

# Effect of Adrenalectomy on Implantation and Maintenance of Pregnancy in Ovariectomized Rabbits

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## Introduction

Wu and Allen<sup>1)</sup> found that if progesterone alone was given twice daily, successful maintenance of pregnancy was observed in ovariectomized rabbits, and it was therefore suggested that oestrogen was not needed for normal implantation and maintenance of pregnancy in the rabbit. However, the adrenal glands may contribute sufficient steroids, particularly oestrogen<sup>2)</sup> to affect the conclusions, rabbits both ovariectomized and adrenalectomized were investigated in order to test the possibility that adrenal source of oestrogen may be required for normal implantation and development of foetuses in the ovariectomized rabbit.

## Materials and Methods

Young virgin albino rabbits (mean body weight: 2.80 kg), aged 5~7 months, were ovariectomized and adrenalectomized the day after insemination on day 1. Ovulation was induced by the injection of 25 IU of HCG. Adrenalectomy were performed by the method of White<sup>3)</sup> and given 2.5mg of cortison(Roussel, London) and 2.5 mg of desoxycorticosterone trimethylacetate (CIBA) just prior to operation. Thereafter they were fed the standard pellet diet given the choice of 3% glucose plus 3% NaCl or tap water to drink.

From ovariectomy to killing the rabbits were injected intramuscularly with progesterone with or without oestradiol, or with vehicle alone as shown in

**Table 1.** Allocation and Treatment of Rabbits Employed in the Experiment, with 9-11 Animals per Group (Animals received twice daily injections of steroid or vehicle alone)

| Treatment Group and Dosages                      | Days after Insemination |      |       |       |       |       |
|--|-------------------------|------|-------|-------|-------|-------|
|  | 2~4                     | 5~9  | 10~14 | 15~19 | 20~24 | 25~29 |
| 1. Ovariectomized and Adrenalectomized           |                         |      |       |       |       |       |
| Progesterone(mg/day)                             | 1                       | 3    | 6     | 6     | 3     | 1     |
| 2. Ovariectomized and Adrenalectomized           |                         |      |       |       |       |       |
| Progesterone(mg/day)                             | 1                       | 3    | 6     | 6     | 3     | 1     |
| Oestradiol( $\mu$ g/day)                         | 0                       | 0.25 | 0.5   | 1.0   | 2.0   | 4.0   |
| 3. Sham Ovariectomized and Adrenalectomized      |                         |      |       |       |       |       |
| Peanut Oil(ml)                                   | 1                       | 1    | 1     | 1     | 1     | 1     |
| 4. Sham Ovariectomized and Sham Adrenalectomized |                         |      |       |       |       |       |
| Peanut Oil(ml)                                   | 1                       | 1    | 1     | 1     | 1     | 1     |

table 1. The rabbits were laparotomized for inspection of implantation on day 10 and killed on day 30. On days 5, 10, 20, and 30 plasma from the ovariectomized-adrenalectomized rabbits receiving progesterone alone was pooled and checked for the absence of oestradiol. Neither free nor bound oestradiol was detected in any sample. At autopsy all rabbits were checked for completeness of adrenalectomy by gross inspection and histological examination of any doubtful-looking tissue. No adrenal remnants or regrowth were found.

The following statistics are used in presenting and discussing results:

$$\text{Implantation \%} = \frac{10^2 \times \text{total No. of implants on day 10}}{\text{total corpus luteum count}}$$

$$\text{Development \%} = \frac{10^2 \times \text{total No. of foetuses on day 30}}{\text{total No. of implants on day 10}}$$

### Results

The object of this experiment was to see whether

the adrenal glands, in which the adrenal cortex is a source of oestrogen, affect implantation and maintenance of pregnancy.

Implantation percentages and other relevant data from rabbits ovariectomized and adrenalectomized on day 2 after mating on day 1 are shown in table 2, from which it is clear that adrenalectomy did not affect the results. Maintenance thereafter is shown in table 3, and these data show that adrenalectomy did not affect foetal development percentages to any marked degree ( $p > 0.05$  for all effects), and that mortality associated with the severe operative procedures was evenly distributed. An effect of oestrogen in assisting survival of foetuses was not apparent in this experiment.

### Discussion

The adrenal glands were thought to be a possible source of sufficient oestrogen to permit implantation

**Table 2.** Implantation in Rabbits Ovariectomized and Adrenalectomized on Day 2 after Mating on Day 1 (9-11 animals per group)

| Treatment                         | OVX <sup>1</sup> +ADX <sup>2</sup> | OVX+ADX                       | Sham OVX<br>+<br>ADX | Sham OVX<br>+<br>Sham ADX |
|-----------------------------------|------------------------------------|-------------------------------|----------------------|---------------------------|
|                                   | P <sup>3</sup>                     | P+E <sub>2</sub> <sup>4</sup> | Peanut Oil           | Peanut Oil                |
| No. of Animals Pregnant on Day 10 | 8/9                                | 8/10                          | 8/10                 | 9/11                      |
| Total No. of Corpora Lutea        | 70                                 | 73                            | 85                   | 98                        |
| No. of Implants on Day 10         | 34                                 | 36                            | 44                   | 51                        |
| Percentage Implantation           | 49                                 | 49                            | 52                   | 52                        |

