Radiolabelled uterine proteins during early pregnancy and pseudopregnancy in mice after unilateral ovariectomy and superovulation

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Summary. Uterine secretions were analysed after labelling *in vivo* with L-[4,5-³H]leucine. The acid-precipitable counts were highest on Day 1 *p.c.*, low on Day 2, lowest on Day 3, and similar to Day 2 values on Day 4 *p.c.* After unilateral ovariectomy the acid-precipitable counts for the horn ((+)ovary) ipsilateral to the remaining ovary were higher than those in the contralateral horn ((-)ovary) for each condition studied. Superovulation yielded slightly higher acid-precipitable counts in the (-)ovary and (+)ovary horns of pregnant mice on Day 3 *p.c.*, and in the (+)ovary horn only for pseudopregnant mice on Day 2 *p.c.* The Day 2 counts for the (+)ovary and (-)ovary horns of the superovulating pseudopregnant mice were higher counts than those for superovulating pregnant mice, but the reverse occurred for (+)ovary horns only on Day 4 *p.c.* No qualitative differences between pseudopregnant and pregnant mice were observed on Day 4 and superovulation appeared to enhance the profiles obtained.

Introduction

Proteins present in the uterine lumen of mice have been closely examined in recent years (Gore-Langton & Surani, 1976; Aitken, 1977a, b; Pratt, 1977; Fishel, 1979). During the first 4 days of pregnancy the protein profiles change dramatically and this reflects the changing ratio of progesterone and oestrogen secretions. These hormone-dependent changes in uterine proteins may play a role in the control of embryo metabolism and implantation (Surani, 1975; Aitken, 1977a; Pratt, 1977; Fishel, 1979). Fishel (1979) showed that particular groups of proteins that could be detected in the uterine lumen at implantation were not present when the milieu was unfavourable for implantation.

This investigation was carried out to observe uterine secretions during pregnancy and pseudopregnancy after unilateral ovariectomy and superovulation, in an attempt to detect changes which may be due to embryonic contribution or gonadotrophin treatment.

Materials and Methods

CFLP mice (Anglia Laboratories) were aged between 6 and 8 weeks and kept under standard animal house conditions with a lighting schedule of 05:00–19:00 h. Day 1 was the day of finding a vaginal plug.

Progesterone (4-pregnene-3,20-dione) and oestradiol benzoate (1,2,5(10)-oestratriene-3,13-

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diol-3-benzoate) were obtained from Koch-Light Laboratories. L-[4,5-³H]Leucine (sp. act. 53 Ci/mmol; Radiochemical Centre, Amersham) was used as the protein precursor. Anaesthesia was induced by tribromoethyl alcohol with amylene hydrate (Avertin: Winthrop Laboratories). PMSG (Folligon: Intervet, Cambs, England) and hCG (Chorulon: Intervet) were used for superovulation.

Experiments

Each animal was anaesthetized by an i.p. injection of tribromoethyl alcohol with amylene hydrate, using 0.2 ml of a 1.2% solution per g body weight. In each animal the right ovary was located through a 0.7 cm dorsal incision and removed by cauterization of the oviduct. For convenience the uterine horns ipsilateral and contralateral to the remaining ovary are subsequently referred to as the (+)ovary and (−)ovary horns.

The animals were left undisturbed for a further 30 days, and the females were then placed with proven fertile males or with vasectomized males. Half of the pregnant and half of the pseudopregnant females were treated with 5 i.u. PMSG and 2.5 i.u. hCG 45 h later to induce superovulation. Ovulation number was increased up to 5-fold in this strain of mouse. The numbers of animals used in the various groups are given in Table 1.

Radioactive labelling of proteins and collection and analysis of uterine secretions have been described previously (Fishel, 1979). Briefly, the mice were killed by cervical dislocation 6 h after the injection of radioactive leucine on Days 1, 2, 3 or 4 *p.c.* The contents of the lumen were flushed from the oviductal end with 0.05 ml 0.01 M-phosphate-buffered saline, pH 7.2. After centrifugation, a buffer concentrate was added to the supernatant to give a final concentration of 0.1% sodium dodecyl sulphate, 0.14 M-2-mercaptoethanol and 10% glycerol. The samples were run on 6% SDS-polyacrylamide gels. After electrophoresis the gels were frozen using solid CO₂ and sliced into 1 mm thick discs, using a gel slicer, before liquid scintillation spectrophotometry.

