The Relationship of Conceptus Number and Fetal Sex to Maternal Serum Testosterone Concentration in the Rat (43126)

V. DANIEL CASTRACANE[1] AND J. LUIS DELA CRUZ

Department of Clinical Sciences and Reproductive Biology, Southwest Foundation for Research and Education, San Antonio, Texas 78264, and Department of Natural Sciences, Incarnate Word College, San Antonio, Texas 78209

Abstract. **On Day 8 of pregnancy, the number of implantation sites in pregnant rats was adjusted to 1, 2, 4, 6, or >10. Blood was collected on Days 11, 12, 15, 18, and 20 for the determination of serum testosterone, progesterone, and androstenedione. Serum testosterone levels exhibited a direct linear relationship with implantation number, increasing from 1 through >10 implants. This linear relationship was particularly evident at Days 12, 15, and 18 of pregnancy. Serum progesterone levels increased from one to four conceptuses and plateaued above this number. There was no apparent relationship between the number of conceptuses and serum androstenedione levels, which may reflect the multiple origins of this steroid in the pregnant rat.**

In a separate group of rats in which the number of conceptuses was adjusted to three on Day 8 of pregnancy, blood was collected on Days 11, 12, 15, 18, and 20. Fetal sex was determined between the last bleeding and the day of parturition. Serum testosterone was determined and results were examined with regard to the number of male/female fetuses in the litter of three. There was no relationship between maternal serum testosterone levels and the number of male fetuses, indicating that the fetal testis does not make a significant contribution to circulating maternal testosterone levels.

[P.S.E.B.M. 1990, Vol 195]

There is an increase in maternal serum androgens during the second half of pregnancy in the rat (1–3). Using classical endocrine ablation techniques, our earlier studies have indicated a predominately uterine (fetal-placental) origin for testosterone in the second half of gestation (4, unpublished observations). A similar conclusion has been reached on the basis of ovarian and uterine vein concentrations of testosterone (5). Conceptus number in the rat exhibits a direct relationship to maternal hormone levels; specifically progesterone (6), relaxin (7), and placental lactogen (8). Moreover, the number of fetuses determined the timing for the termination of the nocturnal prolactin surges in the rat (9). In this study, we have examined the relationship of maternal serum testosterone levels, as well as those of progesterone and androstenedione, to the number of fetal-placental units. In addition, we have also examined the relationship of fetal sex to maternal serum testosterone levels.

Materials and Methods

Female rats (Holtzman, Sprague-Dawley strain; 280–330 g) were maintained on a 14:10-hr light:dark cycle with food and water *ad libitum*. Vaginal smears were examined daily to assess the stage of the estrous cycle. Normally cycling female rats were mated on proestrus with males of proven fertility and the last day of vaginal cornification (estrus) was designated Day 1 of pregnancy. Pregnancy was indicated by sperm in the vaginal lavage on the morning of estrus and was confirmed at laparotomy on Day 8 of pregnancy.

Relationship of Conceptus Number to Maternal Steroid Levels. On Day 8 of pregnancy, at the time of laparotomy, under ether anesthesia to confirm pregnancy, the number of conceptuses was adjusted to 1, 2, 4, 6, or >10 by aspiration of the extra implantations with an 18-gauge needle attached to a low pressure vacuum line. The endometrium was scraped away using

[1] To whom requests for reprints should be addressed at Texas Tech University Health Science Center, Department of Obstetrics and Gynecology, 1400 Wallace Blvd., Amarillo, TX 79106.

Received January 26, 1989. [P.S.E.B.M. 1990, Vol 195]
Accepted May 8, 1990.

0037-9727/90/1951-0109$2.00/0

the bevel of the needle to ensure complete curretage of the implantation site. Rats in the >10 conceptus group were used as surgical controls since areas between the implantation sites were aspirated as a surgical procedure similar to other animals in this study. Rats were laparotomized on Days 12 and 20 to confirm that they had maintained the desired number of implantations throughout the experiment. Rats were bled (0.8 ml) on Days 11, 12, 15, 18, and 20 under ether anesthesia by direct jugular vein puncture. Serum was frozen at −20°C until radioimmunoassayed for progesterone, testosterone, and androstenedione.

