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The Role of Estrogen in the Regulation of Corpus Luteum Function in the Midpregnant Rat

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Abstract. The relationship between conceptus number and serum estradiol levels was studied, and the role of estrogen in the regulation of corpus luteum (CL) function was investigated in midpregnant rats. The number of conceptuses was adjusted to one, or 10 or more on day 7 of pregnancy. Serum estradiol levels, serum progesterone levels, and CL weights in rats bearing one conceptus were significantly lower than those in rats bearing 10 or more conceptuses on day 15 of pregnancy. However, administration of estradiol 0.1μg/day decreased serum progesterone levels and CL weights both in rats bearing one conceptus and in rats bearing 10 or more conceptuses. These results suggest that estrogen itself might be luteolytic in midpregnant rats. Some possible explanations of this luteolytic effect of estrogen are discussed.

Key Words: estrogen, corpus luteum, pregnancy, conceptus number

Introduction

It is well known that corpus luteum (CL) function in pregnant rats is mainly regulated by pituitary hormones in the first half of pregnancy[1,2], while placental substances are essential in the second half of pregnancy[3-5].

Recently, Kato et al[6] reported that there was a quantitative relationship between the number of conceptuses and CL function in pregnant rats. The presence of some luteotropic substances in the placenta has also been reported by several investigators[3-5]. However, it is not yet clear how placental substances regulate CL function. On the other hand, the important role of estrogen in the regulation of CL function has been demonstrated in pregnant rats[7-10]. Estrogen, with or without placental substances, exerted a direct luteotropic effect in pregnant rats hypophysectomized and hysterectomized on day 12 of pregnancy[8]. Intraluteal concentration of estrogen was closely related to progesterone secretion and CL growth after day 12 of pregnancy[9,10].

The present study was designed to investigate the effect of placental substances on circulating estrogen levels by experimentally reducing the number of conceptuses. In addition, the role of estrogen in the regulation of CL function in midpregnant rats was examined.

Materials and Methods

Adult virgin female rats (Sprauge-Dawley strain), 240–280g body weight, were housed with free access to Purina chow and water. Individual cycles were followed by daily vaginal smears obtained between 0900 and 1000h. Groups of proestrous rats were housed with males overnight, and those in which vaginal sperms were found were presumed to be pregnant. The day of insemination was considered day 1 of pregnancy. Pregnancy was confirmed by laparotomy on day 7 by the presence of embryonic swelling in the uterus. All rats used in this study had more than 10 conceptuses.

Adjustment of the number of conceptus

A clean but not aseptic technique was used for all operations. On day 7 of pregnancy, each rat was anesthetized with ether and the uterus was exposed through an abdominal incision. A Pasteur pippet was inserted through a small incision in the antimesometrial surface of an embryonic swelling and the entire conceptus was aspirated. Sham aspirations were done similarly except that the incision was made between the swellings and care was taken not to disturb the neighboring conceptuses. Each rat, therefore, received almost the same amount of surgery, being left with one, or 10 or more conceptuses in the uterus.

Experiment I

Two groups of rats, bearing one conceptus or bearing 10 or more conceptuses, were autopsied on day 7, 9, 12 or 15 of pregnancy. Blood samples were taken by puncture of the abdominal vein at autopsy under light ether anesthesia. The blood was allowed to clot, then centrifuged at 4°C, and next stored at −20°C for estradiol and progesterone assays. The ovaries were also removed and cleaned. 5 or 6 CLs were removed from the ovaries, weighed as a group, and the mean CL weight for each rat was calculated.

Experiment II

Estradiol-17β (0.1μg/0.2ml sesame oil/day) was injected daily into two groups of rats, bearing one conceptus or bearing 10 or more conceptuses, from day 7 to 15 of pregnancy. The estradiol-17β dose was determined by a pilot study in which daily injection of 0.1μg/day estradiol-17β into ovariectomized rats increased serum estradiol levels to 10–30 pg/ml. These levels were equivalent to those found in rats bearing 10 or more conceptuses in Experiment I. All rats were autopsied on day 15 of pregnancy. Blood samples and CLs were taken as in Experiment I above.

Hormone assay

Serum estradiol was determined by a radioimmunoassay using a specific anti-estradiol serum which was kindly supplied by Dr. Akira Kanbegawa (Teikoku hormone MfG. Co.). Each sample of 1.5 ml serum was extracted with 10ml ethylether and subjected to Sephadex LH-20 column chromatography (solvent; Benzen: Methanol = 85 : 15). Fractions which contained estradiol were collected, evaporated under N_2, and used for estradiol determination.

