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

I. MATING BEHAVIOUR AND LITTERING RATES

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Summary. Fasting male mice for up to 30 hr did not affect their libido, but fasting for 36 or 48 hr did so for 2 days after the end of the fast. Fasting male mice for up to 48 hr did not affect their fertility on any of the succeeding days.

Fasting both male and female mice for up to 30 hr did not affect the number and distribution of females mated. Fasting for 36 or 48 hr reduced the number of females mated in both the first 2 days and the first 5 days after the end of fasting and altered the distribution of mating in the first 5 days much more than when only males were fasted. Fasting both males and females significantly reduced the littering rate of the females mated 0 to 2 days before or after the end of 18 to 48 hr fasting.

It is concluded that acute inanition in females for 18 to 30 hr at and about the time of mating can reduce fertility without markedly affecting the manifestation of oestrus and that a more prolonged period of inanition of 36 hr or more also depresses oestrus. The essential factor in acute inanition appears to be an acute energy deficiency.

INTRODUCTION

Mice have been fasted for various periods during early gestation (McClure, 1959, 1961b, c). This was an attempt to understand more of the causes of infertility in a dairy herd, suspected to be due to malnutrition for short periods during early lactation (McClure, 1961a).

Complete fasting for 48 hr during the 4th and 5th days after mating caused the destruction of all of the embryos in all of the mice tested. This effect became less as the fasting period was moved to either side of the apparently critical 4th and 5th days, that is, the period of implantation and early development of the decidua (Snell, 1956). Sucrose or starch offered during the 48-hr period when the normal food was withheld prevented the loss of embryos in some of the mice. Embryonic death appeared to be caused by reduced production or release of pituitary gonadotrophins and failure of secretion of progesterone by the corpus luteum. Bruce (1963), when comparing this effect with the olfactory block to pregnancy caused by exposure of recently mated females to strange male mice, was able to reduce the effective period of fasting to 24 hr.

While these experiments have shown that acute inanition can cause the death of implanted embryos, they have not reproduced the condition that occurs in cattle. In cattle, implantation (cotyledonary attachment) begins at about the 30th to 35th day and is not complete until about the 45th day after fertilization (Winters, Green & Comstock, 1942; Melton, Berry & Butler, 1951; Salisbury & VanDemark, 1961). Many of the reproductive failures in the nutritional infertility syndrome of cattle occur before the end of the 1st month, (McClure, unpublished data) and, therefore, before implantation. Observations on the repeat-breeder syndrome in North America (Casida, 1961) indicate that much of the embryonic loss also occurs before the 25th day. Attempts were made to advance the time at which mice were fasted for short periods and to interrupt an earlier stage of the reproductive process. Preliminary results (McClure, 1963) indicated that a 24-hr fast ending on the day of mating, or up to 2 days before mating, depressed the littering rate.

This paper reports the results of experiments designed to define the length of acute inanition required to cause reproductive failure before implantation, and to find the effective times of fasting relative to mating.

MATERIALS AND METHODS

Ten- to 12-week-old virgin mice, from a closed colony of the Sydney White strain, were used. The experiments were conducted in a series over 12 months. At the beginning of each experiment, five female mice were put at random into a cage measuring $10 \times 5 \times 5$ in., and placed in an environment chamber $6 \times 3 \times 4$ ft maintained at 22° C. The chamber was illuminated only by one 40-watt fluorescent tube for 17 hr from 13.00 hours to 06.00 hours daily, and was in complete darkness for the remaining 7-hr period from 06.00 hours to 13.00 hours. Air was continually circulated by an electric fan. The mice were fed on unlimited 'Rat and Mouse Cubes' (Drug Houses of Australia Pty Ltd) supplemented once weekly with a small quantity of fresh green grass. Water was always freely available. The mice were left in the cages for 10 to 14 days before each trial was started, since Hemmingsen & Krarup (1937) found that the reversal of daylight/dark periods requires about 10 days to be effective. At 14.00 hours every day, after males were introduced, the female mice were examined for copulation plugs, the presence of which was taken as evidence of mating. Three series of trials were performed.

Series A was designed to determine the effect of fasting on the mating behaviour and fertility of male mice. At the beginning of each trial one male was placed in a small sub-cage within each cage containing five females, and was fasted for 0, 24, 30, 36 or 48 hr; the fast always ended at the beginning of the third period of darkness after the introduction of the males. The males were then released from their sub-cages.