Relationship of Fetal Sex to Maternal Testosterone Levels. At laparotomy on Day 8 of pregnancy, the number of conceptuses was adjusted to three per rat as described above with laparotomies on Days 12 and 20. Rats were bled (0.6 ml) on Days 11, 12, 15, 18, and 20 and serum was frozen at −20°C until assayed for testosterone. The sex of the fetuses was determined either immediately postpartum or several days prior to parturition when the mothers were killed and fetuses were examined under a dissecting microscope. Data are only presented for those pregnant rats in which the sex from all three fetuses was ascertained.

Radioimmunoassays. Progesterone was analyzed using a highly specific antiserum generously provided by Dr. Gordon Niswender (GDN 337; Colorado State University) and validation of this procedure has been reported (10). The intra- and interassay coefficients of variation were 7.0 and 15.9%, respectively. Testosterone was assayed with a highly specific antiserum (x-181), generously provided by Dr. P. N. Rao, that had been validated previously (11). The intra- and interassay coefficients of variation were 5.8 and 13.5%, respectively. The antiserum for andostenedione (x-322) was also supplied by Dr. Rao; it has minimal cross-reaction with other androgens (12). Intraassay and interassay coefficients of variation were 7.0 and 9.0%, respectively. In all cases, antisera were specific enough to allow assay following ether extraction without any necessary chromatography. Quality control was monitored regularly as part of a World Health Organization Special Program.

Statistics. Repeated measures analysis of variance was used to compare number of implants and days of pregnancy, and to test for consistency of treatment difference across time. Since there was a significant interaction between treatment and time ($P < 0.0001$) results were analyzed by day using a one-way analysis of variance. Single degree of freedom contrasts for linear response with the number of implantations were computed. A one-way analysis of variance was also used to compare maternal testosterone levels in the study of fetal sex for both Day 15 and Day 18 results.

Results

Relationship of Conceptus Number to Maternal Steroid Levels. *Testosterone.* When analyzed by day, using single degree of freedom contrasts, there was no significance to the slope of testosterone levels versus number of implants on Day 11. On days 12, 15, 18, and 20 however, the slopes were highly significant ($P < 0.0001$ on Days 12, 15, and 18, and $P < 0.008$ on Day 20) (Fig. 1).

Androstenedione. There was no apparent relationship of conceptus number and maternal serum androstenedione concentration (Fig. 2).

Progesterone. In pregnant mothers with a single implantation, progesterone levels did not increase during pregnancy. There was a linear increase in maternal progesterone levels at Day 15 of gestation between one and four implantations, above which serum progesterone levels remained plateaued (Fig. 3).