In a separate experiment, blastocysts were transferred to the (−)ovary horn of 6

Table 1. The amounts of labelled acid-precipitable proteins in the uteri of mice after unilateral ovariectomy and induction of superovulation

Group	Treatment		Labelled proteins (d.p.m./uterus/mouse × 10 ³)			
	Ovary	Super-ovulation	Day 1	Day 2	Day 3	Day 4
Pregnant						
1	a	+	8.88 ± 0.18 ^a (8)	7.29 ± 0.21 ^a (8)	2.22 ± 0.08 ^a (6)	7.89 ± 0.30 ^{ad} (10)
	b	−	6.99 ± 0.13 ^b	4.58 ± 0.24 ^b	1.02 ± 0.14 ^b	3.14 ± 0.18 ^b
2	a	+	8.58 ± 0.25 ^a (8)	7.18 ± 0.32 ^a (4)	2.93 ± 0.22 ^c (4)	7.98 ± 0.15 ^a (6)
	b	−	6.89 ± 0.27 ^b	4.28 ± 0.44 ^b	1.65 ± 0.23 ^d	3.87 ± 0.29 ^b
Pseudopregnant						
3	a	+	9.92 ± 0.21 ^c (6)	6.88 ± 0.33 ^{ad} (4)	2.70 ± 0.30 ^{ac} (4)	7.35 ± 0.24 ^{ad} (6)
	b	−	7.50 ± 0.37 ^b	5.43 ± 0.55 ^{bde}	1.28 ± 0.11 ^{bd}	3.57 ± 0.27 ^b
4	a	+	10.25 ± 0.50 ^{cd} (6)	8.48 ± 0.15 ^c (6)	2.90 ± 0.19 ^c (5)	7.31 ± 0.25 ^{cd} (8)
	b	−	6.83 ± 0.23 ^b	5.55 ± 0.16 ^c	1.36 ± 0.26 ^{bd}	3.89 ± 0.39 ^b
5	(both + ovaries)	−	11.34 ± 0.28 ^d (8)			10.73 ± 0.52 ^e (7)

Values are mean ± s.e.m. for the numbers of animals indicated in parentheses for each group.

Within columns, different superscripts indicate significant differences at at least *P* < 0.05 (Student's *t* test).

pseudopregnant and 6 pregnant females. Half of the mice were killed on Day 19 *p.c.* and the rest allowed to go to term to determine whether the (–)ovary horn could support implantation and maintain pregnancy.

Results

Implantation occurred and pregnancy was maintained in the (–)ovary horn of pseudopregnant females. In pregnant mice, the remaining ovary supported pregnancy in the ipsilateral and contralateral horns. Parturition occurred normally at term.

Text-fig. 1. Representative profile of radiolabelled proteins, analysed on 6% SDS-polyacrylamide gels, in the uterine lumen of intact mice on Day 1 of pseudopregnancy (Group 5). M = myosin; B = bovine serum albumin; P = peroxidase; T = trypsin.

As shown in Table 1 the counts of acid-precipitable radioactivity were highest on Day 1 *p.c.* and dropped to a nadir on Day 3 before rising again on Day 4 to a level similar to that on Day 2. In all conditions studied the (+)ovary horn had significantly higher counts than did the (–)ovary horn, except for Groups 3a and 3b on Day 2. For the electrophoretic gels the results, for 4–10 animals/group, were reproducible.

On Day 1 *p.c.* the values for pseudopregnant animals (Groups 3a and 4a) were significantly higher than those of pregnant animals (Groups 1a and 2a) for the (+)ovary horns. The qualitative profile observed on Day 1 *p.c.* for intact pregnant animals (Fishel, 1979) is very different from those analysed during pseudopregnancy (Text-fig. 1). In Group 5 there was a profile with 7 distinct peaks, but most of the radioactivity was incorporated into the protein with an approximate molecular weight of 64 000 compared to that of 220 000–260 000 in pregnant animals.

On Day 2 *p.c.* the Group 4a horns yielded higher acid-precipitable counts than did those in Group 3a, but there was no detectable difference between Groups 3b and 4b.

On Day 3 *p.c.* the values of Groups 1a and 1b were significantly lower than those of Groups 2a and 2b respectively.

