Stimulus requirements for pregnancy initiation in the golden hamster (*Mesocricetus auratus*) change with age and parity

U. W. Huck, R. D. Lisk* and C. L. Guyton*

Biology Program, Sangamon State University, Springfield, IL 62708, U.S.A.; and *Department of Biology, Princeton University, Princeton, NJ 08544, U.S.A.

Summary. Hamsters were mated repeatedly at 60, 180, 300 and 420 days of age or once during their lifetime at one of these ages. Copulatory stimulation was varied both for number of ejaculations (2 *versus* 14) and amount of vaginocervical stimulation (5 *versus* 50–60 intromissions). Two ejaculations provided sufficient spermatozoa to maximize litter size for all age and parity classes tested. Differences in fecundity depended on the amount of vaginocervical stimulation received. Higher levels of vaginocervical stimulation increased littering success at 300 and 420 days for nulliparous females and at 180, 300 and 420 days for multiparous females. Females which did not deliver litters did not show a cessation of oestrous cycles which characteristically follows the induction of a luteal phase. The decreased fecundity observed with increasing age or parity therefore resulted from a change in sensitivity to the stimulus conditions necessary to activate the neuroendocrine arc for establishment of pregnancy.

Keywords: age; parity; pregnancy initiation; litter size; vaginocervical stimulation; golden hamster

Introduction

Copulations are essential both as mechanisms of sperm transfer and as sources of vaginocervical stimulation for pregnancy initiation in species which are induced ovulators or which do not have a spontaneous luteal phase. Minimal stimulus requirements necessary to ensure a high pregnancy rate vary not only between species, but also within a species depending on the animal's age or previous reproductive condition (Dewsbury, 1978) and when during the receptive period (early, middle or late) mating occurs (Huck *et al.*, 1986).

In some species of rodents, young virgin females require less copulatory stimulation than do older multiparous females for a high probability of pregnancy initiation (e.g. laboratory rats: Davis *et al.*, 1977; deer mice: Dewsbury, 1979; montane voles: Gray *et al.*, 1977). A similar pattern has been noted for golden hamsters (Huck & Lisk, 1985b). Huck & Lisk (1985b) also demonstrated that increased vaginocervical stimulation rather than an increased number of spermatozoa was required for pregnancy initiation in older, multiparous females.

In each of the above studies the effects of age and parity were confounded. Therefore, the following study was designed to examine the relative effects of age and parity on the stimulus requirements for pregnancy initiation in golden hamsters.

Materials and Methods

Animals and husbandry. All 538 females and 68 stud males used were laboratory-born descendants of the LVG strain obtained from Charles River, Inc., Wilmington, MA 01887, U.S.A. Each of the stud males had sired 2 or more

previous litters and was rested (no sexual contact) for at least 1 week between tests. Oestrous condition was determined by extravaginal examination (Orsini, 1961). Animals were maintained in windowless, air-conditioned rooms on a 14-h light:10-h dark photoperiod (lights off at 19:00 h). Each was individually housed in a 35 × 20 × 17.5 cm rack-mounted cage provided with commercial laboratory chow and water *ad libitum*. After mating, females were transferred to plastic breeding boxes with wood shavings for bedding and their oestrous condition checked daily, beginning 4 days later.

Apparatus. All matings were conducted in a semicircular Plexiglas arena with a diameter of 35 cm.

Procedure. Females were either mated once at about (± 2 days) 60, 180, 300 or 420 days of age, or they were mated repeatedly at each of these ages. At each mating age, the once-mated females were assigned to one of three treatment groups. Those in Group LI-LE received 2 ejaculations and an average of 5 intromissions from a rested male (low intromission–low ejaculation). To reduce the number of intromissions received by these females (male hamsters usually engage in 10 short intromissions before their first ejaculation and 2–3 before their second (Huck & Lisk, 1985a)), stimulus males were allowed to achieve intromission with another female 5–8 times before being introduced to the experimental female. Males ejaculating during this pretreatment were replaced. Females in Group HI-LE similarly received 5 short intromissions and 2 ejaculations from one male who was immediately replaced by a second, vasectomized male (high intromission–low ejaculation). The vasectomized male was allowed to copulate until a satiety criterion of 15 min without lordosis or an attack by the female upon the male. Vasectomized males produced an average of 36 short intromissions, 13 behavioural 'ejaculations', and 11 long intromissions.

The females in Group HI-HE were mated to satiety with a single intact male from whom they received an average of 42 short intromissions, 14 ejaculations, and 10 long intromissions (high intromission–high ejaculation). These matings were also terminated when the female attacked the male or after 15 min without lordosis.

