Initiation and control of ovulation in the mouse luteal phases.

Effects of gonadotropins and gonadotropin releasing hormone

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Abstract. The aim of the present study was to examine the induction of ovulation during pregnancy, pseudopregnancy, and suckling-delayed pregnancy in mice using exogenous gonadotropins. The present results demonstrate that there are mature follicles in the ovary which can be induced to ovulate with administration of either exogenous human chorionic gonadotropin (hCG) or luteinizing hormone (LH) during pregnancy (Days 1–12) and pseudopregnancy (Days 4–8) in the mouse. hCG was relatively ineffective in initiating ovulation during suckling-delayed pregnancy, and hCG could not induce ovulation on Days 3–6 in any animals, suggesting that follicular growth is not continuous during suckling-delayed pregnancy in the mouse. Ovulation occurred in pregnant and pseudopregnant mice following injection of gonadotropin releasing hormone (GnRH) in a gelatin delay vehicle. Injection of GnRH in saline did not initiate ovulation in pregnant or pseudopregnant mice. A large release of LH was shown to occur following injection of GnRH in gelatin, but no release occurred after the same dose of GnRH in saline. In conclusion, the experiments demonstrate the existence of mature follicles during murine pregnancy and pseudopregnancy, and the lack of indelible follicles during suckling-delayed pregnancy.

It has been suggested that the growth of ovarian follicles is continuous during pregnancy in the mouse (Pederson & Peters 1971) and that ovulation can be induced at any stage of pregnancy by injection of hCG (Greenwald & Choudary 1969). The lack of spontaneous ovulation during pregnancy may not be due to absence of mature follicles in the ovary, but rather to an inhibition of the processes normally initiating ovulation. Aizuma et al. (1972) found that a brief exposure of the pituitary gland to GnRH following a single injection of GnRH in saline was effective in stimulating LH release, but relatively ineffective in enhancing FSH release. They reported, however, that intravenous FSH over a period of 4 h resulted in a large release of both LH and FSH from the pituitary gland and suggested that prolonged exposure to GnRH was essential for full stimulation of the FSH release.

The aim of the present experiments was to investigate the initiation of ovulation during pregnancy in mice, and to extend the investigation to pseudopregnancy and suckling-delayed pregnancy. hCG was used in attempts to induce ovulation during the luteal phases of the mouse. To confirm that the ovulation inducing effects of hCG were due to its LH-like activity, attempts were also made to induce ovulation with LH. In addition, plasma LH levels and ovulation were investigated following injection of GnRH in a 0.9% saline or 15% gelatin solution used to prolong absorption.
Materials and Methods

Mating procedures

Virgin female mice of the outbred Quackenbush (QS) stock, 8–10 weeks of age and weighing between 25 and 30 g, were used in all experiments. The animals were maintained at 23–27°C and 55%–60% humidity and artificially lit from 06.00 h to 18.00 h. They were in metal stock cages and for experimental use housed in groups of 2–3 per 6 × 6 × 11 inch cage. Pellet feeding was ad libitum. Mating was performed by housing pairs of one male and one female.

Mating was detected by the presence of copulatory plugs, and the day of detection of a plug was regarded as Day 1 of pregnancy. Mice with plugs were placed in 18 × 11 × 5 inch stock cages, up to 12 per cage. For the individual experiments only mice with copulatory plugs from the same morning were used. For post partum mating, pregnant females were placed singly in experimental cages with a male and inspected daily for parturition. When this occurred, litter size was adjusted to 8, and females showing (copulatory) plugs during the next two days were left in situ with the male, whereas the others were sacrificed.

Collection of eggs

Blastocysts were flushed from the uterus by cutting just above the utero-tubal junction at the cervix and flushing each horn with 0.5 ml of 0.9% NaCl and examining the result under a binocular microscope.

Oviducts were flushed for ova by cutting just below the utero-tubal junction and freeing the infundibulum. About 0.2 ml of 0.9% NaCl was then injected via the infundibulum and the flushings examined as above.

