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THE SHORT TERM EFFECT OF LIGATION ON THE VAS DEFERENS ON DISTRIBUTION, MOTILITY AND MORPHOLOGY OF SPERMATOZOA IN THE RAT, MOUSE, GUINEA-PIG AND GERBIL

by

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SUMMARY

The immediate effect of unilateral ligation of the vas deferens on the morphology and motility of speramatozoa in the cauda epididymis and vas was investigated in adult rats (Wistar, Sprague-Dawly, and Hooded strains), gerbil, guinea-pig and mice.

Three days following the ligation of the vas deferens decapitated sperms were observed in the vas of rats and gerbils but not in guinea-pig and mice. However, in the mice decapitation occurred three months later. The motility, on the other hand, was reduced in all the species.

In the epididymis, the appearance of the sperm was normal three days after ligation in all the species investigated. However, the motility was reduced except in the guinea-pig. It is suggested that alteration in blood supply and occlusion of the lumen of vas are responsible for the observed phenomena.

INTRODUCTION

Changes in the distribution of sperm in the excurrent ductular system have been reported following local application of sympathomimetic drugs to the vasa deferentia of rats using a slow-releasing formulation made from medical grade silastic in the form of collars (Ratnasooriya *et al.*, 1979). Therefore, it seemed of interest to know the effect of vas ligation on the distribution of sperm in the genital tract, since some of the effects of collars might have been caused by the blockage of the vas by an ingrowth of fibrous material.

At the same time it was decided to study the effects of ligation of the vas on sperm morphology and motility in several species of rodents, since this procedure is known to cause decapitation of sperm in the vas of Wistar rats (Kuwahara and Frick, 1975).

METHODS

Animals

In this study 15 rats (Wistar-strain : 9, Sprague-Dawly strain : 3, Hooded strain : 3), 3 guinea-pigs, 3 gerbils and 6 mice were used. All of them were sexually mature males and were housed under constant temperature (29 $\pm$ 1 ^{0}C) with free access to food (Diet 41B, Millers) and water.

Vas ligation

The animals were operated upon under light ether anaesthesia using aseptic precautions. The lower abdominal area was cleaned with Hibitane solution. A 2-3 cm mid-line incision was made in the lower abdomen with the animals lying in a supine posture. The right vas deferens was exposed at the prostatic end. Care was taken not to withdraw the testis into the abdominal cavity. A double ligation using 5/0 silk sutures was made at the prostatic end of the vas deferens. The distance between the two ligatures was approximately 10 mm except in the mice where it was about 2-3 mm. The vas was then returned to its natural position in the abdominal cavity. The incision in the abdominal wall was closed with a 2/0 silk suture and sprayed with an antibiotic mixture which contained bacitracin, neomycin and polymyxin (Polybactrin, Calmic Ltd.). The incision in the skin was closed with 12 mm Michel wound clips. In the case of mice the skin incision was closed with fine steel wire. The wound was sprayed with a transparent plastic dressing (Nobecutane, B.D.H. Ltd.) and the animals allowed to regain consciousness.

Sperm counts in the male tract

Sperm counts were made in 6 Wistar rats, 3 days following unilateral vas ligation. The animals were anaesthetized with ether and the excurrent ducts from each side were removed and cleared of adhering tissue and the animals then killed. The tract was divided into vas, caput, corpus and cauda epididymis and sperm content in each of these regions was measured as described elsewhere (Ratnasooriya *et al.*, 1979). The counts were made in duplicate and the average of the two determinations was used. The results were expressed as sperm content (X 10^6).

Assessment of motility and morphology

The rest of the animals were also anaesthetized 3 days or 90 days (only mice) following vas ligation. The vas and the epididymides were examined for any obvious distensions or granulomas. The sperms in the vas and the cauda were obtained by macerating them separately on a small petri dish containing 1 ml warm saline (35–37^{0}C). A drop of the resulting suspension

was placed on a clean microscope slide and mounted with a cover glass. The spermatozoa were then assessed for motility under the microscope, using a motility scale of 0–4 and the estimates were recorded to the nearest whole number. A score of 4 indicates maximum motility (where the microsample became a vigorously swirling mass of cells) and score of O indicates complete absence of any type of movement of spermatozoa. An estimate of the percentage of decapitated sperms were made immediately after the assessment of motility.

Statistics

The differences in distribution of sperms in the treated and control sides of the vas deferens of the experimental animals were analysed using the Wilcoxon signed rank, non parametric test (Seigel, 1956) and evaluated as statistically different at the $P<0.05$ level.

RESULTS

The distribution of spermatozoa in different regions of the genital tract of 6 Wistarrats, 3 days following unilateral vas ligation is summarized in Table 1. No significant change in sperm distribution was evident between the ligated and control side.

Table 2 shows the appearance and the motility of the spermatozoa in the vas and cauda epididymis of 4 species following unilateral vas ligation. In the cauda, the morphology of the sperm appeared normal 3 days following vas ligation in all strains of rats and in the other species investigated. However, in the mouse 58% of the sperm were decapitated 3 months later. The motility on the other hand, was reduced in the mouse and gerbil and to a lesser extent in the rat, but was normal in the guinera-pig.