1 ovariectomized    2 adrenalectomized    3 progesterone    4 oestradiol

**Table 3.** Maintenance of Pregnancy in Rabbits Ovariectomized and Adrenalectomized on Day 2 after Mating on Day 1

| Treatment   | OVX <sup>1</sup> +ADX <sup>2</sup> | OVX+ADX                       | Sham OVX<br>+<br>ADX | Sham OVX<br>+<br>Sham ADX |
|---|------------------------------------|-------------------------------|----------------------|---------------------------|
|   | P <sup>3</sup>                     | P+E <sub>2</sub> <sup>4</sup> | Peanut Oil           | Peanut Oil                |
| No. of Animals Pregnant on Day 10                           | 8                                  | 8                             | 8                    | 9                         |
| No. of Animals Surviving on Day 30                          | 6                                  | 6                             | 5                    | 7                         |
| No. of Implants on Day 10 in<br>Animals Surviving to Day 30 | 25                                 | 27                            | 26                   | 37                        |
| Total No. of Foetuses on Day 30                             | 13                                 | 14                            | 14                   | 23                        |
| Percentage Development of Foetuses                          | 52                                 | 52                            | 54                   | 62                        |

1 ovariectomized    2 adrenalectomized    3 progesterone    4 oestradiol

in the absence of the ovaries, should it in fact be needed for implantation. Another possible oestrogen source may be peripheral conversion of adrenal androgens to oestrogens as reported for the human<sup>6)</sup>. Either could lead the investigator to false conclusions. Desoxycorticosterone, released by the adrenal cortex, resembles progesterone in that it produces progestational proliferation in the endometrium of immature rabbits primed with oestradiol, and induction of the oestrus response in the spayed guinea pig<sup>7)</sup>. Six to ten mg of desoxycorticosterone gave a progestational response approximately equivalent to 1 mg of progesterone and the uteri of the rabbits receiving 3 mg of desoxycorticosterone resembled those of animals receiving 0.5 mg progesterone. In this experiment, therefore, the single injection of 2.5 mg of the long-acting desoxycorticosterone acetate (possibly lasting in effect for about three weeks) may have been not only some help for survival of the adrenalectomized does but also have had a small additive effect on the replacement therapy. The ovaries, adrenal cortex and placenta are the organs which could synthesize oestrogens or transform one steroid to another. In the rabbit, Ainsworth and Ryan<sup>1)</sup> failed to demonstrate oestrogen formation from C-19 substrates following incubation with rabbit placental preparations. In the ovariectomized and adrenalectomized rabbit, therefore, it is unlikely that the long-acting desoxycorticosterone acetate could have been transformed to oestrogen.

It was recently shown that cortisol level remains constant for the first 24 days of gestation in the rabbit, but an abrupt increase begins in day 26 with peak levels on day 30<sup>2)</sup>. Declining progesterone levels plus increased cortisol levels appear to be important preparturient changes in the rabbit. However, it is clear from this experiment that no significant contribution to implantation can be attributed to the adrenal glands. Survival of the adrenalectomized does in this experiment without steroid replacement therapy beyond the operation period (sham-ovariectomized but adrenalectomized controls) may have been aided not only by dietary support provided and some effect of the desoxycorticosterone, but also by the fact that the does were pregnant. It

is well established that the pregnant adrenalectomized rat survives longer than its non-pregnant counterpart<sup>4,6)</sup>.

## Conclusion

Since the adrenal glands may contribute sufficient steroids, particularly oestrogens, to affect conclusions, rabbits both ovariectomized and adrenalectomized were subjected to similar tests in comparison with sham-operated controls.

It was clear from this experiment that no significant contribution to implantation and maintenance of pregnancy can be attributed to these glands and that oestradiol was not required for implantation in the rabbit.

## References

1. Ainsworth, L. and Ryan, K.J.: Steroid hormone transformations by endocrine organs from pregnant mammals. 1. Estrogen biosynthesis by mammalian placental preparations *in vitro*. *Endocrinology* (1966) 79 : 875.
2. Baldwin, D.M. and Stabenfeldt, G.H.: Plasma levels of progesterone, cortisol, and corticosterone in the pregnant rabbit. *Biol. Reprod.* (1974) 10 : 495.
3. Beall, D.: Isolation of oestrone from the adrenal gland. *Nature* (1939) 144 : 76.
4. Davis, M.E. and Plotz, E.J.: The effects of cortisone acetate on intact and adrenalectomized rats during pregnancy. *Endocr.* (1954) 54 : 384.
5. Grodin, J.M., Siiteri, P.K. and MacDonald, P.C.: Source of oestrogen production in postmenopausal women. *J. Clin. Endocr. Metab.* (1973) 36 : 207.
6. McKeown, T. and Spurrell, W.R.: The results of adrenalectomy in the pregnant albino rat. *J. Physiol.* (1940) 98 : 255.
7. van Heuverswyn, J., Collins, V.J., Williams, W.L. and Gardner, W.U.: The progesterone-like activity of desoxycorticosterone. *Proc. Soc. Exp. Biol.* (1939) 41 : 551.

8. White, S.W.: Adrenalectomy in the rabbit. Aust. J. Exp. Biol. Med. Sci. (1966) 44 : 447.  
 9. Wu, D.H. and Allen, W.M.: Maintenance of

pregnancy in castrated rabbits by 17- $\alpha$ -hydroxyprogesterone caproate and by progesterone. Fertil. Steril. (1959) 10 : 439.

## 난소를 제거한 가토의 착상 및 임신유지에 있어서 부신 제거의 영향

권            종            국

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### 국문초록

난소를 제거한 가토에 있어서 부신에서 분비되는 oestrogen이 착상에 충분한 가능성이 있음에 비추어 이 실험에서는 가토에서 oestrogen의 근원이 되는 난소와 부신을 함께 제거하여도 착상이 여전히 일어나며 임신이 유지되는가를 실험하였다. 이 실험결과는 다음 두가지 점을 밝혀주었다.

1. 부신에서는 가토의 착상에 충분한 oestrogen을 분비하지 아니하였다.
2. 가토의 착상과 임신유지는 oestrogen 없이도 가능하였다.