THE EFFECT OF CASTRATION AND TESTOSTERONE REPLACEMENT ON SPERM MATURATION IN THE HAMSTER

C. M. LUBICZ-NAWROCKI*

*Unit of Reproductive Biology, University of Liverpool,
Life Sciences Building, Liverpool L69 3BX*

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Summary. Ligation of the distal corpus epididymidis and ductus deferens for 20 days resulted in low fertilizing ability of spermatozoa isolated in the cauda epididymidis of intact hamsters and castrated animals treated daily with 100 μ g testosterone. Following ligation of the ductuli efferentes and vasectomy in intact hamsters, however, new spermatozoa accumulated in the cauda epididymidis and fertilizing ability was maintained at the control level after 20 days. In the testosterone-treated castrated hamsters, the resulting fertilizing ability was dose-dependent: spermatozoa continued to migrate through the epididymis after twenty daily injections of either 12.5 or 100 μ g testosterone but developed fertilizing ability only after treatment with the higher dose of testosterone. The results show that circulating testosterone is important for sperm maturation in the hamster.

INTRODUCTION

Immature spermatozoa undergo a series of morphological and physiological changes after leaving the testis and acquire the ability to fertilize during their passage through the epididymis (Young, 1931; Bedford, 1966; Orgebin-Crist, 1967). These changes are complete by the time spermatozoa reach the distal cauda epididymidis where they are stored but spermatozoa lose their viability after prolonged storage (Hammond & Asdell, 1926; Young, 1929; Tesh & Glover, 1969; Lubicz-Nawrocki & Glover, 1973).

The hormonal requirements for sperm maturation are not precisely known but it is likely that circulating testicular androgens are important since epididymal function is androgen-dependent (Risley, 1963; Martan, 1969). Recent observations show that castration prevents morphological changes in guinea-pig spermatozoa during epididymal transit (Blaquier, Cameo & Burgos, 1972) and that circulating testosterone is important for the development of fertilizing ability in rabbits and rats (Orgebin-Crist, Dyson & Davies, 1972). In order to evaluate the influence of circulating androgen on sperm maturation, the present

* Present address: Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Massachusetts 01545, U.S.A.

experiments were undertaken to examine the effects of castration and testosterone replacement on the development of fertilizing ability in hamsters.

MATERIALS AND METHODS

Adult (10 weeks old) male and female hamsters, weighing 100 to 120 g, were kept under a constant temperature (26°C) and light (15 hr) regimen and received a balanced diet (41B, Dixon & Sons, Ltd, Ware, Herts) and water.

In order to test the effect of castration and testosterone replacement on sperm maturation, twenty-five males were divided into five equal groups and treated as follows: (1) bilateral isolation of the cauda epididymidis—the distal corpus epididymidis and proximal ductus deferens were ligated, using sterilized braided silk sutures (size 5/0, Ethicon, Ltd); (2) bilateral castration, isolation of the cauda epididymidis on both sides and daily treatment with 100 µg testosterone; (3) bilateral vasectomy and bilateral ligation of the ductuli efferentes (efferentiectomy); (4) and (5) bilateral castration, bilateral vasectomy, bilateral efferentiectomy and daily treatment with 12.5 and 100 µg testosterone, respectively. Castration and bilateral isolation of the cauda epididymidis were performed under sodium pentobarbitone anaesthesia through a scrotal approach (Lubicz-Nawrocki & Glover, 1973) and the ductuli efferentes were ligated close to the testis to avoid disturbance to the arterial supply of the caput epididymidis. The animals in each group were killed after 20 days by cervical fracture and the fertilizing ability of spermatozoa pooled from the cauda epididymidis on both sides of each male was tested by the method of Lubicz-Nawrocki & Glover (1973). The cauda epididymidis was macerated in 4 ml sterile Hanks' solution and the material was filtered through sterile gauze; 0.2 ml of the sperm suspension (filtrate) containing 10 to 15 × 10⁶ spermatozoa was injected into each uterine horn of three oestrous females.

Eggs were flushed from the uterine tubes approximately 36 hr after ovulation (Ward, 1946; Strauss, 1956) and whole mounts were prepared (Chang, 1952); the eggs were examined under a phase-contrast microscope and classified as fertilized by the presence of a spermatozoon in the vitellus of a cleaved egg. The fertilizing ability of each male was assessed in terms of 'fertilization rate' as the percentage of fertilized eggs recovered from three females.

The percentage of live spermatozoa in each suspension was estimated from smears stained with aqueous nigrosin-eosin (Lubicz-Nawrocki & Glover, 1973) and the concentration of spermatozoa injected was determined from haemocytometer counts so that it was possible to estimate the number of live spermatozoa injected into each uterine horn. The number of spermatozoa in each cauda epididymidis 20 days after efferentiectomy and vasectomy (Groups 3, 4 and 5) was estimated from haemocytometer counts.

