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Determination of Sperm Transfer Time and Retention times of different Regions of Hamster Epididymis

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ABSTRACT

Most of the scientific researches deal with the epididymal sperm maturation but not with the storage of sperms in epididymis. The present study was carried out to determine sperm transfer time, sperm retention time and sperm motility in different regions of hamster epididymis after placing ligations. Ligations were made at the initial segment of the epididymes. The total number of sperm was assessed using the haemocytometer counting method and sperm counts were taken on defined time intervals starting from the 3rd day to the 78th day of post-ligation. Total sperm count was decreased 50% by 3 days in caput and corpus regions and by 15 days in cauda region. Yet, there were a few numbers of sperm in all regions of hamster epididymis even after 78 days of post-ligation. By 15 days, sperm motility was decreased rapidlyin all epididymal regions and the majority of sperms were immotile by the 24th day of post-ligation. Both sperm counts; immotile and motile sperms in control side was significantly different compared to that of the ligated side of the epididymis ($p< 0.05$). Sperm emptying time was approximately 18 days in the caput alone, 14 days in the corpus and 46 days in the cauda. It is concluded that in the ligated hamster epididymis sperm transfer takes more than 78 days. The findings of the present study will be vital for future studies on mechanisms of sperm transport and sperm storage in the cauda epididymis in detail.

Keywords*:* Hamster, epididymal sperm, transfer time, retention times, sperm motility

The epididymis has a major role in mammalian reproduction, ensuring sperm concentration, maturation, transport and storage (Bedford, 1975; Brooks, 1983; Cooper, 1986). The proximal regions are mainly devoted to sperm maturation (Temple-Smith *et al.*, 1998), while in the distal region, termed cauda, sperm storage takes place (Foldesy and Bedford, 1982). It is controlled by several factors (Kirchhoff, 1999). The best known is androgens; i.e. testosterone and its metabolite, dihydrotestosterone, provided directly from the rete testis and from the systemic circulation (Danzo *et al.*, 1977; Danzo and Eller, 1979). During

epididymal passage, developing sperm undergoes a maturational process that enables them to acquire the ability to move in a forward direction to fertilize ova (Cooper, 1998; Jones, 1999).

Epididymal sperm transit takes a varying amount of time depending on the species and it is approximately 8 - 15 days (Orgebin-crist, 1965; Bedford, 1975; Robb *et al.*, 1978). In human it may be as short as 2 - 3 days and in hamster it is about 8 - 10 days. During their transit through the epididymis, membrane proteins are remodeled, and this remodeling is critical in the acquisition of sperm motility for spermatozoa to be fully fertile (Cooper, 1986; 1998; Sullivan, 1999). In most species, the maturation process is completed when spermatozoa reached the proximal cauda epididymis (Vreeburg *et al*., 1992; Tulsiani *et al*., 1993; Turner, 1995).

Nevertheless, we consider that there is a long lag of research work on epididymal sperm transfer since the very first experiments (Bedford, 1967). Therefore, the main aim of the present study was to initiate the investigation on sperm storage/ sperm emptying times in the hamster epididymis using the epididymal ligation technique. Sperm passage through the epididymis, i.e. from proximal parts to the distal parts was not blocked since the main aim of this study was to investigate sperm emptying times.

MATERIALS AND METHODS

The investigation was carried out in the Department of Zoology, University of Ruhuna, Matara using common hamsters, *Cricetus cricetus* (age 13 - 14 weeks: weight 135g $\pm$ 15) which were acclimatized in animal house conditions (Temperature 28 $\pm$ 2 °C; RH 90 $\pm$ 10). Animals were selected randomly and used for ligation experiments.