On Day 4 *p.c.* there was no detectable difference between values in Groups 3a and 4a and 3b and 4b, but values in Group 2a were significantly higher than those in Group 4a. The protein profiles obtained on Day 4 *p.c.* during the different experimental conditions are illustrated in

Text-fig. 2. Apart from some minor differences the profiles are essentially very similar. Up to 9 peaks were detected and most of the radioactivity was always incorporated in the fraction corresponding to a molecular weight of 65 000–68 000. In Group 1 (Text-fig. 2a) the protein with an approximate molecular weight of 30 000 was not detected in the (–)ovary horn and the 13 000–14 000 molecular weight protein was not detected in the (+)ovary horn. The 13 000–14 000 protein was also undetected in Group 3 (Text-fig. 2b). Superovulation appeared to enhance the definition of the profiles for pseudopregnant mice but not those of the pregnant animals.

Text-fig. 2. Representative profiles of radiolabelled proteins, analysed on 6% SDS–polyacrylamide gels, in the uterine lumen at day 4 *p.c.* of mice from (a) Group 1, (b) Group 3, (c) Group 2 and (d) Group 4 (see Table 1) according to the presence (○) or absence (●) of the ovary on that side. M = myosin; B = bovine serum albumin; P = peroxidase; T = trypsin.

Discussion

The previous study on radiolabelled uterine-secreted proteins (Fishel, 1979) during the first 4 days of pregnancy analysed only pregnant animals and used pooled samples. In this study radiolabelled uterine proteins were analysed during pseudopregnancy, after unilateral ovariectomy and also after superovulation. These treatments allow comparisons between the presence and absence of spermatozoa in the uterine lumen and any putative sperm products, changes due to increased gonadotrophins, any embryonic contribution, and variations in the endocrine states of particular animals.

The results suggest that the various endocrine states have little effect on the profile of uterine proteins, labelled *in vivo*. On all 4 days the amount of acid-precipitable material in the uterine lumen was higher in the (+)ovary horns than in the (–)ovary horns, regardless of whether the females were pregnant or pseudopregnant or the numbers of eggs ovulated. These lower counts may have been caused by tissue damage inflicted during ovariectomy. However, the protein profiles in the (–)ovary horns were reproducible and the presence of the ovary would appear to have some influence on the proteins secreted into the uterine lumen.

The qualitative differences between pregnant and pseudopregnant animals on Day 1 *p.c.* may be an indication that certain products from spermatozoa are contributing to the uterine milieu, or that spermatozoa induce particular secretions from the uterus. The quantitative difference on Day 2 between pseudopregnant animals ovulating normally or induced to superovulate (Group 4) may be a truer representation of uterine-specific proteins than the results observed for pregnant animals (Group 2).

The induction of superovulation by circulating gonadotrophins did not qualitatively affect the profiles of uterine proteins during early pregnancy (results for Days 2 and 3 *p.c.*, unpublished), in spite of the increased numbers of embryos. On Day 4 *p.c.* however, the induction of superovulation led to a slight, but significant, quantitative difference between the pregnant and pseudopregnant states, suggesting that the embryos may take-up and incorporate some of the injected label and release labelled protein into the milieu. Mouse blastocysts have been shown to synthesize and release glycoproteins into the medium *in vitro* (Fishel & Surani, 1980). Although no embryonic contribution was detected in the protein profiles in the present study this does not preclude its presence; an embryonic contribution may have been masked by the uterine secretions. It is also possible that the presence of embryos induces an increase in uterine secretion (Nilsson, 1974).

As similar radiolabelled protein profiles were obtained in the presence or absence of embryos at the time of implantation it can be concluded that the changes detected on Day 4 *p.c.* are not a result of implantation and therefore not a marker for it. These results confirm earlier work (Aitken, 1977b). The results for the pseudopregnant females on Day 4 *p.c.*, indicative solely of uterine-specific proteins, show an enhancement of profiles with superovulation (Group 4a compared with Group 3a) which has been reported previously (Gore-Langton & Surani, 1976). The rise on Day 4, both quantitatively and qualitatively, of uterine-secreted proteins makes it difficult to regard them as having a passive role, and the possible biological functions of these macromolecules requires investigation. Tzartos & Surani (1979) have used ¹²⁵I-labelled uterine proteins to show that uterine proteins from Day 5 pregnant rats have a higher affinity for rat blastocysts than do uterine proteins from pro-oestrous animals or albumin. Such proteins may help to maintain optimal embryonic metabolism (Fishel & Surani, 1978).

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