Pituitary Hormone Control of Implantation in the Mouse

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Abstract

In hypophysectomized pregnant mice replacement therapy designed to mimic the normal physiological situation showed that FSH in combination with either prolactin or LH, or prolactin plus LH, could initiate implantation in the absence of the pituitary gland. No pituitary hormone was by itself capable of achieving this result. The combination of prolactin with FSH gave better results than a combination of LH with FSH. Prolactin from sheep, cattle or rats was equally effective in combination with rat FSH in initiating implantation. In mice exhibiting suckling-induced delay of implantation this delay was terminated by injection of FSH. GH by itself or in conjunction with other hormones had no significant effect on implantation or on any of the other parameters associated with implantation that were measured.

On the basis of these experimental results it is suggested that prolactin and LH are involved with progesterone production and FSH with oestrogen production, both of which are required for implantation in the mouse.

Introduction

The role of the ovarian hormones in initiating implantation has been well demonstrated. Evidence has shown that a combination of progesterone and oestrogen is required for successful implantation in the mouse (Humphrey 1967a, 1967b; McLaren 1969, 1971, 1973; Bindon 1973). The role of the pituitary hormones in the control of implantation is, however, not fully established.

For implantation, which occurs late on day 5 of pregnancy (day 1 being the day on which the copulatory plug is found), the pituitary gland is essential until about 2400 h on day 3 (Bindon 1969; Bindon and Lamond 1969). If the gland is removed between 2400 h on day 3 and day 11, abortion or resorption of embryos results. Injection of an anti-LH serum into mice before 0600 h on day 4 inhibits implantation (Bindon 1971; Munshi *et al.* 1972). Bindon (1971) also demonstrated that implantation in hypophysectomized mice injected with progesterone could be initiated by injecting them with a mixture of ovine LH and FSH (100 μg of each daily for 3 days). Either of these hormones was ineffective by itself at similar doses. Choudary and Greenwald (1969) suggested, on the basis of experimental work in mice hypophysectomized early in pregnancy, that both prolactin and FSH are required to maintain pregnancy.

From the published work it appeared that the three anterior pituitary hormones LH, FSH and prolactin are involved in implantation. This paper describes a series of experiments carried out in hypophysectomized mice, or mice in which suckling-induced delayed implantation occurred, to ascertain which pituitary hormones are

capable of initiating implantation and their role in this process. The effect of GH was also studied in conjunction with LH, FSH and prolactin.

In most of the experiments hypophysectomy was performed between 1000 and 1200 h on day 2 of pregnancy. This was done because plasma measurements of LH and FSH revealed that LH levels were low at the time chosen for hypophysectomy, then rose during the afternoon of day 2 and that FSH levels were falling during day 2 and rose early on day 3 (Gidley-Baird 1977). Measurements of plasma prolactin by Murr *et al.* (1974) showed an approximate three-fold increase in levels between day 1 and day 2. This information on plasma LH, FSH and prolactin levels suggested that removal of the pituitary gland and initiation of replacement therapy at this time would best approximate the normal situation in intact pregnant mice. After initiation of implantation with pituitary hormones implants were maintained until the time of autopsy with progesterone because of the relatively small amounts of pituitary hormones available.

Although measurements of plasma LH and FSH during normal pregnancy and of LH in hypophysectomized mice receiving exogenous LH have been made (Gidley-Baird and Bindon 1976; Gidley-Baird 1977), no measurements of plasma FSH levels have been made in hypophysectomized mice receiving FSH. This was done in the first experiment and at the same time these samples were analysed for LH content to give an indication of contaminating LH levels in the FSH preparation used for this work. The dose of prolactin used was chosen because this dose was found to maintain plasma progesterone levels at values similar to those found prior to implantation in normal pregnancy (Gidley-Baird 1977).

Materials and Methods

Mice

These were albino mice of the Quackenbush strain (QS), born and reared in this Department. Virgin females aged 8–10 weeks and weighing between 25 and 30 g were used for the experiments. Mice used in suckling-induced delayed implantation experiments were allowed to carry their first pregnancy to term and those which showed a *post partum* copulatory plug were selected for experimental work. These mice all had their litters standardized to eight pups after the *post partum* mating.

