

Termination of Pregnancy in the Rat by the Antiserum to the β Subunit of Ovine Interstitial Cell Stimulating Hormone¹ (38447)

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It has recently been shown that the β subunit of ovine ICSH² alone is capable of inducing ovulation in hamsters (1), and can maintain pregnancy in the rat when injected together with prolactin (2). In addition, it was found that the immunological activity of the hormone resides in the β subunit (3). This report will describe the effectiveness of a rabbit antiserum to ICSH- β in terminating pregnancy in the rat.

Experimental. Ovine ICSH and its subunits were isolated as previously described (4, 5). Rabbit antisera to ICSH and ICSH- β were prepared according to published procedures (3, 6). The cross reactivity of the antiserum to ICSH- β with rat ICSH was ascertained by the quantitative precipitin test (7) using rat pituitary extract as the source of antigen. Fifty rat pituitary glands were homogenized with 5 ml saline solution at 0° for 5 min and the insoluble material was removed by centrifugation in the cold room for 30 min. The clear supernatant was used for the quantitative precipitin test and 0.1 ml of the supernatant represents one rat pituitary equivalent.

Virgin female rats (150–180 g) were allowed to mate with males of proven fertility and the day of finding sperm in the vaginal smear was considered Day 1 of pregnancy. Pregnancy was confirmed by exploratory laparotomy on Day 8 after the smear. The animals were then treated with the test antiserum, given by subcutaneous injection. At the end of the treatment, the animals were autopsied and examined for the presence of

live embryos. The ovaries and uteri in appropriate cases were dissected and weighed. All animals in the control group were autopsied 24 hr after delivery.

Results and Discussion. Figure 1 presents quantitative precipitin curves for ovine ICSH- β and the rat pituitary extract using antiserum to ICSH- β . It is evident that rat ICSH cross reacts to the extent of approximately 50% with the ovine ICSH- β antiserum. Data in Table I show that the antiserum is effective in terminating pregnancy in the rat. At autopsy on Day 18 it was observed that the uterus was clean, devoid of any signs of pregnancy. The ovarian and uterine weights of animals treated with the antiserum

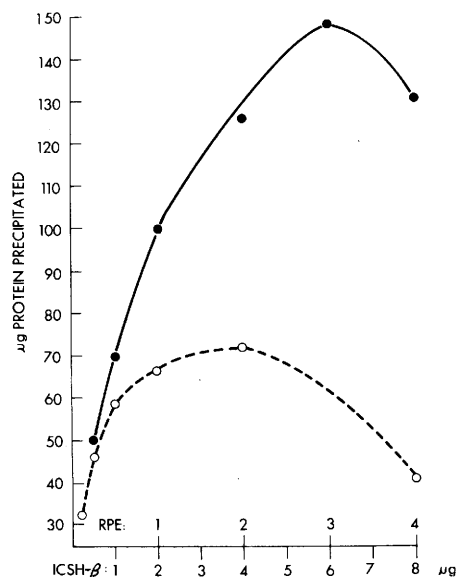


FIG. 1. Quantitative precipitin curves of ICSH- β and rat pituitary extract using antisera to ICSH- β ; ●—●, SR-6 with ICSH- β ; ○---○, SR-6 with rat pituitary extract. RPE, rat pituitary equivalent as defined in the text.

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² Abbreviations: ICSH, ovine pituitary interstitial cell stimulating hormone; ICSH- β , the β subunit of ovine ICSH; TSH, thyroid stimulating hormone; FSH, follicle stimulating hormone; RPE, rat pituitary equivalent.

TABLE 1. Effect of Antiserum to ICSH- β on Pregnancy in the Rat.^a

Group no.	No. of rats	Treatment	Ovary ^e	Uterus ^e	Remarks
1	7	NRS	100 $\pm$ 13.5		All delivered live young on Day 22 (average 11 pups)
2	9 ^c	SR-6 ^b	70 $\pm$ 13.2	278 $\pm$ 90.0	Complete termination in 7 rats
3	2	ICSH a/s ^d	61	189	Bleeding within 24 hr and complete termination.

^a All animals were autopsied on Day 18 except Group 1; NRS, normal rabbit serum; SR-6, antiserum to ICSH- β .

^b Each animal received 1 ml antiserum per day on Days 8–10.

^c Two rats did not show vaginal bleeding, but had dead fetuses.

^d Two-tenths milliliter on Day 8.

^e Weight in mg; mean $\pm$ standard deviation.

were reduced and corpora lutea were regressed. All animals treated with normal rabbit serum delivered normal, live young on Day 22 of pregnancy. However, it was noticed that while the ICSH antiserum-treated group (Table I, Group 3) required only one injection, treatment with its β subunit antiserum (Table I, Group 2) required daily administration over a period of 3 days. Further, the appearance of vaginal bleeding was not seen until 72 hr after treatment with ICSH- β antiserum; no external bleeding was observed in two animals.

The high dosage of antiserum to ICSH- β required to terminate pregnancy is probably due to the fact that antiserum to ICSH- β cross reacts to a lesser extent than the reaction of the antiserum to native ovine ICSH with rat pituitary ICSH. The possibility of contaminating antibodies to the native hormone may be ruled out in view of the fact that the ICSH- β used was highly purified (5) and immunologically characterized (3); it was employed for the elucidation of its primary structure (8). It is also likely that the extent of biological neutralization of circulating ICSH in the rat by the β subunit antiserum may be much less than the immunological cross reactivity observed by the quantitative precipitin test (Fig. 1). It should be pointed out that only two rats were employed for the group treated with antiserum to the intact ICSH as the effects of ICSH antiserum on reproductive processes in the female rat have been thoroughly investigated (9–11).

Recent studies (12, 13) on TSH, ICSH, and FSH indicate that of the two subunits, the β subunit confers hormonal specificity, whereas the α subunit is either identical or nearly so structurally in a given species. The fact that the ICSH- β antiserum is effective in neutralizing endogenous ICSH in the pregnant rat offers the

use of the β subunit as an immunogen instead of intact ICSH. This would reduce the chances of cross reactivity with circulating TSH or FSH in the experimental animal.

The immunological approach may be a simple method for regulating human reproduction. However, it has not progressed to the point of practical application, mainly because of the potential problems of protein sensitization in the recipient such as anaphylaxis following antiserum treatment. It has been recently shown (14) that by using monkey antiserum to ovine ICSH it is possible to disrupt the menstrual cycle and also terminate early pregnancy in the monkey. It may therefore be expected that the use of a highly potent homologous (monkey) antiserum to ovine ICSH- β may greatly facilitate the application of immunological techniques in the control of human fertility.

Summary. It has been shown that rabbit antiserum to the β subunit of ovine ICSH causes termination of pregnancy in the rat when it was injected for three days, beginning on Day 8 of pregnancy.

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