RESULTS

Table 1 shows that fertilizing ability was low 20 days after isolation of the cauda epididymidis in intact animals (Group 1) and in castrated animals treated daily with 100 µg testosterone (Group 2). By contrast, when the whole epididymis

Table 1. The effect of castration and treatment with testosterone on the fertilizing ability of hamster spermatozoa isolated for 20 days in the cauda epididymidis or following isolation of the whole epididymis

Animal group (n = 5)	Treatment	Mean no. of live sperm./uterine horn $\times 10^6$ ($\pm$ S.E.)	Mean no. of eggs recovered/3♀♀ (with ranges)	Mean no. of eggs fertilized/3♀♀	Mean % fertilization rate* ($\pm$ S.E.)
1	C.E. isolated	2.5 (1.3)	39 (31 to 47)	10.2	23.0 (12.1)
2	Castration + C.E. isolated + 100 μ g T/day	1.6 (0.5)	42 (32 to 50)	8.4	20.0 (6.6)
3	Vasectomy + efferentiectomy	3.5 (0.8)	34 (28 to 44)	31.0	91.1 (6.2)
4	Castration + vasectomy + efferentiectomy + 12.5 μ g T/day	2.1 (0.8)	36 (33 to 39)	0	0 (0)
5	Castration + vasectomy + efferentiectomy + 100 μ g T/day	2.6 (0.5)	42 (30 to 39)	40.0	95.2 (4.7)

C.E. = cauda epididymidis; T = testosterone.

* Mean fertilization rate: average of five 'fertilization rates' obtained by estimating the fertilizing ability of each male.

was isolated from the rest of the reproductive system in intact animals (following efferentiectomy and vasectomy, Group 3), the sperm content of the cauda epididymidis increased after 20 days (Table 2) and fertilizing ability was maintained at the control level (Table 1).

It may be seen from Table 1 that the effect of testosterone on the development of the fertilizing ability in castrated hamsters was dose-dependent. Following castration, ligation of the ductuli efferentes and vasectomy, twenty daily consecutive injections of 12.5 μ g testosterone maintained the passage of spermatozoa through the epididymis and prevented sperm death but the spermatozoa were infertile. At the higher dose (100 μ g), however, fertilizing ability was high after 20 days (Table 1) and comparable to that found following vasectomy and efferentiectomy in intact animals (Group 3).

Table 2. The effect of castration and replacement with testosterone on the number of hamster spermatozoa in the cauda epididymidis 20 days after vasectomy and efferentiectomy

Animal group (n = 5)	Treatment	Mean no. of sperm. $\times 10^6$ in right and left cauda epididymidis ($\pm$ S.E.)
Untreated controls	None	245.4 (9.9)
3	Vasectomy + efferentiectomy	410.6 (12.2)
4	Castration + vasectomy + efferentiectomy + 12.5 μ g T/day	401.2 (8.6)
5	Castration + vasectomy + efferentiectomy + 100 μ g T/day	418.8 (15.6)

T = testosterone.

Table 1 shows that there was no relation between the mean number of live spermatozoa injected and fertilizing ability so that the low fertilizing ability of spermatozoa from animals in Groups 1 and 2 and infertility in Group 4 was not due to sperm death.

DISCUSSION

This study shows that the fertilizing ability of spermatozoa isolated in the cauda epididymidis is low after 20 days in intact hamsters and in castrated animals treated daily with 100 μg testosterone, but remains high if new spermatozoa enter the cauda epididymidis. The resulting high fertilizing ability is a reflection of the survival of spermatozoa already present at the time of ligation and maturation and survival of the new spermatozoa, since most spermatozoa in the hamster caput and corpus epididymidis are infertile (Horan & Bedford, 1972).

Maintenance of the fertilizing ability of mature hamster spermatozoa has been shown to be androgen-dependent (Lubicz-Nawrocki & Glover, 1973) and exogenous testosterone prevents loss of fertilizing ability of mature spermatozoa in castrated rats and rabbits (Orgebin-Crist *et al.*, 1972). The present study shows that spermatozoa migrate through the epididymis in castrated hamsters receiving 12.5 or 100 μg testosterone daily but that spermatozoa develop fertilizing ability only during treatment with the higher dose of testosterone. These results confirm recent observations showing that, in rats and rabbits, castration impairs sperm maturation but that exogenous testosterone maintains normal development of fertilizing ability (Orgebin-Crist *et al.*, 1972).

It is still not clear to what extent the epididymal secretions contribute to sperm maturation and to what extent the process is simply due to inherent temporal changes within the spermatozoa. The many different testosterone-dependent enzyme activities in the epididymis (Martan, 1969) suggest that circulating testosterone supports epididymal function which thereby maintains optimal conditions for the development of fertilizing ability. In rats injected with tritiated testosterone, the poor labelling of epididymal spermatozoa compared to epididymal tissue (Blaquier, 1971) supports the possibility of an indirect effect of testosterone on sperm maturation. However, testosterone is metabolized in the epididymis to 5 α -dihydrotestosterone and 3 α -androstenediol (Gloyne & Wilson, 1969; Inano, Machino & Tamaoki, 1969; Blaquier, 1971; Aafjes & Vreeburg, 1972) so it is possible that small amounts of testosterone metabolites may enter the lumen of the duct and have a direct effect on the spermatozoa. Experiments are in progress to test this possibility.

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