Hypophysectomy

The procedure used was the Bindon (1969) modification of the technique of Lamond and Emmens (1959). Using this method the pituitary gland can be observed during extirpation by suction pipette and completeness of removal can be checked visually.

Hormones

The hormones used were: pregn-4-ene-3,20-dione (progesterone), Sigma; prolactin, NIAMDD rat RP-1; prolactin, NIH-P-B3; prolactin, NIH-P-S9; luteinizing hormone, NIH-LH-S 16; follicle stimulating hormone, NIAMDD rat FSH B-1; FSH, NIAMDD rat RP-1; and growth hormone, NIH GH B 17.

Injection of Hormones

Progesterone was dissolved in sesame seed oil and the appropriate dose injected in 0·1 ml of oil. Protein hormones were injected in 0·1 ml of 15% (w/v) gelatin vehicle.

Autopsy

Mice were killed on day 8 of pregnancy. Body weight, uterine and ovarian weight, and number of implants were recorded. When no visible signs of implantation were present the uteri were flushed with 0·9% (w/v) saline and the washings examined microscopically for the presence of blastocysts.

Experimental Procedures and Results

Experiment 1: Measurement of Plasma FSH and LH Levels in Hypophysectomized Mice after Injection of Exogenous FSH

Adult female mice were hypophysectomized and injected 2 days later with FSH in 0·1 ml of 15% gelatin. Two groups each of 25 hypophysectomized mice received 10 or 50 μg of FSH. A third group of 25 hypophysectomized mice received 0·1 ml of gelatin vehicle. Five animals from each group were killed at 1, 2, 4, 8 and 16 h post-injection and the plasma was collected and individually assayed using radioimmunoassay techniques for LH (Gidley-Baird and Bindon 1976) and FSH (Gidley-Baird 1977).
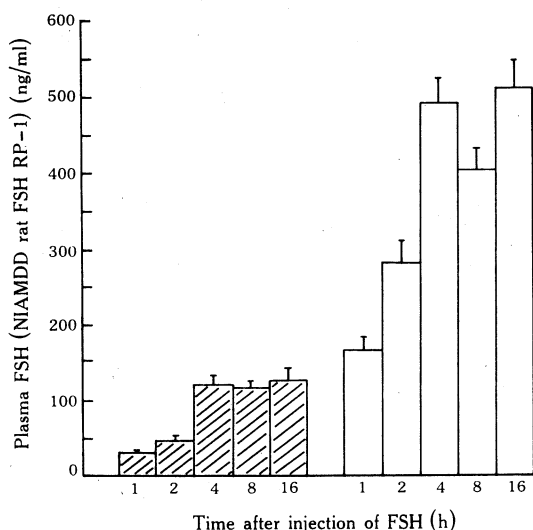

Fig. 1. Mean values and standard errors of plasma FSH for groups of five hypophysectomized mice at various times after injection of NIAMDD rat FSH B-1. Shaded histograms are for mice injected with 10 μg FSH; unshaded histograms are for mice injected with 50 μg FSH.

Fig. 1 shows the mean levels of FSH and standard errors for groups of five mice killed at each time interval. At no time did any of the mice in the group receiving vehicle alone show any value of FSH above the limit of sensitivity of the assay (20 ng NIAMD rat FSH RP-1 per ml plasma). In both groups receiving FSH, plasma FSH levels rose until 4 h post-injection and remained at these levels until 16 h post-injection.

Only in the case of the 50-μg dose of FSH at 1 h post-injection did measurement of LH show values above the limit of sensitivity of the assay, the mean value being 1·0 ng NIH LH S-16 per ml. As a result of this, doses of FSH were incubated in plastic tubes coated with an anti-LH serum prior to use in replacement experiments.