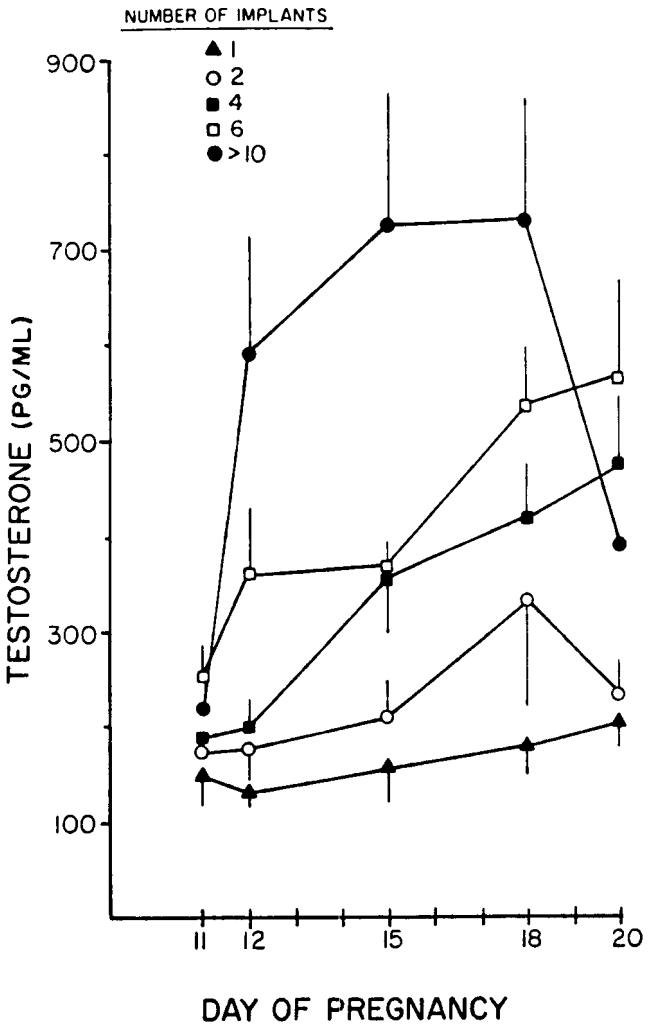

Figure 1. Mean (± SE) testosterone levels in maternal serum of pregnant rats bearing 1, 2, 4, 6, or >10 implantations from Days 11 through 20 of gestation. A blood sample was collected from each pregnant rat at each time period. There were 6 rats with one implant; 7 rats with 2 implants; 5 rats with 4 implants; 8 rats with 6 implants, and 5 rats with 10 or more implants.

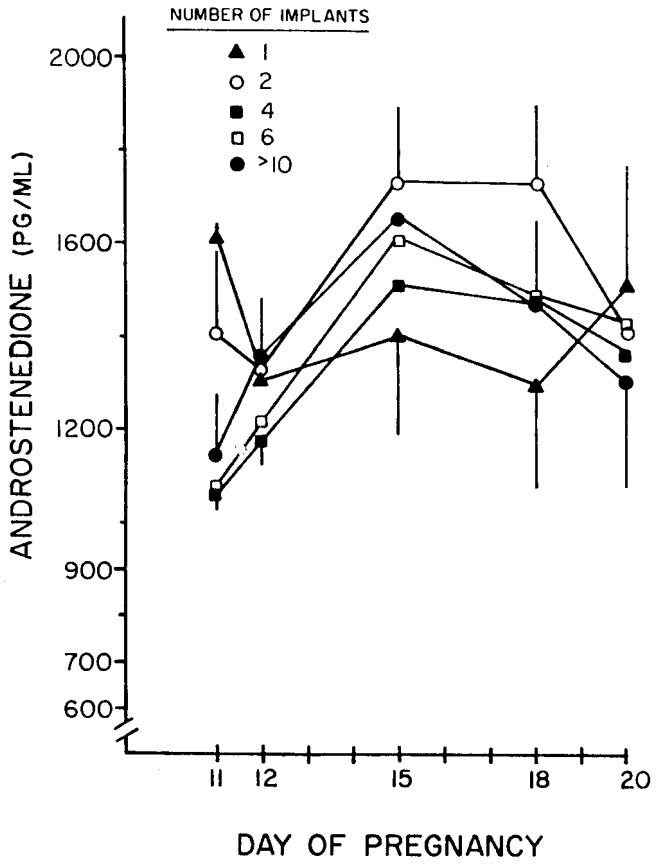

Figure 2. Mean (± SE) androstenedione levels in maternal serum of pregnant rats. The same animals and *n* values, which are described in Figure 1, apply to these results.