Serum progesterone was determined by a radioimmunoassay reported previously[29].

Results

Experiment I

Figure 1 shows serum estradiol levels in rats bearing one conceptus or bearing 10 or more conceptuses. Serum estradiol levels in

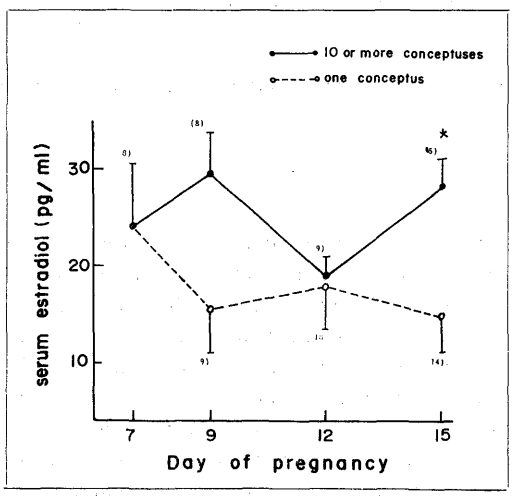

Fig. 1 Changes in serum estradiol levels in rats bearing one conceptus or bearing 10 or more conceptuses during days 7–15 of pregnancy. The graph shows mean values ± SEM, with the number of observations in parentheses.
*P<0.01 versus one conceptus group

rats bearing one conceptus were lower than those in rats bearing 10 or more conceptuses from day 9 of pregnancy onward. Day 15 serum estradiol levels in rats bearing one conceptus were significantly ($P<0.01$) lower than those in rats bearing 10 or more conceptuses.

Figure 2 shows serum progesterone levels and CL weights in rats bearing one conceptus or bearing 10 or more conceptuses. Serum progesterone levels and CL weights in rats bearing 10 or more conceptuses increased rapidly after day 12 of pregnancy. CL weights on day 12 of pregnancy in rats bearing one conceptus were significantly ($P<0.001$) smaller than those in rats bearing 10 or more conceptuses, whereas there were no significant differences in day 12 serum progesterone levels between both groups. On day 15 of pregnancy, both serum progesterone levels and CL weights in the one conceptus group were significantly ($P<0.001$) lower than those in the 10 or more conceptuses group. Day 15 serum progesterone levels in the one conceptus group were significantly ($P<0.05$) lower than day 12 levels.

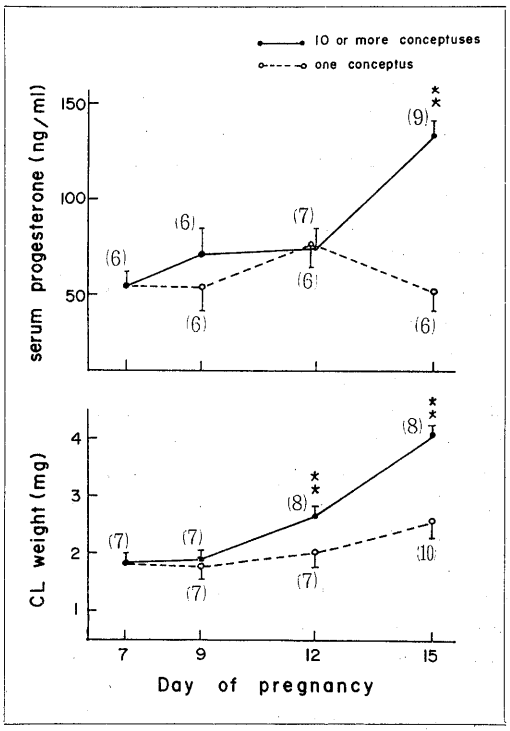

Fig. 2 Changes in serum progesterone levels and CL weights in rats bearing one conceptus or bearing 10 or more conceptuses during days 7–15 of pregnancy. The graph shows mean values ± SEM, with the number of observations in parentheses.
**$P<0.001$ versus one conceptus group

Experiment II

Table I shows the effect of estradiol-17β injection on serum progesterone levels and CL weights at day 15 of pregnancy. Estradiol treatments caused a significant decrease in serum progesterone levels in both the one conceptus and 10 or more conceptuses groups. Day 15 CL weights were also smaller, although not significantly, in the estradiol treated group relative to the control group. In the estradiol treated group, three rats bearing one conceptus aborted. However, there were no significant differences in serum progesterone levels and CL weights between rats which aborted and rats which did not abort. No rats bearing 10 or more conceptuses aborted due to estradiol treatments.

