Aust. J. Biol. Sci., 1974, **27**, 275–83

Hormonal Requirements for Implantation and Pregnancy in the Ovariectomized Rabbit

J. K. Kwun and C. W. Emmens

Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W. 2006.

Abstract

After induced ovulation and insemination on day 1, followed by ovariectomy on day 2, a schedule of 1 mg of progesterone in two injections in oil per day on days 2–4 inclusive followed by 2 or 3 mg per day on days 5–9 inclusive was found to induce implantation in the majority of ovariectomized rabbits. Implantation percentages ($10^2 \times$ total no. of implants on day 10 $\div$ no. of corpora lutea) in different groups of 8–11 rabbits varied between 24 and 61%; sham-operated and vehicle-injected controls gave overall significantly higher percentages of 57–79%. The addition of 0·1–0·5 μg of 3,17β-oestradiol to experimental groups at various times during these procedures resulted in implantation percentages of 49–63%, but did not consistently improve them.

The only seemingly consistent effect of oestrogen was on implantation maintenance from day 10 onwards in does with surviving foetuses. These showed 5·1 implants/doe on day 10 and 2·8 foetuses/doe on day 30 with progesterone alone, and 5·2 implants/doe on day 10 and 3·7 foetuses/doe on day 30 (a significant difference) with added oestradiol, as against 6·0 implants/doe and 3·8 foetuses/doe on days 10 and 30 respectively in control animals.

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. No effects of adrenalectomy or of added oestrogen were apparent, and the results were otherwise similar to those above.

Overall, these findings demonstrate difficulties in producing consistent results and go far towards explaining the contradictory literature that has grown up on this subject.

Introduction

In the rabbit removal of the ovaries or corpora lutea interrupts implantation and pregnancy (Allen and Corner 1929) at any stage, while the administration of luteal extracts can maintain gestation to full term in ovariectomized animals (Allen and Corner 1930). The original success with crude extracts in maintaining pregnancy in rabbits castrated the day after mating was not followed by success with progesterone (Allen and Heckel 1937). This led Allen and Heckel (1937) to think that success with crude extracts may have been due to their having a suitable ratio of oestrogen and progesterone. However, the addition of oestrogen was not found to increase the capacity of progesterone to maintain pregnancy (Allen 1937).

Some later workers have reported that progesterone alone is able to replace ovarian function at implantation and in maintaining pregnancy (Chang 1951; Wu and Allen 1959; Prasad and Kalra 1967; Denker 1972), while others have suggested that oestradiol as well as progesterone is necessary if, variously, normal implantation, gestation and birth are to occur (Chambon 1949; Kehl and Chambon 1949; Hafez and Pincus 1956; Hilliard and Eaton 1971; Arthur and Daniel 1972; Horrell *et al.* 1972).

Wu and Allen (1959) decided that, since 17α-hydroxyprogesterone caproate maintains pregnancy quite well, progesterone itself might be too short-acting unless given more than once per day, and they did in fact achieve some success with divided injections, but in very small numbers of animals (three or four per group).

The problem is two-fold: what are the requirements of progesterone and oestradiol (i) for implantation, and (ii) for the maintenance of pregnancy thereafter in ovariectomized animals? Neither of these seems to have been satisfactorily determined, and most if not all recent workers have failed to check normality of implantation by carrying pregnancy through to term. These aspects of implantation and gestation in the rabbit have therefore been studied in a series of experiments with ovariectomized does given progesterone with or without oestradiol. It must be stressed that when replication with adequate numbers of animals is practised, some degree of heterogeneity is frequent in animal work. This cannot be seen unless investigators have used large enough groups and have repeated their experiments, as in the current work.

Materials and Methods

Young virgin albino rabbits (mean body wt ± S.D. 2·84 ± 0·44 kg), aged 5–7 months, were obtained from the University Animal House and kept in individual cages in a room maintained at 21–27°C, receiving artificial light from 0600 to 1800 h.

On day 1 each doe in all experiments was intravenously injected with 25 i.u. of human chorionic gonadotrophin (HCG; Pregnyl) and inseminated 30 min later with 0·1–0·2 ml of freshly collected semen. The semen was collected by artificial vagina. The does were then randomized to experimental groups; imbalance in final numbers was due to exclusion of animals found at ovariectomy to have been pseudopregnant on the day of insemination, or to intercurrent deaths (particularly in adrenalectomized subjects).