After ovulation, the eggs take approximately 72 h to reach the uterus (Humphrey 1968; Bindon 1973). It is therefore necessary to distinguish between eggs from an initial ovulation (primary eggs) and those experimentally induced within this time (secondary eggs). Animals were autopsied 24 h after injection, and the eggs from induced ovulation could be recognized as unfertilized or 1-cell eggs in contrast to the 2–4 cell eggs of the original mating. The cell mass of 1-cell eggs after fertilization is appreciably larger than that of unfertilized eggs, and these too can be differentiated. In the pseudopregnant mouse, all eggs will be unfertilized. To avoid confusion, induced ovulation during pseudopregnancy was investigated only on Days 4–8, before and after which eggs from natural ovulation might be found in the oviducts.

Hormones

Protein hormones were injected sc in 15% aqueous gelatin solution or in 0.9% saline. The hormones used were: Human chorionic gonadotropin, hCG (Pregnyl, Organon). Luteinizing Hormone NIH LH S-19 (National Institute of Health, MD). LH/FSH-RH (GnRH) (National Institute of Arthritis, Metabolic and Digestive Diseases, National Institute of Health, MD).

Collection of blood samples

Blood samples were collected from the tail vein of the mice into small heparinized plastic tubes. The tubes were centrifuged and the plasma stored at −25°C until analysis.

LH assay

LH was measured using a solid-phase radioimmunoassay as previously described (Gidley-Baird & Bindon 1976). The assay utilized an antiserum to ovine LH and ovine LH standards, and it measures LH levels in 20 µl of plasma with a sensitivity of less than 0.6 µg/l.

Replicate reliability typically showed a variation of 5% or less. The mean and SD for the index of precision (λ) for 10 assays was 0.049 ± 0.021. Ovine TSH showed about 12% cross-reaction in the assay, whilst rat FSH and Prl and ovine FSH, Prl and GH showed no cross-reaction.

Experimental design

Administration of hCG during pregnancy. In groups of three, 216 female mice were housed with a single male, and female mice showing copulatory plugs on the same morning were allocated into groups of six animals. The six mice in each group were injected on the required day (Days 1–12 of pregnancy) with either 0.1 ml of 0.9% NaCl (controls) or 5 IU or 10 IU of hCG in 0.1 ml 0.9% NaCl.

Administration of hCG during pseudopregnancy. In groups of three, 90 female mice were housed with a single vasectomized male, and female mice showing copulatory plugs on the same morning were allocated into groups of six animals. The six mice in each group were injected on the required day (Days 4–8 of pseudopregnancy) with 0, 5 or 10 IU of hCG as in 'administration of hCG during pregnancy'.

Administration of hCG during suckling-delayed pregnancy. One hundred and fourty-four female mice showing copulatory plugs on the same morning were allocated with their offspring into groups of three mice. Administration of hCG was carried out on Days 1–12 of suckling-delayed pregnancy using 0.1 ml 0.9% NaCl (controls) or 10 IU hCG in 0.1 ml 0.9% NaCl. Each treatment was given to six mice.

Administration of LH during pregnancy. Seventy-two mice with copulatory plugs on the same morning were allocated into 3 groups. Animals in group 1 received either 0, 2, 10 or 50 µg of LH in saline on Day 3 of pregnancy. Groups 2 and 3 received similar injections on Days 4 and 5 of pregnancy, respectively.

Administration of GnRH during pregnancy and pseudopregnancy. Pregnant mice and pseudopregnant mice were
prepared as described in the previous experiments. The mice were injected with GnRH in 0.9% NaCl or 15% gelatin solution on Days 2, 4, 6 and 8 of pregnancy and pseudopregnancy. The doses were 0, 0.1 or 1.0 µg of GnRH, other details being the same as in the previous experiments.