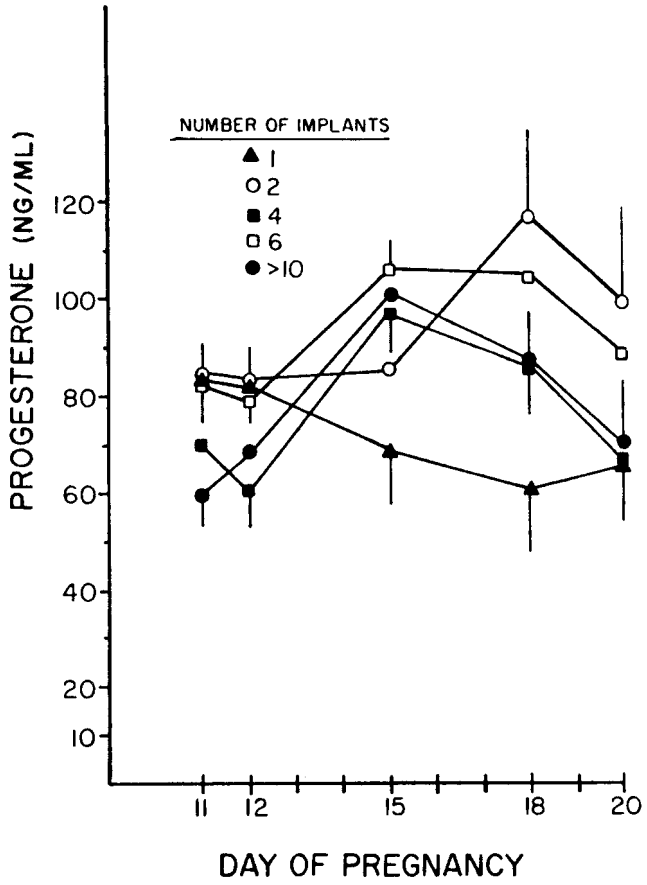

Figure 3. Mean (± SE) progesterone levels in maternal serum of pregnant rats. The same animals and *n* values, which are described in Figure 1, apply to these results.

Relationship of Fetal Sex to Maternal Testosterone Levels. The distribution of male/female fetuses in a controlled litter of three fetuses was without effect on the maternal serum testosterone levels as judged by one-way analysis of variance. Data are only presented for testosterone levels on Day 15 ($P = 0.54$) and Day 18 ($P = 0.33$) of pregnancy. In those litters in which the sex of only two fetuses could be confirmed, testosterone results are in good agreement (data not presented) with those for litters in which the sex of three fetuses was ascertained (Fig. 4).

Discussion

The results of the first study demonstrate a direct linear relationship between conceptus number and maternal testosterone level. This linear relationship is especially evident on Days 12, 15, and 18 of pregnancy. In intact, full complement pregnancy, testosterone levels increase from Day 12 to peak levels at Days 18–20 of gestation (1–4). A similar relationship between the number of conceptuses and maternal serum testosterone levels has been reported in mice (13). If circulating testosterone levels were to effect placental testosterone production, we would expect significant deflections from the linear relationship of testosterone to implant number. The linearity of this comparison suggests that testosterone does not exert any regulatory effect on placental testosterone production, but further studies are required to clarify this relationship.

We have also found a linear relationship between one and four conceptuses and corresponding serum progesterone levels on Day 15 of pregnancy as previously reported by Kato *et al.*, (6). The absence of such a linearity at gestational times other than Day 15 may be due to the fact that the maximum serum level of progesterone in full complement pregnancies is found at this time (14). The plateau of serum progesterone in rats bearing four or more implants suggests a maximal luteotropic stimulus at this number of fetal-placental units. Kato *et al.* (6) have reviewed the literature and concluded that rat placental lactogen is not the placental hormone responsible for this linear progesterone relationship. Since testosterone is an important component of the luteotropic complex, it is possible that increasing testosterone levels, through luteal aromatization, may serve this function. Alternatively, the possibility of a chorionic gonadotropin of placental origin exists in the rat (15–17). In a previous report we have presented data which indicates production of a placental chorionic gonadotropin and that placental numbers