At various times after insemination the rabbits were ovariectomized and/or adrenalectomized under fluothane anaesthesia, using a Fluotec mark 2 anaesthetizing machine (oxygen, 2·5 l/h; fluothane, 2·5%). Sham-operated control rabbits were laparotomized and the ovaries exposed to allow the number of ovulation points to be counted. After each operation the rabbits received 500 000 i.u. of Triplopan (benethamine penicillin G, 0·188 g; procaine penicillin G, 0·1 g; sodium penicillin G, 0·12 g).

From ovariectomy to killing the rabbits were injected intramuscularly with progesterone with or without 3,17β-oestradiol, or with vehicle alone. Each steroid was given in 0·5 ml of peanut oil by separate injection twice daily, at 0830–0900 and 1730–1800 h. The total daily dose of each is given in the Results section below. The rabbits were killed on day 10 (experiment 1), or laparotomized for inspection of implantation on day 10 and killed on day 30 (experiments 2 and 3).

Rabbits subjected to adrenalectomy or sham-adrenalectomy were operated on by the method of White (1966) and given 2·5 mg of cortisone acetate just prior to operation. Thereafter they were fed the standard pellet diet, but given the choice of 3% glucose plus 3% NaCl or tap water to drink. 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: implantation percentage $= 10^2 \times$ total no. of implants on day 10 $\div$ total corpus luteum count; and survival percentage $= 10^2 \times$ total no. of foetuses on day 30 $\div$ total no. of implants on day 10. All χ^2 tests of significance were conducted with correction for small numbers and further corrected if necessary for heterogeneity between animals. Frequently, χ^2 for main treatment effects was insignificant even if unadjusted for heterogeneity and so no further tests were needed.

Results

Pilot Trials

Although some of the facts discussed above appeared to be firmly established, it was felt desirable to check their application to our own animals, and to determine dosage limits within which useful further work should lie. Over 100 ovariectomized does and controls were therefore used to confirm that:

1. Rabbits ovariectomized on days 2, 4, 6, 7 and 8 after ovulation and insemination on day 1, and left without replacement therapy, failed to undergo implantation.
2. Rabbits ovariectomized on days 2 or 5 and given an excess of progesterone from the day after ovariectomy onwards (8 mg/day) exhibited implantation. Those ovariectomized on day 5 showed implantation at a rate not significantly less than in controls, 28 of 39 (72%) *v.* 34 of 39 (87%) of corpora lutea being represented by implants in the two groups respectively. Not more than 1 mg/day of progesterone is in fact needed after ovariectomy on day 2 for more than 50% implantation to occur. Three successive experiments gave 16 of 31, 18 of 30 and 20 of 39 corpora lutea represented by implants following progesterone injections of 1 mg/day, compared with 28 of 42 in controls (five animals per group).
3. The addition of 2·5 or 10 μg/day of oestradiol from the day after ovariectomy lowered or abolished the implantation rates seen under (2) in all instances, while the addition of 0·5 μg/day or less, although not improving implantation rates significantly, did not lessen them.

It is not felt that these expected results merit further presentation or discussion, except to note that not all implants in the experimental groups appeared normal on gross examination.

Table 1. Effect of progesterone and oestradiol on implantation in rabbits ovariectomized on day 2 after insemination on day 1 (experiment 1)

Animals received two injections per day, commencing on the day of ovariectomy, of progesterone (P, dose expressed as mg/day) and oestradiol (E2, dose expressed as μg/day). There were 8–10 animals per group

Injections and doses given: Days 2–4	Days 5–9	No. of animals pregnant	Total no. of corpora lutea	No. of implants on day 10	No. of implants appearing normal	Gross percentage implantation
Nil	P (2)	0/10	76	0	0	0
Nil	P (2), E2 (0·2)	0/10	80	0	0	0
P (1)	P (2)	7/9	69	29	23	42
P (1)	P (2), E2 (0·2)	7/8	66	41	34	62
P (1), E2 (0·1)	P (2), E2 (0·2)	9/10	79	50	49	63
Controls (sham-operated)		8/8	75	59	59	79

Experiment 1

In this experiment groups of 10 animals were ovariectomized on day 2 and injected daily with either 0 or 1 mg of progesterone on days 2–4, followed by 2 mg of progesterone with or without oestradiol on days 5–9 (Table 1). The design was an incomplete factorial. The percentage implantation did not fall significantly below that in the controls in the groups receiving 1 mg of progesterone per day on days 2–4, with or without 0·1 μg of oestradiol (P just greater than 0·05), followed by 2 mg of progesterone with 0·2 μg of oestradiol on days 5–9, although not all implants appeared normal. No implantation occurred without administration of progesterone on days 2–4 and progesterone alone throughout the test period did not give a satisfactory percentage implantation in comparison with controls ($\chi^2_{(1)} = 20 \cdot 9$, $P < 0 \cdot 001$).