**LH release in pregnant mice following injection of GnRH in saline or in gelatin.** Over an 8-h period following injection of GnRH, blood samples were collected from 18 mice which had shown copulatory plugs on the same morning. The 18 mice were divided into three equal groups and received the following treatment on Day 4 of pregnancy: Group 1: 0.1 ml of 0.9% NaCl + 0.1 ml of 15% gelatin solution. Group 2: 1.0 µg of GnRH in 0.1 ml of saline. Group 3: 1.0 µg of GnRH in 0.1 ml of 15% gelatin solution.

Blood samples were taken at 0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 h after injection, and the animals were autopsied 24 h after injection.

**Statistics**

Analysis of significance was performed using Fisher’s exact test.

**Results**

**The effect of administration of hCG during pregnancy, pseudopregnancy, and suckling-delayed pregnancy**

No secondary eggs were recovered from the control animals treated with 0.1 ml of saline at different days of pregnancy, pseudopregnancy or suckling-delayed pregnancy. By contrast, administration of hCG, 5 as well as 10 IU, induced ovulation of secondary eggs in all treated mice at Days 1–12 of pregnancy ($P < 0.01$).

In addition, administration of hCG (5 or 10 IU) also induced ovulation of secondary eggs in all treated mice but one at Days 4–8 of pseudopregnancy ($P < 0.05$).

Induction of secondary eggs by hCG in suckling-delayed pregnant mice was not as efficient as the induction of ovulation of secondary eggs in pregnant mice. In suckling-delayed mice, hCG induced ovulation in only 4 mice out of 6 on Day 1 ($P < 0.05$) and in 2 on Day 2 ($P > 0.05$). No ovulations were induced on Days 3–6, and only one mouse ovulated on each of Days 7 and 8 ($P > 0.05$). After Day 8, mice which ovulated in response to 10 IU of hCG either had implants in the uterus or blastocysts in conjunction with developing implantation sites, demonstrating that these mice were not longer in a state of ‘delayed pregnancy’, but should be considered merely as pregnant mice.

**The effect of administration of LH on Days 3, 4 and 5 of pregnancy**

No animal ovulated in response to 0 or 2 µg of LH, whereas animals receiving 10 µg ($P < 0.01$) or 50 µg ($P < 0.05$) of LH ovulated secondary eggs (Table 1). There was no significant difference between the number of animals ovulating on Days 3, 4 and 5 ($P > 0.05$).

**The effect of administration of GnRH during pregnancy and pseudopregnancy**

No pregnant mice and only one pseudopregnant animal ovulated in response to GnRH in saline. However, administration of GnRH in 15% gelatin had an ovulatory effect in both pregnant and pseudopregnant mice (Table 2). At 0.1 µg of GnRH, no mice ovulated at Day 2 of pregnancy or pseudopregnancy, and only a few animals ovulated at later stages when treated with 0.1 µg GnRH. By contrast, administration of 1.0 µg of GnRH in gelatin on Days 4, 6 or 8 induced

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<th>Day</th>
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<td>50</td>
<td>6***</td>
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</table>

* The numbers of mice ovulating refer to the ovulations obtained as a result of LH treatment (i.e. secondary ovulations).
** Significantly ($P < 0.01$) different from 0 µg of LH.
*** Significantly ($P < 0.05$) different from 0 µg of LH.
ovulation of secondary eggs in the majority of pregnant and pseudopregnant animals.

Comparison of LH release in pregnant mice following injection of GnRH in saline or in gelatin

Fig. 1 shows a comparison of the plasma levels of LH in the three groups. No significant release of LH occurred in animals injected with GnRH in saline when compared to the controls (P > 0.05), but a sharp peak at 1 h was seen in those injected with the same amount of GnRH in gelatin solution.