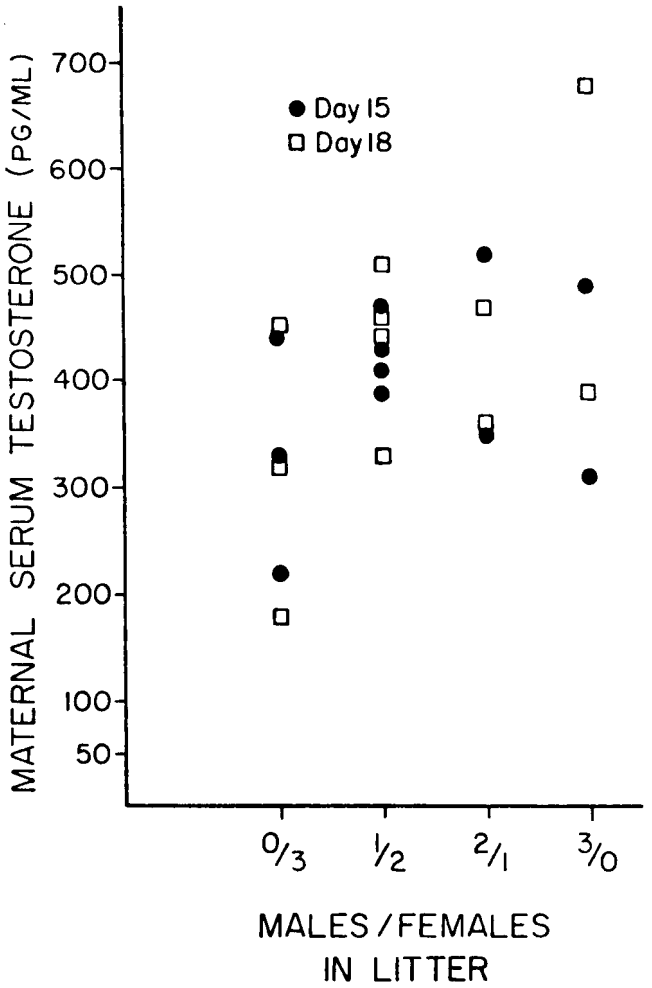

Figure 4. Individual serum testosterone levels in pregnant rats bearing three fetuses and plotted according to the number of male/female fetuses in each litter. Testosterone values are presented for Days 15 and 18 of gestation since they are both days of elevated maternal testosterone levels.

may be critical in supplying enough of this putative gonadotropin for ovarian stimulation (18).

An important distinction exists between the regulation of progesterone and testosterone levels in the pregnant rat after Day 12. Progesterone is a luteal product that is regulated by placental luteotropins. Placental testosterone is an important product in this complex as it provides precursors for intraluteal estrogen production which has been demonstrated to be an essential component of the luteotropic complex in this species. Conversely, the production of testosterone during the second half of pregnancy is from placental sources and may involve some fetal contribution of precursors for placental conversion. It is therefore not unexpected that regulation of progesterone and testosterone in pregnancy with different numbers of implantations is markedly different. Control of luteal progesterone production requires placental luteotropic production and seems to be maximal at four or more implants (6). Placental testosterone represents production by the placenta and seems to be directly related to placental mass and therefore increases directly with placental number. The distinct difference in the regulation of progesterone and testosterone is reflected in the results of this study.

There is no relationship between conceptus number and serum androstenedione levels. This finding was not unexpected since our previous studies (4) have indicated that androstenedione has multiple origins and that endocrine ablation studies could not delineate a major source for this androgen in the maternal serum of the pregnant rat.

In the second part of this study, we have examined the relationship of fetal sex to maternal serum testosterone levels. It has been reported that maximal testosterone production by the fetal rat testes occurs on about Day 18 of gestation (19–21), at which time testosterone levels in female fetuses are identical to those in the maternal circulation (21). Days 18–20 are also the time of the peak maternal levels of androgens (1, 3, 4). Furthermore, a demonstration of placental testosterone production *in vitro* also indicates Day 18 as the time of maximal production (22). These observations suggest that, in the absence of placental aromatase in the rat (23), fetal testicular steroids may cross the placenta in late pregnancy and contribute to elevated maternal levels. Observations in other species give little support for this possibility and in the human there is generally no correlation between the sex of the fetus and maternal testosterone levels (24–27). Similarly, in the pregnant mare, plasma testosterone levels were unrelated to fetal sex (28). Maternal serum testosterone levels in mice with a predominance of male fetuses were not different from testosterone levels in mothers with predominantly female fetuses (29). In the present study we have examined serum testosterone levels with regard to the distribution of male and female fetuses in a litter controlled at three implantations. There is no relationship of maternal testosterone level to the number of male fetuses. While the number of observations in our study is small, the physiologic results are in agreement with other species. Further studies are required to determine which aspects of the fetal-placental relationship in the rat are most important in the increase of maternal testosterone levels.

These studies were supported by NIH grant R01-HD-11806 to VDC and the Minority Biomedical Research Program (NIH) Grant 5-506-RR-08170-03 (J. L. C.) administered through Incarnate Word College.

The generous gifts of antisera from Drs. Gordon Niswender and P. N. Rao are acknowledged. We are grateful for the expert assistance of Mary Louise DeLeon and Elizabeth Wood in animal studies, Edna Wright and Daniel Martinez in steroid radioimmunoassays, and of Valerie Arneson in statistical analysis. The secretarial work of Debbie Pallmeyer is greatly appreciated.

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