Experiment 2

This experiment was of the design shown in Table 2, replicated three times with 10 or 11 animals per group. Injection schedules were based on previous experience and on the literature, and were clearly a somewhat fortunate guess, in that pregnancy was often maintained in these ovariectomized animals.

Table 2. Allocation and treatment of rabbits employed in experiment 2

Animals (10–11 per replicate group) received twice daily injections of steroids or vehicle alone

Treatment group and dosages	Days after insemination: 2–4	5–9	10–14	15–19	20–24	25–29
1. Ovariectomized						
Progesterone (mg/day):	1	3	6	6	3	1
2. Ovariectomized						
Progesterone (mg/day):	1	3	6	6	3	1
Oestradiol (μg/day):	0	0·5	1·0	1·5	2·0	2·5
3. Sham-operated controls						
Peanut oil (ml/day):	1	1	1	1	1	1

Since progesterone output from the rabbit ovary was known to be highest between days 15 and 20 of pregnancy (Mikhail *et al.* 1961; Hilliard *et al.* 1968), the daily dose of progesterone was increased from 1 mg in the early days of pregnancy to 6 mg on days 10–19, and decreased thereafter. The amount of oestradiol needed to interrupt pregnancy increases according to the stage of pregnancy (Greenwald 1957; Schofield 1962). Therefore the daily dose of oestradiol was increased gradually from nil on days 2–4 to 2·5 μg on days 25–29, as indicated in Table 2.

Table 3. Effect of progesterone and oestradiol on implantation in rabbits ovariectomized on day 2 after insemination on day 1 (experiment 2)

Animals (10–11 per replicate group) were treated according to the schedule in Table 2. P, progesterone; E2, oestradiol; S.O., sham-operated controls; I/C = no. of implants on day 10÷no. of corpora lutea; I (%) = percentage implantation

	Group 1 (P): I/C	I (%)	Group 2 (P+E2): I/C	I (%)	Group 3 (S.O.): I/C	I (%)
Replication 1	32/59	54	33/68	49	61/80	76
Replication 2	15/63	24	43/84	51	43/75	57
Replication 3	42/69	61	48/84	57	40/96	42
Total or mean	89/191	47	124/236	53	144/251	57

Implantation percentages (Table 3) did not differ significantly ($\chi^2_{(2)} = 4 \cdot 7$, $P > 0 \cdot 05$) between treatment groups, and, as there was also significant interaction, the extremes of 47% (progesterone alone) and 57% (controls) cannot be regarded as different.

Table 4. Effect of progesterone and oestradiol on percentage survival of foetuses on day 30 in rabbits ovariectomized on day 2 after insemination on day 1 (experiment 2)

Treatment details as for Table 3

	Group 1 (P): Foetuses/ implants[A]	Survival (%)	Group 2 (P+E2): Foetuses/ implants	Survival (%)	Group 3 (S.O.): Foetuses/ implants	Survival (%)
Replication 1	17/32	53	22/33	67	35/61	57
Replication 2	7/15	47	27/43	63	28/43	65
Replication 3	18/42	43	14/48	29	29/40	73
Total or mean	42/89	47	63/124	51	92/144	64

[A] No. of foetuses on day 30/no. of implants on day 10.

Survival percentages shown in Table 4 differed significantly in total between treatment groups ($\chi^2_{(2)} = 8 \cdot 1$, $P < 0 \cdot 05$), but interaction was just significant. However, it cannot in any case be concluded that the addition of oestradiol increased survival of foetuses since $\chi^2_{(1)}$ for this effect was only $0 \cdot 15$ ($P > 0 \cdot 05$).

