malnourished rats.
Total Circulating Estrogens

The data in Table 1 indicate that total estrone and estradiol are also somewhat lower in undernourished rats than in the control rats, but the differences are small relative to the variability.

Discussion

The trends in plasma progesterone concentrations obtained in the ad libitum fed group of the current study conform to findings previously reported, which suggested that peak levels of progesterone occur at about day 15 of pregnancy and decline after day 19 (20-22). Although some workers have reported a drop in progesterone levels between days 5 and 12 (9) or between days 12 and 19 (23), these changes are not always reported (24, 25) and were not observed in this study.

The time pattern of estrogen blood levels observed here is also consistent with earlier studies of estradiol in rat serum (23) and of estradiol and estrone secretion rates in pregnant rats as determined from ovarian venous plasma concentrations (26).

The present results in the malnourished animals suggest that nutrition may influence maternal plasma levels of estrogens and progesterone during the period of most rapid fetal growth. Generally speaking, estrogen increases are enhanced and peak progesterone concentrations are reduced in undernourished animals. The effects were small, but consistent in the three models studied.

Although the tendency for plasma progesterone levels to be lower in low protein fed rats was not observed here until day 19, other workers have noted a significant decrease in plasma progesterone as early as day 9 in rats fed a zero protein diet (10). Higher levels of plasma progesterone were observed as the level of dietary protein increased to 6% and then to 18% (10), which would be consistent with our results.

It has previously been shown that rats fed a protein-free diet during gestation will fail to produce live young, but can be made to do so if injected daily with estrone and progesterone (8). It is interesting to note that progesterone alone was more successful than estrone alone in supporting pregnancy in these undernourished rats (8). Furthermore, injections of prolactin and estrone from days 5 to 12 can also prevent the abortions which occur in rats fed no protein, and progesterone levels are
concomitantly increased (27). These reports are consistent with our observations that plasma progesterone, but not estrone, tended to be reduced by undernutrition, and was also directly related to plasma volume and fetal growth. Previous workers have documented that fetal growth declines when progesterone is lowered with an anti-progesterone antibody, but effects on plasma volume were not measured (28). Taken together with the current study, the data suggest that altered hormone levels occur during undernutrition in pregnancy and may affect the mother's adjustments to pregnancy including plasma volume changes. In extreme conditions, hormone levels may be affected early in pregnancy and abortion may be a consequence. Under milder conditions hormone levels may be affected later and fetal growth retardation may result.

It is possible to propose several ways by which undernutrition may alter hormone production in pregnant rats. Reduced blood levels of lutenizing hormone (LH) may play a role in the hormone changes observed here. It has been shown that administration of LH increases ovarian blood flow in the ewe (29) and in the rat (30), and also raises systemic progesterone concentration in the ewe (29). Removal of LH with anti LH antibodies reduces ovarian blood flow and lowers plasma progesterone.

If nutritional deprivation alters blood flow to the ovary, it also would be likely to affect plasma progesterone. This is particularly true because in the rat the ovary is the primary source of progesterone (21) although some placental production can occur late in pregnancy (21, 31). Moreover, in early pregnancy (until day 8) in the rat, removal of LH results in termination of pregnancy, which is preventable with progesterone but not estrogen or prolactin administration (32). Whether nutritional deprivation alters LH levels in rats or not, nutrition may affect ovarian blood flow by other mechanisms. Reduced ovarian blood flow at term has been reported in 50% food restricted rats (15). Although some workers have not observed any decrease in ovarian blood flow in 50% food restricted rats (33), they also did not observe the increase in ovarian blood flow observed by others (34) over the course of gestation. Whether food restriction would effect ovarian blood flow directly, or indirectly through changes in LH, cannot be stated. Since LH can be affected by undernutrition in nonpregnant humans (7), reduced LH production could be participating in mediating the dampened rise of progesterone. On the other hand, LH
may not be important in inducing the progesterone changes observed here, since factors other than LH probably also influence progesterone production late in rat pregnancy (21, 23).