Discussion

These experiments demonstrate that there are mature follicles in the mouse ovary which can be induced to ovulate during pregnancy and pseudopregnancy using administration of hCG. This supports the works of Greenwald & Choudary (1969) with the pregnant mouse and of Taya & Sasamoto (1977) with the pregnant rat and the demonstration of Pederson & Peters (1971) that the growth of follicles is continuous during pregnancy in the mouse. The present results indicate that this is also the case during pseudopregnancy. Continued follicular growth implies that the secretion of FSH from the pituitary gland is continuous during these periods of luteal activity. Kovacic & Parlow (1972), Murr et al. (1974), and Gidley-Baird (1977) showed that there are significant amounts of FSH in the plasma of the mouse during these periods.

Bindon (1971) and Gidley-Baird & Emmens (1975) also showed that FSH is required for implantation in the mouse, and the works of Whitten (1955, 1958), Bindon (1970, 1971), Gidley-Baird & Emmens (1978) and Gidley-Baird (1981) show that the cause of suckling-delayed implantation is a suppression of the FSH release from the pituitary gland. In the present study, hCG was relatively ineffective in initiating ovulation on Days 1 and 2 of delayed pregnancy and incapable of causing ovulation in any animals between Days 2 and 6. After Day 8 only animals which showed signs of incipient implantation ovulated in re-

<table>
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</table>

* The numbers of mice ovulating refer to the ovulations obtained as a result of GnRH treatment (i.e. secondary treatment).

** Significantly (P < 0.01) different from 0 µg of LH.

*** Significantly (P < 0.05) different from 0 µg of LH.
sponse to hCG. This suggests that follicular growth is not continuous during suckling-induced delay of implantation, presumably as a consequence of the inhibition of pituitary FSH release during this period (Gidley-Baird 1981).

Injection of 10 and 50 µg of LH in saline on Days 3, 4 and 5 of pregnancy induced ovulation on these days, thus confirming the results of the hCG experiments. Gidley-Baird & Bindon (1976) showed that injection of 10 µg of LH in saline in hypophysectomised mice resulted in plasma LH levels comparable to levels measured at the time of ovulation in the pseudopregnant mouse, and 50 µg of LH resulted in plasma LH levels well in excess of those required for ovulation. Murr et al. (1974) and Gidley-Baird & Bindon (1976) found that there is a pre-implantation rise in endogenous LH levels around Days 3–5 which does not, however, initiate ovulation. In the present work, the LH was given on Days 3–5 to coincide with the relatively high endogenous LH levels and thus the increase in circulating levels initiated ovulation.

Injection of 1.0 µg of GnRH in saline into pregnant mice generally did not cause ovulation, although two mice which received two injections of 1.0 µg of GnRH within an hour had a single unfertilised egg in the oviduct (results not shown). Castro-Vasquez & McCann (1975) found that the priming effect of a second injection of GnRH was evident at pro-oestrus only and suggested that this priming effect was induced by prior elevation of oestrogen titres in early pro-oestrus. The number of blastocysts recovered from both control and GnRH-treated animals was low, probably due to difficulties in collection associated with compression of the blastocysts in the uterine crypts at this time.

Gidley-Baird & Bindon (1976) showed that after injection of LH in a 15% gelatin in hypophysectomised mice, plasma LH was maintained at a more constant level for a longer period of time than after injection in saline or oil. Consequently, GnRH was injected in a 15% gelatin solution to investigate whether ovulation could be initiated by prolonging the exposure of the pituitary gland to a high level of GnRH. GnRH, 1.0 µg, in gelatin caused ovulation on Days 4, 6 and 8 in the majority of pregnant and pseudopregnant mice, but was relatively ineffective in initiating ovulation on Day 2. The endogenous LH level has been shown to be low on Days 1 and 2 of pregnancy and pseudopregnancy in the mouse by Murr et al. (1974), Gidley-Baird & Bindon (1976), and Gidley-Baird (1977). This may account for the reduction in numbers of mice ovulating at this time. No mouse exhibiting suckling-induced delay of implantation ovulated in response to GnRH in either saline or gelatin, not even around the time of implantation (results not shown). This may be due to decreased pituitary responsiveness at this time or more likely to a lack of mature follicles as the earlier experiments suggest.

References


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