Table 5. Effect of progesterone and oestradiol on the number of animals pregnant on days 10 and 30 in rabbits ovariectomized on day 2 after insemination on day 1 (experiment 2)

See Table 2 for details of treatment schedules

	Replication: 1	2	3	Total	Percentage pregnant	Implants or foetuses per pregnancy
Group 1 (progesterone)						
No. of animals	8	10	9	27		
No. pregnant on day 10	6	5	9	20	74	4·5
On day 30	4	3	8	15	56	2·8
Group 2 (progesterone + oestradiol)						
No. of animals	10	11	10	31		
No. pregnant on day 10	8	8	9	25	81	5·0
On day 30	5	7	5	17	55	3·7
Group 3 (sham-operated controls)						
No. of animals	9	9	9	27		
No. pregnant on day 10	9	9	6	24	89	6·0
On day 30	9	9	6	24	89	3·8

The numbers of animals pregnant on day 30 differed significantly between treatment groups ($\chi^2_{(2)} = 6 \cdot 3$, $P < 0 \cdot 05$), but quite apart from the fact that there were significant replication differences and interaction, it is clear that, as before, the main difference was between controls and the rest and that the addition of oestradiol did not help (Table 5). In the controls no rabbit pregnant on day 10 lost a litter although 36% of foetuses were lost, but 13 complete litters were lost in the experimental groups. These lost litters often had low implantation percentages and thus represented marginally pregnant animals, whereas in controls the three rabbits that were never pregnant had 42 corpora lutea between them and their non-pregnant state suggests some particular fault in the batch of controls in replication 3.

Overall, it would seem from this experiment that any effect of oestradiol on implant survival was confined to the possibility that it increased survival of foetuses on day 30 in those animals that remained pregnant. Thus, the 15 rabbits pregnant on day 30 after treatment with progesterone alone had 5·1 implants/doe and 2·8 foetuses/doe, the 17 rabbits pregnant on day 30 after combined treatment had 5·2 implants/doe and 3·7 foetuses/doe ($\chi^2_{(1)} = 4·6$, $P < 0·05$), and the 24 control animals had 6·0 implants/doe and 3·8 foetuses/doe. Failure to demonstrate the earlier effect of oestradiol seen in experiment 1 was possibly due to an increase of dosage on days 5–9.

Experiment 3

In this experiment groups of 9–11 animals were ovariectomized and adrenalectomized, or sham-operated. Two groups were treated as in experiment 2, one with daily progesterone alone, in the same dosage schedule (Table 2), and the second with the addition of oestradiol, varying somewhat from Table 2 as it was felt as stated above that excess oestrogen may have been given in the early phases. The new schedule was: days 2–4, 0; 5–9, 0·25; 10–14, 0·5; 15–19, 1·0; 20–24, 2·0; and 25–29, 4·0 μg of oestradiol per day. There were two control groups, (i) sham-ovariectomized and adrenalectomized and (ii) sham-ovariectomized and sham-adrenalectomized, as indicated in Table 6. The object of the experiment was to see whether the adrenal glands affect implantation and the maintenance of pregnancy.

Table 6. Implantation in rabbits ovariectomized and adrenalectomized on day 2 after mating on day 1 (experiment 3)

See text for details of treatment schedule

Treatment	No. of animals pregnant on day 10	Total no. of corpora lutea	No. of implants on day 10	Percentage implant-ation
1. Ovariectomized + adrenalectomized, progesterone	8/9	70	34	49
2. Ovariectomized + adrenalectomized, progesterone + oestradiol	8/10	73	36	49
3. Sham-ovariectomized + adrenalectomized, peanut oil	8/10	85	44	52
4. Sham-ovariectomized + sham-adrenalectomized, peanut oil	9/11	98	51	52

Table 7. Maintenance of pregnancy in rabbits ovariectomized and adrenalectomized on day 2 after mating on day 1 (experiment 3)

Treatments are as defined in Table 6

Measurement	Treatment: 1	2	3	4
No. of animals pregnant on day 10	8	8	8	9
No. of animals surviving to day 30	6	6	5	7
No. of implants on day 10 in the animals that survived to day 30	25	27	26	37
Total no. of foetuses on day 30	13	14	14	23
Percentage survival of foetuses	52	52	54	62

Implantation percentages and other relevant data are shown in Table 6, from which it is clear that adrenalectomy did not affect results. Maintenance thereafter is shown in Table 7, and these data show that adrenalectomy did not affect survival percentages to any marked degree ($P > 0\cdot05$ 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 experiments reported here have been a series of 'sighting' shots at finding the requirements for the induction of implantation in the ovariectomized rabbit and its maintenance to term. They must clearly be followed by other tests, but seem worth reporting since, with all their deficiencies, a good measure of success was achieved. They also demonstrate the variability of responses obtained despite care over experimental design and procedure.