Discussion

Surgical reduction of conceptus number prevented the rapid increase of CL growth and progesterone levels normally observed after day 12 of pregnancy. Interestingly, on day 12 of pregnancy, CL weights in rats bearing one conceptus were significantly smaller than those in rats bearing 10 or more conceptuses. These findings fit with the previous report[6] which indicated that serum progesterone levels and CL weights are apparently related to conceptus number, and that they are regulated in different manners.

Table 1 Effect of estradiol-17β on serum progesterone levels and CL weights on day 15 of pregnancy in rats bearing one conceptus or bearing 10 or more conceptuses. Each rat received daily injections of 0.1μg estradiol-17β during days 7-15 of pregnancy, and was autopsied on day 15.

		number	serum progesterone (ng/ml±SE)	CL weights (mg/CL±SE)
one conceptus group				
	E_2 0	6	36.6± 5.1	2.6±0.1
	E_2 0.1	7§	8.2± 1.8*	2.2±0.2
10 or more conceptuses group				
	E_2 0	4	129.4±13.9	3.9±0.1
	E_2 0.1	7	75.0± 3.5*	3.6±0.2

§ Three rats aborted, but there were no significant differences in serum progesterone levels and CL weights between rats which aborted and rats which did not abort.

* $P<0.01$ versus E_2 0 group

The present results also demonstrate that serum estradiol levels in rats bearing one conceptus are lower than those in rats bearing 10 or more conceptuses. Several investigators[11,12] have reported changes in serum estradiol levels in intact pregnant rats. Similar chages were also observed in rats bearing 10 or more conceptuses in the present study. It has been reported that the CL can convert androgen to estrogen[13-16], and that the rate of conversion mainly depends upon the amount of precursor androgen, rather than the activity of aromatase enzyme[16]. The source of androgen is not yet clear. However, Chan et al[17] and Rembiesa et al[18] have reported that the rat placenta can produce androgen. Therefore, the difference in serum estradiol levels in rats bearing one conceptu-s or bearing 10 or more conceptuses might be due to the difference in the amount of androgen from the placenta. On the other hand, Fortune et al[19] demonstrated the production of androgen in theca cells stimulated by LH. In addition, it has been reported that the rats placenta secretes LH-like substances during pregnancy[3-5,20-22]. Although there are some controversial findings in which the pituitary (or LH) appeared to have little effect on estrogen production during the second half of pregnancy[23], we can not exclude the possibility that some LH-like substances from the placenta may regulate serum estrogen levels. Presumably, they would affect the production of androgen at the ovary.

It is well known that estrogen has luteotropic effects when prolactin or some placental substances are present[13]. Gibori et al[8] and Takayama et al[24] reported that estrogen increased serum progesterone levels in rats hypophysectomized and hysterectomized on day 12 of pregnancy. Estrogen also prevented the luteolytic effect of LH-antiserum between day 9 and day 12 of pregnancy[7]. However, administration of physiological doses (0.1μg/day) of estradiol-17β, unexpectedly, decreased serum progesterone levels both in rats bearing one conceptus and in rats bearing 10 or more conceptuses (Table I). These findings clearly indicate that the differences in serum progesterone levels and CL weights between rats bearing one conceptus and rats bearing 10 or more conceptuses were not due only to differences in serum estradiol

levels. It was interesting to note that estrogen treatments in intact pregnant rats on day 11 blocked the increase in serum progesterone levels on day 15 usually found in normal pregnant rats[7]. These findings, together with the present data, strongly suggest that estrogen might not be luteotropic, but rather luteolytic in the presence of pituitary and gravid uterus. It was not clear how estrogen caused luteolysis in the present study. It is also difficult to explain why the physiological dose of estrogen caused abortions in rats bearing one conceptus. In our preliminary studies, we found that estradiol affected the LH-RH content of the hypothalamus in pregnant rats (Yamashita et al, unpublished findings). Some possible actions of estrogen at the uterus have also been demonstrated. Ham et al[25] reported that estradiol caused a rise in prostaglandin levels in the uterus, although this was blocked by progesterone. It is well known that prostaglandin has luteolytic and abortive effects in pregnant rats[26,27]. Alexandrova et al[28] showed that the concentration of oxytocin receptor in the uterus was proportional to the ratio of plasma estradiol to progesterone. It is likely, therefore, that since rats bearing one conceptus showed significantly lower serum progesterone levels, even a small amount of estrogen might cause luteolysis and abortion in rats bearing one conceptus.

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