Experiment 2: Initiation of Implantation with LH and FSH

In this and the following four experiments (numbers 3, 4, 5 and 6) mice showing evidence of mating were hypophysectomized between 1000 and 1200 h on day 2 of pregnancy. Pituitary hormones were administered on days 2 and 3 as indicated in the individual experiments and all mice received 1 mg of progesterone on days 4, 5, 6 and 7 and were killed and examined on day 8.

Eighty mice showing copulatory plugs on the same morning were hypophysectomized and divided into eight groups, with treatments as in Table 1a and injections of LH at 1200 and 2400 h on each of days 2 and 3 and FSH at these times only on day 3.

Table 1*a* shows that at both dose levels tested LH alone was not capable of initiating implantation, but a combination of LH with FSH was successful. Although the best result for implantation was obtained when the ratio of FSH to LH was 5 to 1 by weight, a chi-squared test revealed that this result was not significantly different from the results in other groups which received LH plus FSH in different ratios. No blastocysts were recovered from mice which received only FSH at the 50-μg dose.

Table 1. Initiation of implantation in hypophysectomized mice with (*a*) LH and FSH, and (*b*) prolactin plus LH and/or FSH

Treatment group (dose per injection)	No. of mice	No. of mice with implants	Mean ± s.e. of implants per mouse	No. of mice with blastocysts	Percentage of mice with implants
(*a*) LH and FSH					
1. 10 μg LH	7	0		7	0
2. 10 μg LH+ 10 μg FSH	9	0		8	0
3. 10 μg LH+ 50 μg FSH	8	6	12·0±1·0	2	75
4. 50 μg LH	8	0		7	0
5. 50 μg LH+ 10 μg FSH	8	3[A]	9·0±2·6	6[A]	37
6. 50 μg LH+ 50 μg FSH	8	4	9·3±2·1	4	50
7. 10 μg FSH	7	0		6	0
8. 50 μg FSH	9	0		0	0
(*b*) Prolactin plus LH and/or FSH					
1. 50 μg Pro	9	0		8	0
2. 50 μg Pro+ 10 μg LH	8	0		6	0
3. 50 μg Pro+ 50 μg LH	8	0		8	0
4. 50 μg Pro+ 10 μg FSH	8	4	12·6±0·9	4	50
5. 50 μg Pro+ 50 μg FSH	8	8	12·0±1·0	0	100
6. 50 μg Pro+ 5 μg LH+ 5 μg FSH	7	0		7	0
7. 50 μg Pro+ 10 μg LH+ 10 μg FSH	8	6	14·1±0·7	2	75

[A] One mouse in this group had implants in one uterine horn and blastocysts in the other.

Experiment 3: Initiation of Implantation with Prolactin, LH and FSH

Sixty-three mice showing copulatory plugs on the same morning were hypophysectomized and divided into seven groups. Mice in all groups received 50 μg of NIH sheep prolactin S-9 at 1200 and 2400 h on days 2 and 3. In addition at 1200 and 2400 h on day 3 the groups also received FSH and/or LH treatments as in Table 1*b*.

Table 1*b* indicates that prolactin alone or in combination with LH did not initiate implantation, but a combination of prolactin with FSH was successful. With the

highest dose of FSH 100% of mice showed successful implantation. With the lowest dose of FSH and a constant amount of prolactin fewer mice showed implants and a combination of this FSH dose and an equal amount of LH did not give a significant increase in the number of mice with implants. There were no significant differences between the mean number of implants per mouse in any of the groups in which implantation occurred.

Experiment 4: Comparison of the Efficiency of LH or Prolactin in Combination with FSH to Initiate Implantation

Sixty mice showing copulatory plugs on the same morning were hypophysectomized and divided into six groups. Mice were injected with hormones (LH, FSH and prolactin NIH S-9) as shown in Table 2a at 1200 and 2400 h on the days indicated.