A dose of 1 mg of progesterone per day was found by Pincus and Werthessen (1938) to be sufficient for normal implantation, and a dose of 0·3 mg of progesterone per day was found by Hafez and Pincus (1956) to be insufficient. However, daily dosage of 1 mg of progesterone alone on days 2–4 and 2 mg of progesterone alone on days 5–9 gave a poor percentage implantation (experiment 1; see Table 1). The quantity of 0·2 μg of oestradiol administered on days 5–9 appeared in this experiment to have improved the percentage implantation (or its maintenance) irrespective of earlier oestradiol treatment, in agreement with a slight natural rise in oestradiol release reported between days 4 and 6, which may play some role in the process of implantation or maintenance thereafter (Hilliard and Eaton 1971). It was of interest that the percentage implantation in these rabbits did not differ significantly from that of controls, although falling below them.

In experiment 2 the results obtained in the first replication were in general agreement with those of previous pilot experiments, except that no effect of oestradiol could be seen on percentage implantation, so it was repeated three times rather than proceeding to further variations. The overall results were somewhat heterogeneous, but indicate fairly clearly that any effect of oestradiol is small, although it may have improved the percentage survival of foetuses in those rabbits that remained pregnant. A curious and unexplained difference between experimental and control groups was a frequent loss of whole litters in the former but no loss of whole litters in the latter, although frequent losses of individuals within litters occurred.

The adrenal glands were thought to be a possible source of sufficient oestrogen to permit implantation in the absence of the ovaries, should it in fact be needed for implantation. This could lead the investigator to false conclusions. However, it is clear from experiment 3 that no significant contribution to implantation can be attributed to these glands. Survival of the adrenalectomized does in this experiment without steroid replacement therapy (sham-ovariectomized but adrenalectomized controls) may have been aided not only by the dietary support provided 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 (McKeown and Spurrell 1940; Davis and Plotz 1954).

Implantation percentages and survival rates so far obtained in ovariectomized rabbits are in general less satisfactory than in controls, and further work is needed.

The results do not sufficiently strongly support the idea that oestrogen is needed in pregnancy in the rabbit for any such conclusion to be drawn, and they also demonstrate the degree of variability that may be encountered even *within* one laboratory. This variability cannot be seen in the unreplicated results of previous workers, but it helps to explain their discrepancies, and there is no evidence that repetition within any one laboratory would have given consistent answers.

In the reflexly ovulating rabbit, 20α-hydroxyprogesterone appears to have a special functional significance (Hilliard *et al.* 1967), and it is produced in approximately 10-fold greater amounts than progesterone in the period between coitus and ovulation (Hilliard *et al.* 1963). Implantation occurs in rabbits ovariectomized after mating followed by the administration of either 20α- or 20β-hydroxyprogesterone (Rennie and Davis 1965). Investigation of the role of hydroxyprogesterone, together with further exploration of progesterone and oestrogen dosage at various stages of pregnancy, may pave the way to successful and consistent maintenance of normal pregnancies in as high a percentage of ovariectomized does as in controls.

It should be noted that the sham-operated controls in experiment 2 had about one young per litter less than untouched mothers in the animal house from which they came, but the various procedures to which they had been subjected could well account for this difference. Implantation percentages in general were low, even in controls, but may be characteristic of induced ovulation and artificial insemination as performed routinely in this work.

Acknowledgments

This work was supported by a grant from the Australian Research Grants Committee. One of us (J.K.K.) is a postgraduate student under the Colombo Plan. We wish to thank Dr D. A. Shutt for testing plasma for the presence of oestradiol, and Dr T. G. Kennedy for helpful criticism and suggestions.

References

Allen, W. M. (1937). Some effects of estrin and progestin in the rabbit. *Cold Spring Harbor Symp. Quant. Biol.* **5**, 66.

Allen, W. M., and Corner, G. W. (1929). Physiology of the corpus luteum. III. Normal growth and implantation of embryos after very early ablation of the ovaries, under the influence of extracts of the corpus luteum. *Am. J. Physiol.* **88**, 340.