Series B was designed to determine the effects of fasting both male and female mice on their mating behaviour and littering rates. Since the results of Series A indicated that fasting males for up to 30 hr had no effect on libido or on fertility, and that the longer periods had only a slight effect on libido and none on fertility, both male and females were fasted together in order to reduce

possible nervous effects upon behaviour and fertility. At the beginning of each trial one male mouse was introduced to each cage of females. Mice were transferred to clean cages with fresh sawdust and not fed for 0, 12, 18, 24, 30, 36 or 48 hr. The fasts ended at 08.00 hours, except for that of 30 hr which ended at 14.00 hours. The periods of fast were staggered with the object of getting reasonably equal numbers in each group with end of fasting to mating intervals of 0, 1, 2, 3 and 4 days (that is, about one oestrous cycle length). Other groups of mice were fasted for similar periods, ending 1 or 2 days after the finding of copulation plugs. In planning the time to end the periods of fasting, use was made of Whitten's (1956) observations that, after introduction of males to groups of virgin females, more than half of the females are mated on the 3rd night.

Series C was designed to determine the constituent of the diet the absence of which, during fasting, was responsible for causing the infertility observed in Series B. The food was withheld from the mice for a 30-hr period, during which they were given an unlimited supply of 75% w/v solution of D-glucose instead of drinking water.

RESULTS

Series A. Effect of fasting on mating and fertility of males

The distribution of matings of unfasted females paired with fasted males during the first 5 days after the end of fasting (that is, during the 3rd to 7th days after the introduction of the male into the sub-cages) is shown in Table 1. Fasting male mice for 24 or 30 hr did not affect the number or proportion of

TABLE 1
DISTRIBUTION OF MATINGS OF UNFASTED FEMALE MICE PAIRED WITH FASTED MALES

Interval between end of fast and mating (days)	Days after introduction of males into sub-cages	No. of females mated by males fasted for:						
		0 hr	24 hr	30 hr	36 hr	48 hr	24+30 hr	36+48 hr
0	3	12	10	10	8	6	20	14
1	4	5	13	3	2	6	16	8
2	5	2	3	2	1	5	5	6
3	6	3	2	0	3	4	2	7
4	7	3	3	2	7	3	5	10
No. of females paired with males		40	50	35	39	52	85	91
No. of females mated on Days 3 to 4*		17					36	22
As percentage of No. mated on Days 3 to 7*		68.0 (a)					75.0(b)	48.9(c)
As percentage of total No. paired		42.5 (d)					42.4(e)	24.2(f)
No. of females mated on days 3 to 7*		25					48	45
As percentage of total No. paired		62.5 (g)					56.5(h)	49.5(i)

* After introduction of males into sub-cages.

TESTS OF SIGNIFICANCE $df = 1$

- (a) versus (b), $P = 0.5$ NS.
- (a) versus (c), $P > 0.05$ NS.
- (d) versus (e), $P = 1.0$ NS.
- (d) versus (f), $P < 0.05, > 0.02$.*
- (g) versus (h), $P > 0.5$ NS.
- (g) versus (i), $P > 0.2$ NS.

TABLE 2
DISTRIBUTION OF MATINGS OF FASTED FEMALE MICE PAIRED WITH FASTED MALE MICE

Interval between end of fast and mating (days)	Days after introduction of males	No. of females mated in groups fasted for :									
		0 hr	12 hr	18 hr	24 hr	30 hr	36 hr	48 hr	12+18 hr	24+30 hr	36+48 hr
0 1 2 3 4	1	4	0	4	5	6	4	5	4	11	9
	2	10	0	7	8	5	8	6	7	13	14
	3	20	7	14	11	10	5	4	21	21	9
	4	12	2	5	8	11	7	0	7	19	7
	5	7	1	5	12	1	6	1	6	13	7
	6	1	1	4	8	2	8	8	5	10	16
4	7	5	1	3	7	0	11	11	4	7	22
No. of females paired with males		104	25	80	104	45	104	104	105	149	208
Mean day of mating 3- to 7-day period S.D.		4.1 1.30 (a)	3.9 1.33	4.3 1.28	4.7 1.37	3.8 0.85	5.4 1.42	5.8 1.41	4.2 1.38 (b)	4.5 1.39 (c)	5.6 1.44 (d)
No. of females mated on Days 3 to 4 As percentage of No. mated on Days 3 to 7		32 71.1 (e)	9 75.0	19 61.3	19 41.3	21 87.5	12 32.4	4 16.7	28 65.1 (f)	40 57.1 (g)	16 26.2 (h)
As percentage of total No. paired		30.8 (i)	36.0	23.8	18.3	46.7	11.5	3.8	26.7 (j)	26.8 (k)	7.7 (l)
No. of females mated on Days 3 to 7 As percentage of total No. paired		45 43.3 (m)	12 48.0	31 38.8	46 44.2	24 53.3	37 35.6	24 23.1	43 41.0 (n)	70 47.0 (o)	61 29.3 (p)