Table 2. Initiation of implantation in hypophysectomized mice with (a) LH or prolactin with FSH, and (b) sheep, cattle or rat prolactin with FSH

Treatment group (dose per injection)	No. of mice	No. of mice with implants	Mean ± s.e. of implants per mouse	No. of mice with blastocysts	Percentage of mice with implants
(a) LH or prolactin with FSH					
1. 50 μg LH × 2, days 2, 3	9	0		8	0
2. 50 μg LH × 2, days 2, 3 + 50 μg FSH × 2, day 3	8	4	9·6 ± 2·4	4	50
3. 50 μg Pro × 2, days 2, 3	7	0		7	0
4. 50 μg Pro × 2, days 2, 3 + 50 μg FSH × 2, day 3	9	9	12·7 ± 2·4	0	100
5. 50 μg FSH × 2, days 2, 3	9	0		0	0
6. 50 μg Pro × 2, days 2, 3 + 50 μg LH × 2, day 3	9	0		8	0
(b) Sheep, cattle or rat prolactin with FSH					
1. 50 μg sheep Pro + 50 μg FSH	8	7	12·1 ± 1·0	0	88
2. 50 μg cattle Pro + 50 μg FSH	8	8	10·7 ± 1·1	0	100
3. 50 μg rat Pro + 50 μg FSH	10	10	9·0 ± 1·5	0	100

The results shown in Table 2a confirm those of experiments 2 and 3 showing that a combination of LH and FSH, or prolactin and FSH, is required to initiate implantation. These results also show that the combination of prolactin and FSH was more successful than LH and FSH ($\chi^2_{(1)} = 8·99$, $P < 0·01$).

Experiment 5: Initiation of Implantation using Sheep, Cattle or Rat Prolactin with FSH

Thirty mice showing copulatory plugs on the same morning were hypophysectomized and divided into three groups. All mice received 50 μg of prolactin at 1200 and 2400 h on days 2 and 3 and 50 μg of FSH at 1200 and 2400 h on day 3.

The results demonstrate that prolactin from three different species was equally effective in combination with FSH in initiating implantation (Table 2b). There were no significant differences in the mean number of implants per mouse between groups.

Experiment 6: The Effect of GH with Prolactin and FSH on the Initiation of Implantation

Sixty-three mice showing copulatory plugs on the same morning were hypophysectomized, divided into seven groups and injected with NIH-prolactin S-9 and/or GH and/or FSH at 1200 and 2400 h on the days and with the doses shown in Table 3a.

Table 3. Initiation of implantation in hypophysectomized mice with (a) GH with prolactin and FSH, and (b) prolactin, LH, FSH and no progesterone maintenance

Treatment group (dose per injection, day of treatment)	No. of mice	No. of mice with implants	Mean ± s.e. of implants per mouse	No. of mice with blastocysts	Percentage of mice with implants
(a) GH with prolactin and FSH					
1. 50 μg Pro × 2, days 2, 3	8	0	0	8	0
2. 50 μg Pro × 2 + 50 μg GH, days 2, 3	6	0	0	6	0
3. 50 μg Pro × 2, days 2, 3 + 50 μg FSH × 2, day 3	7	7	9·4 ± 1·1	0	100
4. 50 μg GH × 2, days 2, 3	9	0	0	9	0
5. 50 μg GH × 2, days 2, 3 + 50 μg FSH × 2, day 3	8	0	0	8	0
6. 50 μg FSH × 2, days 2, 3	7	0	0	1	0
7. 50 μg Pro × 2 + 50 μg GH, days 2, 3 + 50 μg FSH × 2, day 3	8	8	10·4 ± 1·6	0	100
(b) Prolactin, LH, FSH and no progesterone					
1. 100 μg Pro, days 1–6	8	0		6	0
2. 100 μg Pro, days 1–6 + 50 μg LH, days 4, 5, 6	8	0		6	0
3. 100 μg Pro, days 1–6 + 100 μg LH, days 4, 5, 6	7	0		4	0
4. 100 μg Pro, days 1–6 + 50 μg FSH, days 4, 5, 6	7	7	9·4 ± 0·9	0	100
5. 100 μg Pro, days 1–6 + 100 μg FSH, days 4, 5, 6	6	4[A]	8·5 ± 1·7	0	67
6. 100 μg FSH, days 4, 5, 6	8	0		0	0

[A] Both mice in group 5 which did not have implants or blastocysts showed signs of aborted implantation.