Similar procedures were used with the repeatedly-mated females. At 60 ± 2 days of age, 224 females were mated to satiety with an intact stud male. These females were then allowed to raise their litters until the young were 25 days old, when they were removed and the dams were returned to metal cages. At 180 ± 2 days of age these females were divided into three groups and mated in the manner previously described for the females mated once. Only animals that were mated *ad libitum* with an intact male and subsequently delivered litters were remated at 300 days of age. The same procedure was used to obtain females for the 420-day matings.

Vasectomies were performed under anaesthesia induced by pentobarbitone sodium by making a small incision in the lower abdominal wall anterior to the scrotal sacs and tying off each ductus deferens in two places. The section between the ties was then removed. Seminal vesicles were also tied and removed at this time. At 2 weeks after surgery, vaginal lavages were taken from females which had received intromissions and behavioural ejaculations from a vasectomized male. The absence of spermatozoa and coagulated seminal fluids was used to validate the surgical procedure.

Results

Littering success and mean litter sizes are summarized in Table 1. Wilcoxon 2-sample tests and Mann–Whitney paired tests were used to compare litter sizes, and χ^2 tests to compare littering success. Only differences of $\alpha < 0.05$ are reported. Since ejaculation frequency had no effect on littering success or litter sizes, Groups HI-LE and HI-HE were combined for statistical analysis (and are henceforth referred to as high-intromission females) and compared to Group LI-LE (low intromission).

Percentage of females littering

Littering success was significantly higher for females receiving more intromissions. This effect was statistically significant for nulliparous females mated once at 300 ($\chi^2_{(1)} = 30.95$, $P < 0.001$) and at 420 ($\chi^2_{(1)} = 35.42$, $P < 0.001$) days of age. The effect was also significant for repeatedly mated females at 180 ($\chi^2_{(1)} = 98.93$, $P < 0.001$), 300 ($\chi^2_{(1)} = 79.84$, $P < 0.001$) and 420 ($\chi^2_{(1)} = 12.90$, $P < 0.001$) days of age.

There was no significant effect of age on littering success for once-mated and repeatedly mated animals receiving a high number of intromissions. However, for females of both categories receiving a low number of intromissions, littering success declined consistently with increasing age. Littering success for low-intromission nulliparous females declined significantly between 180 and 300 days ($\chi^2_{(1)} = 11.55$, $P < 0.01$) and a similar (but only marginally significant) pattern was observed between 300 and 420 days of age ($\chi^2_{(1)} = 2.75$, $P < 0.1$). For low-intromission, repeatedly mated females, littering success declined significantly between 180 and 300 days ($\chi^2_{(1)} = 10.53$, $P < 0.01$) and between 300 and 420 days ($\chi^2_{(1)} = 44.27$, $P < 0.001$).

Table 1. The effects of number of copulations and amount of vaginocervical stimulation on pregnancy rate and litter size in hamsters in relation to age and parity

Group*	60 days				180 days				300 days				420 days			
	Females delivering litters		Litter size		Females delivering litters		Litter size		Females delivering litters		Litter size		Females delivering litters		Litter size	
	No.	%	Mean	s.e.	No.	%	Mean	s.e.	No.	%	Mean	s.e.	No.	%	Mean	s.e.
Mated once																
LI-LE	25	92	10.1	0.4	35	86	12.4	0.3	25	36	11.2	1.0	25	12	8.6	0.6
HI-LE	25	100	10.3	0.6	25	96	12.0	0.4	25	92	11.6	0.5	25	88	9.0	0.7
HI-HE	25	100	10.7	0.7	27	100	12.9	0.5	27	100	11.8	0.6	25	84	8.7	0.5
Mated repeatedly																
LI-LE	—	—	—	—	30	37	12.3	0.3	40	18	11.0	0.3	40	5	7.3	1.8
HI-LE	—	—	—	—	30	90	13.1	0.4	25	76	12.1	0.4	25	44	8.2	1.0
HI-HE	224	100	10.6	0.2	164	99	12.7	0.2	98	92	11.7	0.5	25	36	8.7	0.9

*Group LI-LE, 5 intromissions and 2 ejaculations; Group HI-LE, 5 intromissions and 2 ejaculations, plus mating to satiety with vasectomized male (~60 intromissions); Group HI-HE, mating to satiety with intact male (~52 intromissions and 14 ejaculations).

Comparison of the repeatedly-mated and once-mated females revealed only two significant differences. For low-intromission females mated at 180 days, littering success was significantly higher for the nulliparous than for the repeatedly-mated females ($\chi^2_{(1)} = 14.69$, $P < 0.001$). For high-intromission females mated at 420 days, littering success was also higher for the nulliparous females ($\chi^2_{(1)} = 22.69$, $P < 0.001$).

Except for one primiparous female mated at 300 days and two multiparous females mated at 420 days, all females which failed to deliver litters continued to display regular 4-day oestrous cycles.

