Effects of male accessory sex gland secretions on early embryonic development in the golden hamster

W. S. O, H. Q. Chen* and P. H. Chow

Anatomy Department, Faculty of Medicine, Hong Kong University, 5 Sassoon Road, Hong Kong

Summary. The ventral prostates, dorsolateral prostates, coagulating glands, seminal vesicles and/or ampullary glands were bilaterally removed from adult male hamsters. Removal of these glands did not affect the fertilization rate and cleavage of the embryos at 48 h post coitum (p.c.). Air-dried preparations of the embryos showed a delay in cleavage at 72 h p.c. and a significant number of degenerated embryos was also found in females mated with males from which all the male accessory sex glands had been removed. A significant implantation loss was also observed at 122 h p.c. The results suggest that, in the golden hamster, removal of the male accessory sex gland causes a slower cleavage rate in embryonic development and a significant embryonic loss during pregnancy.

Keywords: accessory sex glands; embryo; cleavage; implantation; hamster

Introduction

Male accessory sex gland secretions contribute the bulk of the ejaculate. These secretions are known to have a variety of actions on the gametes and the female reproductive tract (see Mann & Lukwak-Mann, 1981). Removal of some or all of the accessory sex glands has been shown to affect fertility in guinea-pigs (Lawlah, 1930), rats (Queen et al., 1981), mice (Pang et al., 1979) and golden hamsters (Chow et al., 1986).

In the golden hamster, total removal of the male accessory sex glands or ablation of the ventral prostate alone was reported to reduce female fertility (Chow et al., 1986), although it was not clear how this was brought about. Studies of sperm distribution in the female reproductive tract showed that only the males from which all the accessory sex glands had been removed deposited significantly fewer spermatozoa in the female tract, and fewer spermatozoa reached the oviduct at 1-5 h post coitum (p.c.) following such matings compared with the control group (P. H. Chow & W. S. O, unpublished data). In the present study, we have investigated fertilization, early embryonic development and implantation following matings with male from which various accessory sex glands had been removed.

Materials and Methods

All the golden hamsters (Mesocricetus auratus) used in the study were randomly bred and maintained in the Laboratory Animal Unit, Faculty of Medicine, University of Hong Kong, under 14 h light:10 h dark cycle (lights on 11:00-01:00 h). All female hamsters were checked daily for vaginal secretion for at least 2 consecutive normal cycles before mating.

Surgery. Various accessory sex glands were removed from 10-week-old male hamsters as described by Chow et al. (1986) to give the following four experimental groups: SH—sham-operated animals; AGX—bilateral excision of the

*Present address: Shanghai Institute of Planned Parenthood Research, Shanghai, People’s Republic of China.
ampullary glands; VPX—bilateral excision of the ventral prostate glands; TX—bilateral excision of the ampullary glands, ventral prostates, dorsolateral prostates, coagulating glands and seminal vesicles. The operated males were used for mating 4 weeks after surgery and the success of the operation was checked post mortem.

Embryonic cell counts. Each female hamster was mated with one operated male on the day of oestrus at 09:00 h for 15 min; 32 females were killed at 48 h p.c. and 56 females were killed at 72 h p.c. Embryos were flushed from oviducts and/or uterine horns using Eagles' Minimal Essential Medium and the numbers of corpora lutea in the ovaries were recorded. Embryonic cells were dispersed on a glass slide using the method described by Fujimoto et al. (1975) with a hypotonic treatment for 12 min for 48-h embryos and 16 min for 72-h embryos at room temperature. The slides were stained with Giemsa and the total number of cells was counted.

Embryo viability test. Embryos were recovered from 32 females at 48 h and from 32 females at 72 h after mating with the operated males. The embryos were tested for viability using the fluorescein diacetate (FDA) method (Mohr & Trounson, 1980). The FDA was used at a final concentration of 5 μg/ml TALP medium. The embryos were observed for fluorescence and the result recorded photomicrographically.

Implantation sites. Females (N = 56) mated with experimental males were anaesthetized at 122 h p.c. and injected via the inferior vena cava with 0.2 ml 0.5% pontamine blue solution in 0.9% (w/v) NaCl, and were killed by an overdose of pentobarbitone sodium 10 min after injection. The uterine horns were examined for implantation sites and the number of corpora lutea on the ovaries was recorded.

Statistics. The results from the four groups were compared with the Kruskal–Wallis test, and the Mann–Whitney test was used for comparison between groups.

Results

As shown in Table 1, there was no difference in cell numbers at 48 h p.c. between different groups of animals, but by 72 h p.c. embryos derived from males without ampullary glands (AGX) had significantly fewer cells than the control group (P < 0.01), an effect that was even more pronounced in the VPX and TX groups (P < 0.001).

Embryos with cells containing pyknotic nuclei in the air-dried preparations were classified as degenerating embryos. There was no significant difference in the proportion of degenerated embryos at 48 h p.c., but at 72 h p.c. there was a significantly greater number of degenerated embryos in the TX group (Table 2).

Table 1. The effects of excision of ampullary glands (AGX), ventral prostate glands (VPX), or all the accessory sex glands (TX) on the numbers of cells in preimplantation embryos in the golden hamster

<table>
<thead>
<tr>
<th>Time p.c.</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SH (controls)</td>
</tr>
<tr>
<td>48 h</td>
<td>No. of animals</td>
</tr>
<tr>
<td></td>
<td>No. of embryos</td>
</tr>
<tr>
<td></td>
<td>Mean cell no./embryo</td>
</tr>
<tr>
<td>72 h</td>
<td>No. of animals</td>
</tr>
<tr>
<td></td>
<td>No. of embryos</td>
</tr>
<tr>
<td></td>
<td>Mean cell no./embryo</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.
*P < 0.01, **P < 0.001 compared with Group SH.