In the vas, varying proportions of spermatozoa were decapitated in the 3 strains of rats and in the gerbil. However, the sperm were normal in appearance in the guinea-pig and in the mouse. However, 3 months later a larger porportion of headless sperms were found in the mouse. A complete lack of motility was seen in Wistar rat (3 days) and in the mouse (3 months) while in all the other groups there was a marked reduction in motility.

No obvious distension or granulomas were evident either in the vas or in the epididymis in any of the animals investigated, indicating that there is no massive accumulation of spermatozoa.

DISCUSSION

A redistribution of sperm is evident in the vas deferens and in epididymis of Wistar rats, 3 days following local application of sympathomimetic drugs to the vas using a slow-releasing formulation (Ratnasooriya *et al.*, 1979).

It has been suggested that such a distribution could result either from a mechanical block as with methoxamine or by a sustained spasm of vas under the collar as in the case of tyramine (Ratnasooriya *et al.*, 1979). However, the present experiment suggests that this alone could not account for the effect on sperm distribution seen with collars, since no significant change in the sperm distribution was seen following vas ligation for the same length of time. Ligation was found to cause decapitation of sperm in the vas of rats (in agreement with Kuwahara and Frick, 1975) and in other species except the guinea-pig within 3 days. Decapitation with sympathomimetic treatment was much less, indicating that effects of collars cannot be entirely explained by a blockage of the vas deferens.

Another effect seen with ligation was a reduction in motility of the spermatozoa in the vas but not in the cauda epididymis of all the species tested except in the guinea-pig. In contrast, sympathomimetics caused total immotility in the sperms of the cauda, again indicating that occlusion of the vas plays at most a small part in the effect of sympathomimetic-containing collars.

Some degree of damage to sperm in the vas was observed in all the species. The exact mechanism however, for such a damage *in vivo* is still unknown. With the obstruction of vas, accumulation of sperm could take place which might change factors like pH, osmotic pressure, ionic balances or gas tensions, which would affect the physiology of sperm (see Mann, 1964). However, this seems an unlikely explanation since there was no significant rise in sperm numbers in rat following 3 days vas ligation. Alternatively, diminished blood supply to the vas due to the occlusion of the vasal artery could be responsible for the decapitation. A change in the blood supply would bring about noteworthy alterations in the vasal physiology resulting perhaps in a hostile environment for the sperms stored in the vas deferens. On the other hand, maintenance of normal integrity of epididymal sperm reported here and by Kuwahara *et al.* (1975) may have resulted by minimal alterations in blood supply to it since epididymis of mammals receives a dual blood supply (Gunn and Gould, 1970).

TABLE 1

Effect of unilateral vas ligation on the sperm distribution in the genital tract of male rats. The sperm numbers in different regions of the ligated side were compared with those of unligated side. Each result is the mean ± s.e.mean from 6 rats

	Total sperm number (millions)	
	Control (left vas deferens)	*Ligated (right vas deferens)*
Caput epidiymis	102 ± 15	96 ± 22
Corpus epididymis	19 ± 2	15 ± 3
Cauda epididymis	249 ± 28	272 ± 39
Whole epididymis	370 ± 38	382 ± 60
Vas deferens	22 ± 6	24 ± 9

TABLE 2

Effect of unilateral vas ligation on the morphology and motility of spermatozoa in the vas and cauda epididymis of rat, gerbil, guinea pig and mouse. Quantitative results are expressed as mean ± S.E. mean

Species/strain	Cauda epididymis				vas deferens			
	Morphology % decapitated		Motility Score		Morphology % decapitated		Motility Score	
	control	ligated	control	ligated	control	ligated	control	ligated
Rat								
Wistar strain	normal	normal	4.0 ± 0	3.3 ± 0.6	normal	75 ± 25	4.0 ± 0	0 ± 0
Sprague-Dawly	normal	normal	4.0 ± 0	3.6 ± 0.3	normal	10 ± 10	4.0 ± 0	2.3 ± 0.3
Hooded	normal	normal	3.6 ± 0.3	2.3 ± 0.8	normal	20 ± 5	3.6 ± 0.3	1.6 ± 0.3
Gerbil	normal	normal	4.0 ± 0	2.0 ± 0	normal	8.3 ± 8.3	4.0 ± 0	2.0 ± 0.5
Gu nea-pig	normal	normal	4.0 ± 0	4.0 ± 0	normal	normal	4.0 ± 0	1.6 ± 0.3
Mice	normal	normal	4.0 ± 0	1.6 ± 0.3	normal	normal	3.6 ± 0.3	1.6 ± 0.3
Mice (3 months)	normal	58 ± 8.3	4.0 ± 0	0.3 ± 0.3	normal	91 ± 8.3	4.0 ± 0	0 ± 0

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