INFERTILITY IN MICE CAUSED BY FASTING AT ABOUT THE TIME OF MATING

II. PATHOLOGICAL CHANGES

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Summary. The internal genitalia of female mice, which had been fasted for one 30-hr period at about the time of mating, were examined 0 to 7 days after mating.

The major causes of infertility of mice fasted before mating were failure of ovulation and death of ova before implantation. Ovulation of primary oöcytes, and the presence of 'supplementary' spermatozoa in ova, were observed, but these were too infrequent to be the results of the treatment.

When the fast ended 2 to 4 days after mating the major cause of infertility was death of the ova before implantation. Much less frequently implantation failed.

INTRODUCTION

Previous work (McClure, 1966) has shown that fasting female mice for 18 to 48 hr at about the time of mating markedly reduces the proportion that produce litters. Glucose prevents this effect. Experiments described below are designed to find out the exact time at which the failure occurs, and to identify the accompanying pathological changes in the female internal genitalia.

MATERIALS AND METHODS

Mice of the same strain were kept under the conditions described in the earlier work (McClure, 1966). The experimental animals were fasted for 30 hr, that is, the period which causes the greatest fall in littering rates without significantly inhibiting oestrus. The fast always ended at 14.00 hours, 1 hr after the end of the daily 7-hr period of darkness. Mating usually occurs 3 days after the introduction of a male to a group of virgin females (Whitten, 1956). This fact was used to arrange fasting periods so that many females were mated on the day fasting ended or up to 2 days after the end of fasting. Other mice, chosen at random, were fasted for a 30-hr period ending 2 to 4 days after mating.

Mated mice were killed between 14.00 and 18.00 hours 0 to 7 days after the finding of copulation plugs. The internal genitalia were examined immediately by the following methods: (a) macroscopically; (b) under the dissecting microscope; (c) if within 4 days of mating, the ovaries were separated from the Fallopian tubes, with the utmost care not to rupture follicles, and were fixed in 12.5% formal-saline, sectioned, stained with haematoxylin and eosin and with Oil Red O and examined microscopically; the Fallopian tubes were gently teased in and the uterine horns flushed with 0.9% sodium chloride solution and the washings searched for ova under the scanning lens of the microscope—when found, the ova were mounted in the saline under a coverslip ringed with soft petrolatum, and were examined under the low and high power objectives; (d) if more than 4 days after mating, one horn and Fallopian tube were examined as under (c), and the others were fixed in 12.5% formal-saline, sectioned and stained with haematoxylin and eosin and with Oil Red O and examined microscopically.

Controls were not fasted. They were killed at comparable times and examined in a similar manner.

RESULTS

Categories of abnormality

Macroscopic and microscopic examination revealed various abnormalities which have been grouped into six categories (Table 1).

Failure of ovulation. Fifteen mice, killed 0 to 3 days after mating, had ovaries in which follicles, but no corpora lutea, either white or hyperaemic, were visible macroscopically, and in which no large corpora lutea were apparent microscopically. No ova were found in the Fallopian tubes or uterine horns. These pregnancies were considered abnormal. All mice of this group had been fasted before mating.

Ovulation of primary oöcytes. In six mice, killed 0 to 5 days after mating, some or all of the ova found in the Fallopian tubes were primary oöcytes (cf. Austin & Braden, 1954). The primary oöcytes were free of coronal cells. Usually only one or two primary oöcytes were found. In only two were other ova present; one was a mouse, fasted after mating, in which the three ova present were fragmenting; the other was a control from which four normal 4-cell ova were also recovered. The other four mice of this group had been fasted before mating; in these, the major lesion was probably ovulatory failure. The pregnancies of this group, except that of the control mouse, were considered abnormal.

Ova containing 'supplementary' spermatozoa. In two mice one or more of the ova contained many spermatozoa free in the perivitelline space (cf. Austin & Braden, 1953; Braden, Austin & David, 1954). Both were mated 0 to 2 days after the end of fasting. In one mouse, one of the four ova recovered contained many 'supplementary' spermatozoa, and in the other, two out of four. No polyspermy was seen, but this may have been due to the density of the cytoplasm of these ova. The pregnancies of the mice in this group were considered normal.

