

**The effect of pinealectomy on serum progesterone and oestradiol levels during the last stage of pregnancy in rats**

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**Summary.** Pinealectomy leads to significantly higher levels of progesterone (on days 19 and 20 of gestation) and oestradiol (on days 21 and 22) in the serum of pregnant rats. These results indicate that during the last phase of pregnancy the pineal gland may be modifying the levels of gonadal hormones, although not affecting the sequence of the physiological events.

The pineal gland may be helping to regulate the balance of hormones during the prenatal period. Much evidence indicates that the pineal – by way of its indoleamines and polypeptides – may be affecting the synthesis and release of reproductive hormones either by central action at the level of the hypothalamus, or through direct action on the gonads<sup>1,2</sup>. In earlier experiments<sup>3</sup> we found serum LH levels were higher in pinealectomized than in sham-operated control rats during the last 2 days of pregnancy, as a result of increased LH release from the pituitary. At the same time, prolactin levels in hypophysis and serum were reduced, suggesting decreased synthesis and release of that hormone. It was interpreted from these findings that during the last phase of pregnancy the pineal gland is involved in the modification of hormone balance by depressing the release of LH and increasing the synthesis of prolactin. Since an interrelationship between pituitary gonadotrophins and gonadal hormones has been established, it was considered necessary to determine whether any changes analogous to those in gonadotrophins occur in the level of gonadal hormones in pinealectomized rats during the same stage of pregnancy. Levels of blood progesterone and oestradiol were therefore measured in pinealectomized and sham-operated control rats during the last 4 days of pregnancy. Changes in levels of gonadal hormones and the exact timing of their occurrence should indicate whether the effects exerted by the pineal substances on the pituitary hormones are primary (direct) or secondary to the changes taking place in the gonads.

**Materials and methods.** Female rats of the Hebrew University's 'Sabra' strain were pinealectomized or given sham-operations when 22–24 days old and housed in airconditioned (23–24 °C) quarters lit from 07.00–19.00 h by overhead 40-W 'daylight' tubes. 6 or 7 weeks later they were mated. The morning on which a sperm-positive vaginal

smear was obtained was termed day 1 of gestation. The rats were decapitated between 10.00 and 12.00 h on days 19, 20, 21 and 22 of gestation. Their blood was collected, centrifuged and the sera stored frozen at –20 °C. Only sera from dams with 4 or more living foetuses were taken into consideration. Sera were assayed with radioimmunoassay kits for oestradiol and progesterone purchased from Isodan Ltd, Jerusalem.

**Results.** Serum progesterone levels, which in rats are high during pregnancy, remain so on days 19 and 20 but begin to decline towards day 21 and fall more abruptly before parturition on day 22 of gestation (table 1).

In the pinealectomized rats, serum progesterone levels are significantly higher than in respective sham-operated rats during days 19 and 20 of gestation. On day 21, although a rapid decline is taking place, serum progesterone is still higher in the pinealectomized than in sham-operated group. Before parturition (on day 22 of gestation) the difference between the 2 groups disappears and very low levels of serum progesterone are seen in both (table 1). In contrast to those of progesterone, the serum levels of oestradiol show a sharp increase during days 21 and 22 of gestation when compared to the preceding days' levels, the increase being significantly greater in the pinealectomized than in the sham-operated animals (table 2).

**Discussion.** The results of this study demonstrate that pinealectomy-induced changes in gonadotrophin levels of pregnant rats during the period preceding parturition<sup>3</sup> are accompanied by changes in serum concentrations of progesterone and oestradiol.

It is interesting that, after pinealectomy, while progesterone was found to be enhanced during days 19 and 20 of gestation, oestradiol showed an increase only towards the end of pregnancy, during days 21 and 22. Pinealectomy could, therefore, be acting as a booster to the physiological

Table 1. The effect of pinealectomy on serum progesterone levels (ng/ml ± SEM) in rats during late pregnancy

Treatment	Days after conception 19	20	21	22
Sham-operation	80.7 ± 3.9 (18)	86.1 ± 3.1 (21)	60.0 ± 6.1 <sup>c</sup> (18)	13.2 ± 1.3 <sup>d</sup> (20)
Pinealectomy	99.4 ± 3.1 <sup>b</sup> (16)	99.7 ± 3.6 <sup>a</sup> (20)	68.2 ± 6.3 <sup>c</sup> (17)	12.0 ± 1.1 <sup>d</sup> (21)

<sup>a</sup>  $p < 0.01$ ; <sup>b</sup>  $p < 0.001$  compared to sham-operated group. <sup>c</sup>  $p < 0.02$ ; <sup>d</sup>  $p < 0.001$  significance of differences within each group between consecutive days. Number of animals in parentheses.