Regardless of its cause, lower progesterone may be contributing to the lower plasma volumes we observed. A progesterone metabolite (5-alpha dehydroprogesterone) (35) has been reported to reduce vascular responsiveness to angiotension II (36, 37), a hormone that induces vasoconstriction. This reduced responsiveness may be crucial for the expansion of the vascular system which must occur if plasma volume is to increase, especially since angiotension II concentrations rise during pregnancy (37). In the presence of lowered progesterone concentrations and lower total circulating progesterone, the production of this metabolite may also decrease, contributing to reduced plasma volume expansion and thereby effecting fetal growth.

The significance and causes of the generally higher estrogen levels observed on day 19 in our malnourished rats are also unknown. They may reflect increased enterohepatic circulation of estrogen secreted in bile (38). Alternatively, urinary excretion may be reduced, as it may be in humans (13), because of a reduction in conjugation by the liver.

Higher estrogen levels could participate in reducing progesterone levels, since under some circumstances estrogen enhances conversion of progesterone to reduced products (in ovariectomized, pseudopregnant rats) (35). Progesterone seems to be important in maintaining uterine blood flow, since ovariectomy decreases uterine blood flow (days 7-11 of rat gestation) and progesterone prevents the decrease (39). Earlier studies have demonstrated reduced plasma volume (16, 17) and uterine blood flow (15, 33) in undernourished rats.

Furthermore, the increased estrogen levels may affect fetal growth in other ways. Estrone tends to reduce average intrauterine oxygen tension in nonpregnant rats, a variable dependent on the patency of the uterine vessels (40). Thus, if estrone is increased and progesterone simultaneously reduced, intraluminal oxygen tension may be lowered, particularly if uterine blood flow is low due to a failure of plasma volume expansion. Reduced oxygen availability could, by itself, impair fetal growth. This factor may contribute to the reduced birth weight observed at high altitudes.
Another mechanism that may be responsible for reduced serum progesterone is suggested by the observation that luteal tissue (in the cow) secretes more progesterone in the presence of serum (41). It has been hypothesized that steroid-binding proteins may facilitate transport of steroids into the blood. We saw only small changes in total plasma protein, which were measured in the animals studied (data not shown), which would not support such an explanation. However, large differences in the specific proteins for steroid transport could remain unnoticed in a total protein assay.

The significance of the observed hormonal changes may be many. Progesterone is an important determinant of the mother's metabolic adjustments, increasing pancreatic insulin production, storage of carbohydrate in the liver, decreasing the sensitivity of adipocytes to insulin (encouraging fat deposition), and stimulating lipoprotein lipase in the breast, aiding in preparation for breastfeeding (4). If these adjustments were reduced, the overall effect would be to curtail the gestational changes in substrate use, which are usually thought to be protective of fetal growth.

Some actions of estrogen normally antagonize the effects of progesterone. For example, estradiol increases insulin sensitivity in adipose tissue and reduces adipocyte cell size. Therefore high estrogen levels would augment the effect of low progesterone levels. Recent work indicates that estradiol levels are higher in first pregnancies than in second pregnancies, suggesting a possible relationship to the smaller infant size observed in primiparous women. Similarly, the hormonal differences we observed may be responsible for the fetal growth retardation in the three models of malnutrition studied here.

References


Table 1. Hormone Levels in Pregnant Rats (Mean±SEM)

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<th>Initial Wt</th>
<th>Carcass Wt</th>
<th>Plasma Estrone</th>
<th>Estradiol</th>
<th>Progesterone</th>
<th>Total circulating amounts:</th>
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<td>250.6±6.3</td>
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<td>250.4±1.5</td>
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<td>178±14</td>
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<td>250.7±12.0</td>
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<td>183±31</td>
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<td>FOOD RESTRICTED</td>
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Carcass wt = eviscerated carcass + (liver, kidney, adrenals and mls blood drawn)