None of the three hormones prolactin, GH or FSH could alone initiate implantation in hypophysectomized mice (Table 3a). Combinations of GH with prolactin or GH with FSH were also unsuccessful. The only successful combinations were prolactin with FSH and prolactin with GH and FSH. There was no significant difference between the groups receiving prolactin with FSH and prolactin with GH and FSH with regard to body weight, ovarian weight, number of implants, and implant weight. In the group of mice which received 50 μg of FSH twice daily on days 2 and 3, in only one mouse were blastocysts recovered at autopsy.

Experiment 7: Initiation of Implantation in Hypophysectomized Mice using Prolactin, LH, FSH and no Progesterone Maintenance

Fifty mice which showed copulatory plugs on the same morning were hypophysecto-

mized between 1000 and 1100 h on that morning (day 1) and divided into five groups. Mice in groups 1–5 (inclusive) received 50 μg of NIAMDD rat prolactin RP-1 at 1200 and 2400 h on days 1–6 (inclusive) of pregnancy. In addition mice in groups 2 and 3 received 50 and 100 μg respectively of LH at 1200 h on days 4, 5 and 6. Mice in group 4 received 50 μg and mice in groups 5 and 6, 100 μg respectively of FSH at the same time on these days.

Alterations in the timing of FSH replacement in hypophysectomized mice given prolactin in this experiment did not prevent the initiation of implantation (Table 3b). Again the results demonstrated that a combination of prolactin and LH did not initiate implantation. Prolactin was able to substitute for progesterone in maintaining implanted embryos.

Table 4. Initiation of implantation in mice exhibiting suckling-induced delay of implantation with (a) single injections of FSH on day 4 or on days 4, 5 and 6, and (b) FSH given on days 3 and/or 4

Treatment group (dose per injection, day of treatment)	No. of mice	No. of mice with implants	Mean ± s.e. of implants per mouse	No. of mice with blastocysts	Percentage of mice with implants
(a) Single injections of FSH on day 4 or on days 4, 5 and 6					
1. 0·1 ml gelatin vehicle, 0600 h, day 4	8	0		8	0
2. 10 μg FSH, 0600 h, day 4	8	0		8	0
3. 50 μg FSH, 0600 h, day 4	7	1	17	4	14
4. 0·1 ml gelatin vehicle, 0600 h, days 4, 5, 6	8	0		7	0
5. 10 μg FSH, 0600 h, days 4, 5, 6	8	2	8·0 ± 1·8	6	25
6. 50 μg FSH, 0600 h, days 4, 5, 6	8	4	10·3 ± 1·6	2	50
(b) FSH given on days 3 and/or 4					
1. 0·1 ml gelatin vehicle × 4, days 3, 4	8	0		8	0
2. 50 μg FSH, day 3	8	0		8	0
3. 50 μg FSH × 2, day 3	7	3	11·3 ± 1·9	0	43
4. 50 μg FSH, day 4	8	0		4	0
5. 50 μg FSH × 2, day 4	8	4[A]	12·1 ± 1·8	0	50
6. 50 μg FSH, days 3, 4	8	4	10·8 ± 1·4	1	50
7. 50 μg FSH × 2, days 3, 4	8	6[B]	14·6 ± 1·7	1	75

[A] The implants of two mice in this group were degenerating.
[B] One mouse in this group had degenerating implants.
Mice in both of these groups without implants or blastocysts showed signs of aborted implantation.

Experiment 8: Initiation of Implantation with FSH in Mice Exhibiting Suckling-induced Delay of Implantation

This experiment was performed in two parts.

Part A. Forty-eight mice which showed *post partum* copulatory plugs on the same morning were divided into six groups and injected once per day as shown in Table 4a. All mice were killed on day 8 and examined for implants or blastocysts. At autopsy 50% of mice injected with 50 μg of FSH on days 4, 5 and 6 showed successful implantation.