Table 2. The effect of pinealectomy on serum oestradiol levels (pg/ml ± SEM) in rats during late pregnancy

Treatment	Days after conception 19	20	21	22
Sham-operation	44.7 ± 4.7 (18)	42.3 ± 4.9 (18)	62.6 ± 4.5 <sup>c</sup> (20)	62.3 ± 4.9 (20)
Pinealectomy	40.1 ± 2.8 (20)	53.3 ± 8.1 (18)	80.0 ± 3.9 <sup>a,c</sup> (20)	84.3 ± 6.1 <sup>b</sup> (19)

<sup>a</sup>  $p < 0.02$ ; <sup>b</sup>  $p < 0.01$  compared to sham-operated group. <sup>c</sup>  $p < 0.005$  compared to the same group on the previous day. Number of animals in parentheses.

processes, i.e. further enhancing progesterone and oestradiol levels while they are high, though not by preventing their decline nor advancing their rise. Consequently, the pineal may be able to modify the levels of gonadal hormones but not affect the sequence of the physiological events. In a previous study<sup>3</sup> in which we investigated changes in pituitary hormones during pregnancy in pinealectomized rats, an enhanced increase in serum LH and decreased serum prolactin rise were observed during the 2 days before parturition. Thus, in the absence of the pineal, the increase in the gonadal hormone progesterone precedes the changes in pituitary hormone levels, which would rule out a direct effect of the pituitary on progesterone secretion. Moreover the enhanced levels of LH in serum do not affect the timing of the sharp decline in progesterone that occurs in the same animals at the end of pregnancy. This is in accord with the proposal made by Gibori and Richards<sup>4</sup>, that in the 2nd half of pregnancy the pituitary has an inhibitory effect on luteal cell function and that this may be being regulated by a placental-pituitary feedback. They suggest that the placenta may be regulating corpus luteal cell LH receptor content and progesterone production in the 2nd half of pregnancy by 2 of its hormones, 1 luteotrophic prolactin-like, the other LH-like chorionic gonadotrophin. The increased progesterone levels induced by pinealectomy may therefore result from enhanced production of placental hormones, as has been indicated by the prolactin-like luteotrophin which is at its peak on day 18 of pregnancy<sup>5</sup>.

Furthermore, it was demonstrated that during the prenatal period adrenal steroidogenesis contributes significant amounts of progesterone to the total maternal pool<sup>6</sup>. On day 22 of pregnancy, adrenal secretion of progesterone reaches a level severalfold that produced by the ovary or at any time during the oestrous cycle. Since the pineal gland is functioning as an inhibitory modulator of the adrenal

cortex in rats with normal oestrous cycle<sup>7</sup>, it is possible that the enhanced adrenal function brought about by pinealectomy is largely responsible for the increased progesterone levels in pregnant pinealectomized rats.

The sharp decline in progesterone that takes place ante partum in pinealectomized rats coincides with a surge of LH<sup>3</sup>. The possibility exists that the higher levels of LH in serum of pinealectomized animals contribute to the lowering of the progesterone peaks, bringing them down on day 21 to levels almost as low as those of the controls. This postulation may be substantiated by the finding that the ante partum fall in serum progesterone requires the presence of the pituitary<sup>8</sup>.

Regarding the changes in oestradiol brought about by pinealectomy, it seems likely that the pineal hormones normally exert an inhibitory effect on oestrogen production via the placental-pituitary complex mentioned previously. The pinealectomy-potentiated increase in serum oestradiol levels during the period preceding parturition coincides with the enhanced rise in serum LH<sup>3</sup> and could be a result of a positive feedback of that hormone.

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## The effects of vitamin E-deficiency on serum prolactin and serum luteinising hormone levels in the pregnant rat<sup>1</sup>

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**Summary.** No significant differences were observed between the serum prolactin or serum LH levels of vitamin E-deficient or vitamin E-replete rats during the first 12 days of gestation. It is suggested that pituitary dysfunction is not the cause of the characteristic foetal resorption observed in vitamin E-deficient rats.

Vitamin E-deficiency in the female rat is characterized by foetal resorption<sup>3</sup> which can be prevented by administration of adequate doses of the vitamin as late as the 11th day of the pregnancy<sup>4</sup>. Since hypophysectomy in the rat up to day 11 of gestation also results in termination of the pregnancy<sup>5</sup>, it is not surprising that several investigators have sought to explain the characteristic foetal resorption in vitamin E-deficient rats in terms of pituitary dysfunction. However, the results of such investigations have provided conflicting results. Thus Nelson<sup>6</sup> observed no histological differences between the pituitaries of vitamin E-deficient or vitamin E-replete rats at various stages of gestation. In contrast, Barrie<sup>7</sup> reported a degranulation of the basophils of the pituitary in vitamin E-deficient rats suggesting a decreased gonadotrophin concentration. Similarly, estimations of the gonadotrophin content of the pituitary of pregnant vitamin E-deficient rats by biological assay have indicated that the concentration is decreased<sup>8</sup>, increased<sup>9,10</sup>,

or is unchanged<sup>11</sup>. During the first half of gestation in the rat, the corpus luteum is maintained by the actions of the pituitary hormones prolactin and luteinising hormone (LH)<sup>12-16</sup>. The present study was undertaken to establish whether or not foetal resorption in the vitamin E-deficient rat is associated with changes in the circulating levels of prolactin and LH.

**Materials and methods.** The animals used in this study were female wistar rats bred in this laboratory. An experimental group of rats were weaned at 21 days of age on a vitamin E-deficient diet composed of purified casein 25%, dried yeast 10%, lard 5%, codliver oil 5%, sucrose 49%, vitamin premix (no vitamin E) 1%, and a mineral premix 5% (Cooper Nutrition Ltd.). A control group of animals were weaned on an identical diet with the exception that vitamin E was added at a concentration of 250 IU/kg. All animals were housed individually in a controlled environment (22 °C; 14/10 light/dark schedule), and allowed access to food and