Degenerating ova. Twenty mice had ova most of which showed structural signs

TABLE I
REPRODUCTIVE STATE OF FASTED AND CONTROL MICE

	Interval between mating and examination (days)	No. of mice with detected abnormalities						Total pregnancies		
		Not pregnant: cause unknown	Ovulatory failure	Primary oöcytes	Ova containing 'supplementary' sperms	Degenerating ova	Implantation failure	Abnormal	Uncertain	Normal
Mated after fasting	0	—	3	—	—	—	—	3	1	1
	1	—	4	3	1	4	—	8	1	4
	2	1	6	1	1	3	—	9	0	7
	3	—	2	—	—	—	—	2	0	0
	4	6	—	—	—	5	—	10	1	1
	5	1	—	—	—	—	—	1	1	1
	6	4	—	—	—	—	—	4	0	2
	Total	12	15	4	2	12	0	37 (67.3%)	2 (3.6%)	16 (29.1%)
Mated before fasting	2	—	—	—	—	1	—	1	0	0
	3	2	—	—	—	1	—	3	0	0
	4	4	—	—	—	3	—	5	2	0
	5	3	—	1	—	3	1	6	1	0
	Total	9	0	1	0	8	1	15 (83.3%)	3 (16.7%)	0
Fasted	Total	21	15	5	2	20	1	52	5	16
Controls (not fasted)		8	0	1	0	0	0	8 (36.4%)	0	14 (63.6%)

of degeneration (Plate I, Figs. a, b and c). Usually the ova were fragmented, but sometimes cytoplasm seemed to have leaked into the perivitelline space; rarely, cleavage had failed (that is, there were unicellular ova 2 days or more after mating). There were degenerated ova at all stages from a single cell to a blastocyst. They were present in mice of both pre- and post-mating fasting groups, but not in controls.

Failure of implantation. One mouse, killed 5 days after mating, had blastocysts in the uterus but there were no signs of decidual reaction. This mouse had been fasted after mating.

Not pregnant, cause unknown. Twenty-nine mice were not pregnant, did not contain deciduomata and gave no evidence of ova or degradation products. These negative findings could have resulted from ovulatory failure, failure to recover ova, rapid passage and excretion of ova, rapid degeneration and resorption of ova or marked loss of structure rendering the ova unidentifiable. Twenty-four of the mice in this group were examined 4 or more days after mating—perhaps after the time when it was possible to determine the type of failure.

Controls

Of the twenty-two control mice comparable to those in the fasted groups, fourteen (63.6%) contained sufficient normal ova, morulae, blastocysts or implanted embryos to indicate normal pregnancy. One mouse had one structurally abnormal ovum (a primary oocyte) together with four apparently normal 4-cell ova. The ovaries of all fourteen mice contained hyperaemic corpora lutea. None of the remaining eight mice was pregnant or showed any sign of ova or deciduomata. The ovaries of two non-pregnant mice examined within 3 days of mating contained hyperaemic corpora lutea. The remaining six non-pregnant mice were examined 5 to 7 days after mating.

Fasted mice

The ovaries, ova and uteri of the fasted mice were often abnormal. The signs ranged from absence of ovulation to failure of implantation: they included ovulation of primary oocytes, failure of cleavage, 'supplementary' spermatozoa in the perivitelline space, and degeneration of ova and blastocysts. The incidence of abnormality is shown in Table 1.

The ovaries of only seven of the twenty mice from which degenerating ova were found contained hyperaemic corpora lutea. The ovaries of the remaining thirteen mice, and those of the one with implantation failure, contained only white non-hyperaemic corpora lutea.

Ovarian histology

Histological examination of sections of ovaries with follicles but no corpora lutea confirmed the presence of many medium-sized unruptured follicles and the absence of large corpora lutea. The white, non-hyperaemic corpora lutea proved to be small and rather shrunken, and not the distended structures of the unfasted control mice.

PLATE 1

(a) Fragmenting ovum taken from the Fallopian tubes of a mouse 2 days after mating. The mouse was fasted for 30 hr ending on the day of mating. $\times 700$.

(b) and (c) Fragmenting ova taken from the Fallopian tubes of mice fasted for 30 hr after mating. $\times 700$.

(Facing p. 390)

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