Allen, W. M., and Corner, G. W. (1930). Physiology of the corpus luteum. VII. Maintenance of pregnancy in the rabbit after very early castration by corpus luteum extracts. *Proc. Soc. Exp. Biol. Med.* **27**, 403.

Allen, W. M., and Heckel, G. P. (1937). Failure of progesterone to prevent resorption of embryos in rabbits castrated in very early pregnancy. *Science* (*Wash. D.C.*) **86**, 409.

Arthur, A. T., and Daniel, J. C. (1972). Progesterone regulation of blastokinin production and maintenance of rabbit blastocysts transferred into uteri of castrated recipients. *Fertil. Steril.* **23**, 115.

Chambon, Y. (1949). Besoins endocriniens qualitatifs et quantitatifs de l'ovoimplantation chez la lapine. *C. R. Seances Soc. Biol.* **143**, 1172.

Chang, M. C. (1951). Maintenance of pregnancy in intact rabbits in the absence of corpora lutea. *Endocrinology* **48**, 17.

Davis, M. E., and Plotz, E. J. (1954). The effect of cortisone acetate on intact and adrenalectomized rats during pregnancy. *Endocrinology* **54**, 384.

Denker, H. W. (1972). Blastocyst protease and implantation: effect of ovariectomy and progesterone substitution in the rabbit. *Acta Endocrinol.* **70**, 591.

Greenwald, G. S. (1957). Interruption of pregnancy in the rabbit by the administration of oestrogen. *J. Exp. Biol.* **135**, 461.
Hafez, E. S. E., and Pincus, G. (1956). Hormonal requirements for implantation in the rabbit. *Proc. Soc. Exp. Biol. Med.* **91**, 531.
Hilliard, J., and Eaton, L. W. (1971). Estradiol-17β, progesterone and 20α-hydroxypregn-4-en-3-one in rabbit ovarian venous plasma. II. From mating through implantation. *Endocrinology* **89**, 522.
Hilliard, J., Archibald, D., and Sawyer, C. H. (1963). Gonadotropic activation of preovulatory synthesis and release of progestin in the rabbit. *Endocrinology* **72**, 59.
Hilliard, J., Penardi, R., and Sawyer, C. H. (1967). A functional role of 20α-hydroxypregn-4-en-3-one in the rabbit. *Endocrinology* **80**, 901.
Hilliard, J., Spies, H. G., and Sawyer, C. H. (1968). Cholesterol storage and secretion during pregnancy and pseudopregnancy in the rabbit. *Endocrinology* **82**, 157.
Horrell, E., Major, R. W., Kilpatrick, R., and Smith, B. M. (1972). Progestational steroids during pseudopregnancy in the rabbit. *J. Endocrinol.* **55**, 89.
Kehl, R., and Chambon, Y. (1949). Synergie progesterofolliculinique d'ovoimplantation chez la lapine. *C. R. Seances Soc. Biol.* **143**, 1169.
McKeown, T., and Spurrell, W. R. (1940). The results of adrenalectomy in the pregnant albino rat. *J. Physiol.* (*Lond.*) **98**, 255.
Mikhail, G., Noall, M. W., and Allen, W. M. (1961). Progesterone levels in the rabbit ovarian vein blood throughout pregnancy. *Endocrinology* **69**, 504.
Pincus, G., and Werthessen, N. T. (1938). The maintenance of embryo life in ovariectomized rabbits. *Am. J. Physiol.* **124**, 484.
Prasad, M. R. N., and Kalra, S. P. (1967). Mechanisms of anti-implantation action of anti-oestrogens. Proc. 8th Int. Conf. IPPF, Santiago. 9-15, p. 413.
Rennie, P., and Davis, J. (1965). Implantation in the rabbit following administration of 20α-hydroxy-pregnen-3-one and 20β-hydroxypregnen-3-one. *Endocrinology* **76**, 535.
Schofield, B. M. (1962). The effect of injected oestrogen on pregnancy in the rabbit. *J. Endocrinol.* **25**, 95.
White, S. W. (1966). Adrenalectomy in the rabbit. *Aust. J. Exp. Biol. Med. Sci.* **44**, 447.
Wu, D. H., and Allen, W. M. (1959). Maintenance of pregnancy in castrated rabbits by 17α-hydroxy-progesterone caproate and by progesterone. *Fertil. Steril.* **10**, 439.

Manuscript received 18 October 1973