The embryo viability test using the FDA method showed that 84.3–91.7% of the embryos at 48 h p.c. and 83.0–94.5% at 72 h p.c. showed fluochromasia, accumulation of bright green fluorescence in the cells (Rotman & Papermaster, 1966), and were therefore considered to be viable.

Downloaded from Bioscientifica.com at 12/27/2018 08:02:06AM via free access
embryos. Cells of viable embryos at different stages of cleavage did not show any difference in the ability to accumulate fluorescence in the cytoplasm.

No difference was observed in the fertilization efficiency, expressed as percentage of embryos recovered per corpus luteum, between the operated males at 48 h and 72 h p.c. However, the pontamine blue test conducted at 122 h p.c. showed that the implantation rates were significantly lower than in the control (SH) group (Table 2).

### Discussion

Our results show that removal of the male accessory sex glands does not affect the percentage of ova fertilized and the initial cleavage rate of embryos, but embryos from females mated with AGX, VPX and TX males had fewer cells at 72 h p.c. than did controls but they showed fluorochromasia in the embryo viability test. The slow division rate of the embryos may partly account for the low implantation rate as shown by the pontamine blue implantation test. It is known that timing of preimplantation embryonic development is crucial for successful implantation, and a slower rate of cleavage in preimplantation embryos is associated with greater implantation loss (Gates, 1965).

Genetic factors are also known to affect preimplantation embryonic development in the mouse by influencing the timing of activation of the ova and the formation of the pronuclei at fertilization (McLaren & Bowman, 1973) or at the rate of cleavage (Whitten & Dagg, 1962). In the mouse, the rate of preimplantation development is governed by genes within the H-2 complex, and modified by other genes (Warner et al., 1984). Both the maternal (Larsen & Generoso, 1984) and paternal genome (Mann & Lovell-Badge, 1987) are also known to influence the incidence of peri-implantation embryonic mortality. This is the first report of an effect of male accessory sex glands on cleavage and implantation loss. However, it is not clear how these glandular secretions could affect embryonic development.

Other factors such as the maternal environment and immunological factors also cause prenatal loss of embryos (for review, see Wilmut et al., 1986). Chaykin & Watson (1983) showed that mice inseminated with only 5% of the number of spermatozoa normally present in an ejaculate significantly reduced the yield of 2-cell embryos and the number of implantations. It was suggested that the sperm protein kinase may serve as an intermediary in the transmission of sperm-dependent stimuli that control preimplantation embryonic development (Watson et al., 1983). Bellinge et al. (1986) reported that insemination improved implantation in a human in-vitro fertilization and

### Table 2. The effects of excision of ampullary glands (AGX), ventral prostate (VPX) or all the accessory sex glands (TX) on the fertilization efficiency (embryo per CL), embryo degeneration (degenerating embryos per CL) and implantation rate (implantation sites per CL) in golden hamsters

<table>
<thead>
<tr>
<th>Time p.c.</th>
<th>Group</th>
<th>SH (controls)</th>
<th>AGX</th>
<th>VPX</th>
<th>TX</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h</td>
<td>No. of animals</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>% total embryos per CL</td>
<td>78.2 ± 4.2</td>
<td>77.9 ± 7.5</td>
<td>83.7 ± 7.0</td>
<td>76.8 ± 10.3</td>
</tr>
<tr>
<td></td>
<td>% degenerated embryos/CL</td>
<td>0.7 ± 0.7</td>
<td>2.9 ± 1.2</td>
<td>3.8 ± 1.7</td>
<td>6.5 ± 6.0</td>
</tr>
<tr>
<td>72 h</td>
<td>No. of animals</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>% total embryos per CL</td>
<td>75.0 ± 4.3</td>
<td>63.6 ± 3.4</td>
<td>75.0 ± 3.9</td>
<td>75.8 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>% degenerated embryos/CL</td>
<td>9.4 ± 4.5</td>
<td>22.8 ± 7.6</td>
<td>30.1 ± 8.9</td>
<td>46.9 ± 7.6*</td>
</tr>
<tr>
<td>122 h</td>
<td>No. of animals</td>
<td>9</td>
<td>18</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>% implantation sites/CL</td>
<td>68.2 ± 10.1</td>
<td>38.5 ± 11.0**</td>
<td>35.8 ± 13.8**</td>
<td>31.6 ± 11.2**</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.

*P < 0.01, **P < 0.001 compared with Group SH.
embryo transfer programme, while Carp et al. (1984) also showed it in rats. In hamsters, only females mated with males from which all the accessory sex glands had been removed (TX group) had significantly reduced numbers of spermatozoa in the reproductive tract (P. H. Chow & W. S. O, unpublished data). In the present study, a significantly greater number of degenerated embryos were found at 72 h p.c. and fewer embryos had implanted on Day 6 after mating. However, a reduced implantation rate was also found in the groups of females mated with APX and VPX males. The results lead us to believe that there are developmental mechanisms related to the effects of the accessory sex gland secretions, presumably via the spermatozoa and perhaps via maternal physiology.

We conclude from this study that male accessory sex gland secretions can affect the rate of cleavage of embryos and their implantation, although the mechanism involved remains a mystery.

We thank Miss May, P. L. Cheung and Mr U. L. Lui for assistance and Dr G. Shaw for his help in statistical analysis of the results. H.Q.C. was a Beijing–Hong Kong Academic Exchange Centre Fellow. The experiments were supported by Hong Kong University Grant No. 335/031/0013 and a Medical Faculty Research Grant from Hong Kong University No. 311/030/8040/39.

References


Received 16 February 1988