TESTS OF SIGNIFICANCE

Distribution of mating in 3- to 7-day period after introduction of males

(a) versus (b), $P < 0.5$ NS.(a) versus (c), $P = 0.1$ NS.(a) versus (d), $P < 0.001$.***Proportions of females mated $\frac{\text{No. on Days 3+4}}{\text{No. on Days 3 to 7}}$ (e) versus (f), $P > 0.5$ NS.(e) versus (g), $P > 0.05$ NS.(e) versus (h), $P < 0.001$.***Proportion of females mated $\frac{\text{No. on Days 3+4}}{\text{Total No. paired}}$ (i) versus (j), $P > 0.5$ NS.(i) versus (k), $P < 0.5$ NS.(i) versus (l), $P > 0.001$.***Proportion of females mated $\frac{\text{No. on Days 3 to 7}}{\text{Total No. paired}}$ (m) versus (n), $P > 0.5$ NS.(m) versus (o), $P > 0.3$ NS.(m) versus (p), $P < 0.02$, > 0.01 .*

unfasted females mated during the first 2 days or the first 5 days after the end of fasting, nor did it influence the distribution of matings during the 5-day period. Fasting of the males for 36 to 48 hr reduced the number and proportion of females mated in the first 2 days after the end of fasting ($P \leq 0.05$). There was also a smaller number and proportion mated in the first 5 days, and a change in distribution of matings during the first 5 days after the end of fasting; but neither of these was statistically significant. Evidently some of the females not mated in the first 2 days after the end of fasting of the males were mated at their next oestrus on the 4th or 5th day when, presumably, the libido of the males had recovered.

Fasting the males for up to and including 48 hr did not depress their fertility in the first 2 days or the first 5 days after the end of fasting. Seventy-four out of ninety-eight (76%) females mated to fasted males and twenty out of twenty-six (77%) females, mated to control males, littered.

Series B. Effects of fasting males and females on mating and littering rates

The numbers and proportions of females mated during the first 7 days after introduction of males and 5 days after the end of fasting are shown in Table 2.

TABLE 3
LITTERING RATES OF FASTED FEMALES MATED TO FASTED MALES AND FASTED
MICE SUPPLEMENTED WITH GLUCOSE

Interval between end of fast and mating (days)	Littering rates $\frac{\text{No. littering}}{\text{No. mated}}$							
	Length of fast (hr) of males and females							
	0	12	18	24	30	36	48	30+ Glucose
Before 5	3/3	0/1	3/4	3/6	0/1	6/8	5/7	1/1
mating 4	1/1	2/2	3/3	4/6	—	5/8	8/10	2/2
3	6/7	4/5	10/14	10/13	1/1	10/17	8/9	
2	12/12	1/3	6/8	4/10	1/1	2/6	1/1	1/2
1	8/10	3/3	5/8	3/3	2/15	2/7	—	1/2
0	7/8	8/12	7/10	5/14	3/13	0/9	0/5	9/11
After 1	7/7	—	3/10	2/13	1/5	1/3	2/6	4/4
mating 2	2/2	0/1	1/9	2/7	1/2	1/7	3/11	3/3
Total 0 to 2 days before mating	27/30 90% (a)	12/18 66.7% (b)	18/26 69.2% (c)	12/27 44.4% (d)	6/29 20.7% (e)	4/22 18.2% (f)	1/6 16.6% (g)	11/15 73.3% (h)
Grand total	46/50 92.0%	18/27 66.7%	38/66 57.6%	33/72 45.8%	9/38 23.7%	27/65 41.5%	27/49 55.1%	21/25 84.0%

TESTS OF SIGNIFICANCE

- (a) versus (b), $P < 0.2, > 0.1$ NS.
- (a) versus (c), $P = 0.05$.*
- (a) versus (d), $P < 0.001$ ***
- (a) versus (e), $P < 0.001$ ***
- (a) versus (f), $P < 0.001$ ***
- (a) versus (g), $P < 0.01, > 0.001$ **
- (a) versus (h), $P < 0.20, > 0.10$ NS.
- (e) versus (h), $P < 0.001$ ***

Fasting both sexes for 12, 18, 24 or 30 hr did not reduce the number and proportion of females mated during the first 2 or the first 5 days after the end of fasting; nor did it influence the distribution of matings over the first 5-day period. Fasting both sexes for 36 or 48 hr reduced the number and proportion of mice mated during the first 2 and the first 5 days after the end of fasting; it also altered the distribution of matings during the first 5-day period. The numbers and proportions of females littering are shown in Table 3.

There was a progressive decline in fertility as the length of fast increased from 0 to 30 hr. Thereafter, up to 48 hr, the littering rate increased, mainly because more of the mice were mated at a longer period after the end of fasting when their fertility had recovered. The mice seemed particularly sensitive to the effects of fasting when the fast ended during the period from up to 2 days before mating to at least 2 days after mating (Table 3). Littering rates of mice mated on the day fasting ended, or up to 2 days after the end of fasting, fell significantly ($P \leq 0.05$) with the 18-hr fast; the effect was highly significant after longer periods of inanition.

Series C. Effect of replacing food pellets and water with glucose solution

In these experiments the pelleted food and the water were replaced for 30 hr by a 75% w/v solution of D-glucose. The littering rate of the mice in the group given glucose did not differ from that of the control group (Table 3).

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