Litter sizes

Treatment (i.e. the amount of vaginocervical stimuli or number of ejaculations) had no significant effect on litter size (Wilcoxon 2-sample tests). Similarly, for same age groups, litter sizes for females mated once and females mated repeatedly did not differ significantly (Wilcoxon 2-sample tests). However, for all females, litter size increased between 60 and 180 days of age, remained unchanged between 180 and 300 days, and decreased significantly at 420 days of age (Wilcoxon 2-sample tests for females mated once and Mann-Whitney paired tests for females mated repeatedly).

Discussion

This study has separated the relative effects of age and parity on fecundity and litter size. Differences in the percentage of females of different age and parity classes successfully delivering a litter depended entirely on the amount of vaginocervical stimulation (i.e. number of intromissions) received. Both with increasing age, beginning at 300 days and with increasing parity, beginning with the second litter (180 days of age), significantly more females receiving the higher number of intromissions delivered litters. Previous work has shown that females mating late in the receptive period also failed to deliver litters unless they received a high number of intromissions (Huck *et al.*, 1986). Therefore, littering success in the hamster (except for young females first entering the breeding population) is highly dependent upon an adequate level of vaginocervical stimulation. Such stimulation may be provided by a sperm-depleted male as a result of the long intromission pattern

which is highly successful for induction of a pseudopregnancy (if unaccompanied by ejaculations) (Huck & Lisk, 1985b).

Neither ejaculation frequency nor intromission frequency had an effect on litter size in the present study. This is in keeping with earlier studies indicating that two ejaculations provide sufficient spermatozoa to ensure maximal litter size (Huck & Lisk, 1985b). The increase in litter size observed between 60 and 180 days in all females is consistent with earlier findings regarding age/parity-related changes in litter size (Huck *et al.*, 1988). However, the lack of difference between 420-day old nulliparous and multiparous females appears at odds with the results of the earlier study. A likely explanation is that in the present study experimental females were allowed 120 days between matings whereas 50 days were allowed in the previous study. Thus, 120 days between matings may provide sufficient time for the female to regain her body reserves. Since nulliparous and multiparous females show a similar decrease in litter size at 420 days, this effect appears to be the result of age and indicates that these females are past their reproductive prime.

In the present study, nearly all of the females which did not deliver litters also did not show a cessation of oestrous cycles which characteristically follows the induction of a luteal phase. This finding indicates that the decreased fecundity observed in the ageing female or in the multiparous female probably does not result from changed physiology of the reproductive tract which interferes with fertilization or implantation. More likely the reduction in reproductive success of these classes of females results from a change in sensitivity to the stimulus conditions necessary to initiate the neuroendocrine arc for establishment of a successful pregnancy.

This research was supported by National Science Foundation Grant BNS-8607258.

References

- Dewsbury, D.A. (1978) The comparative method in studies of reproductive behavior. In *Sex and Behavior*, pp. 83–112. Eds T. E. McGill, D. A. Dewsbury & B. D. Sachs. Plenum Press, New York.
- Dewsbury, D.A. (1979) Copulatory behavior of deer mice (*Peromyscus maniculatus*). III. Effects of pregnancy initiation. *J. comp. Physiol. Psychol.* **93**, 178–188.
- Davis, H.N., Jr, Gray, G.D. & Dewsbury, D.A. (1977) Maternal age and male behavior in relation to successful reproduction by female rats (*Rattus norvegicus*). *J. comp. Physiol. Psychol.* **91**, 281–289.
- Gray, G.D., Kenney, A.M. & Dewsbury, D.A. (1977) Adaptive significance of the copulatory behavior pattern of male meadow voles (*Microtus pennsylvanicus*) in relation to the induction of ovulation and implantation in females. *J. comp. Physiol. Psychol.* **91**, 1308–1319.
- Huck, U.W. & Lisk, R.D. (1985a) Determinants of mating success in the golden hamster (*Mesocricetus auratus*). I. Male capacity. *J. comp. Psychol.* **99**, 98–107.
- Huck, U.W. & Lisk, R.D. (1985b) Determinants of mating success in the golden hamster (*Mesocricetus auratus*). II. Pregnancy initiation. *J. comp. Psychol.* **99**, 231–239.
- Huck, U.W., Lisk, R.D. & Thierjung, C. (1986) Stimulus requirements for pregnancy initiation in the golden hamster (*Mesocricetus auratus*) change with time of mating during the receptive period. *J. Reprod. Fert.* **76**, 449–458.
- Huck, U.W., Pratt, N.C., Labov, J.B. & Lisk, R.D. (1988) Effects of age and parity on litter size and offspring sex ratio in golden hamsters (*Mesocricetus auratus*). *J. Reprod. Fert.* **83**, 209–214.
- Orsini, M.W. (1961) The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation, and the anestrus hamster (*Mesocricetus auratus*). *Proc. Anim. Care Panel* **11n**, 193–206.

Received 11 February 1988