Part B. Fifty-six mice which showed *post partum* copulatory plugs on the same morning were divided into seven groups and injected at 0600 or 0600 and 1800 h on the days and with treatments as shown in Table 4*b*. The best result for implantation was achieved by giving FSH twice daily on days 3 and 4, 75% of this group of mice showing successful initiation of implantation.

Discussion

This work has shown that FSH in combination with either prolactin or LH, or prolactin plus LH, can initiate implantation in hypophysectomized mice. Neither pituitary hormone by itself achieved this result nor did any combination of the three hormones GH, LH and prolactin, nor any combination of GH with FSH, LH or prolactin. A combination of prolactin and FSH was more efficient than a combination of LH and FSH in initiating implantation. The results show that implantation can be initiated by doses of hormones which give plasma levels comparable with the levels of these hormones found prior to implantation (Gidley-Baird and Bindon 1976; Gidley-Baird 1977). This is in marked contrast to the levels of LH and FSH shown by Bindon (1971) to be necessary to initiate implantation. The difference between these results appears to be due to the different vehicles used for injection. Bindon injected in 0·9% (w/v) saline whereas hormones in this study were injected in 15% (w/v) gelatin, which has been shown (Gidley-Baird and Bindon 1976) to sustain plasma hormone values at a more constant level for a longer period of time.

In hypophysectomized mice Bindon (1969) showed that both progesterone and oestrogen are necessary for implantation. It is suggested that FSH is primarily responsible for stimulation of oestrogen production by the ovary while prolactin and LH are concerned with the production of progesterone.

The evidence for oestrogen stimulation by FSH is, first, that injection of FSH into mice exhibiting suckling-induced delayed implantation initiated implantation. Whitten (1955, 1958) and McLaren (1968) have shown that suckling-induced delayed implantation can be terminated by injecting oestrogen. Second, in mice which received only prolactin, LH or FSH at 50-μg doses, healthy blastocysts were found only in the uteri of animals which received prolactin or LH. Only in one case were blastocysts recovered from any mouse receiving only 50 μg of FSH. Humphrey (1968) showed that injection of oestrogen on day 2 of pregnancy into intact mice or injection of oestrogen without accompanying progesterone into mice ovariectomized after ovulation caused accelerated transport of ova from the fallopian tubes through the uterus and loss into the vagina. Third, in groups of hypophysectomized mice receiving prolactin, or in mice exhibiting suckling-induced delayed implantation, daily doses of 100 μg of FSH for 2 or more days caused abortion of implants in a number of mice. A similar effect occurs if excessive doses of oestrogen are given at this time.

The similarity of results achieved in these experimental situations with oestrogen or FSH suggests that FSH may exert its effect by stimulating ovarian oestrogen. This effect of FSH appears to be independent of any LH action because direct measurements of LH in plasma after administration of FSH show LH contamination to be very low, and to overcome any possible LH effect the FSH preparation was absorbed with an anti-LH serum prior to use. This finding is contrary to results in the rat (Lostroh and Johnson 1966; Armstrong 1968; Madhwa Raj *et al.* 1968; McDonald *et al.* 1969; Schwartz and Ely 1970; Lindner *et al.* 1974) and the rabbit (Spies and Quadri 1967;

Eaton and Hilliard 1971; Hilliard *et al.* 1971; Mills *et al.* 1971) which indicate that in those species LH is required for oestrogen stimulation from the ovaries.

Prolactin and LH in combination did not initiate implantation although either could do so when administered with FSH. As FSH appears to be primarily responsible for oestrogen stimulation then by inference either prolactin or LH could stimulate progesterone production. This inference for prolactin is supported by the results of experiment 6 which demonstrated that prolactin was capable of substituting for progesterone in maintaining the implants until the time of autopsy. Prolactin appears to be more important than LH in stimulating and maintaining necessary progesterone levels, as prolactin in combination with FSH gave a greater implantation percentage than LH plus FSH. Although the results of experiments using prolactin plus FSH show that LH was not required to achieve implantation it appears that in normal pregnancy LH does in fact have a necessary function, as administration of an anti-LH serum prior to implantation prevented it from occurring (Bindon 1971; Munshi *et al.* 1972).

Acknowledgments

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