Under Ether anesthesia, left side of the testicular sac of the hamster was opened and extreme proximal part of the epididymis was cleared. Once the junction between the initial segment and caput was cleared, a silk thread (0.25mm) was passed through it and two knots were placed to ensure blockage of sperm from rete -testis to the epididymis and testicular sac was closed. The right epididymis was left unaltered. Tetracycline hydrochloride ointment was applied at the surgical area and 0.1 ml of Oxytetracycline injection was given to experimental animals. These animals were kept for further investigations. On days 3, 6, 12, 15, 18, 24, 28, 32, 40, 48, 56, 62, 66, 72 and 78, animals from each group (n = 4) were used for epididymal sperm assessment both in the left and the right epididymes.

On the selected days, epididymes were removed from hamsters and thoroughly wiped to remove any blood stain. Both left and right epididymes were used for sperm motility assessment. For sperm motility assessment, different regions of the

epididymis were identified and separated. Epididymal tissue of the selected regions was minced thoroughly and diluted in BWW medium (Biggers *et al*., 1971) and incubated at 37 °C. A drop of sperm preparation (20µl) was used for assessment of sperms. Spermatozoa were scored according to the WHO laboratory Manual (World Health Organization, 1999) as follows: Immotile, twitching, slow moving/ slow motile and highly motile.

The total number of sperm was assessed using the haemocytometer counting method. Results were statistically analyzed by using SPSS 10 for windows. Means were compared by ANOVA.

RESULTS AND DISCUSSION

The present study was carried out to investigate sperm emptying times of hamster epididymis. In order to attain this, sperms entry to the epididymis from the initial segment of the epididymis was blocked by way of ligation. It was believed that blocking the sperm at the initial segment would stop sperm entering to the epididymis. Several investigators have used the same method (Bedford, 1967; Horan and Bedford, 1972).

Total number of sperms (total/ml) in the different ligated epididymal regions and controls are given in table 1. Both sperm counts; immotile and motile sperms in control side was significantly different compared to that of the ligated side of the epididymis ($p< 0.05$).

In the control side of the caput region I, there were 11 x $10^6 \pm$ 3414 sperms. On the 3rd day of post-ligation, the total number of sperm was reduced to 6 x $10^3 \pm$ 2.5. It was gradually increased; on day 6 it was 316 x $10^3 \pm$ 78 and on day 12 it was 366 x $10^3 \pm$ 348. With slight variations the total number of sperms in caput I started to decline from day 15 and there was a 15 fifteen-fold reduction observed in day 72 (table 1).

Similarly, in caput II, a reduction of sperm number started from day 3 (6.44 x $10^3 \pm$4.8) and 95% reduction was observed on 72 day of post-ligation. Majority of the sperm decreased (below half = 70%) by day 6. The reduction of sperm number in caput II region was quite rapid than that of caput I.

As sperm concentration reduced from 50.03 x $10^3 \pm$ 35 on day 3 to 0.75 x $10^3 \pm$ 0.7 on day 15, in caput III it could be stated that the sperm concentration in caput III dropped rapidly and faster than those of in caput I and caput II. In caput III by 24th day of post-ligation, sperm concentration was zero. Overall data shows that the reduction of sperm number in each caput I, II and III regions were extremely high compared to the control data (Table 1).

In the proximal corpus, sperm reduction was highly prominent on day 18 and it was continued until 78th day of post-ligation. In the distal corpus region, reduction

Table 1: Summarized data for total sperm motility and immotility recovered from the different regions of the epididymis of hamster in different days of post-ligation. Values are sperm concentration x 10^3/ml. Control data were for the non-ligated side of the epididymis from all the ligated animals and has been given as an average value. Caput region comprised three basic regions defined as Caput I, Caput II and Caput III, P. Corpus: Proximal Corpus, D. Corpus: Distal Corpus, P. Cauda: Proximal Cauda and D. Cauda: Distal Cauda.

Days Post ligation		Caput I	Caput II	Caput III	P. Copus	D. Copus	P. Cauda	D. Cauda
3	Immotile	5.7 ± 2	118.6±116	50.0 ± 34	56.4 ± 25	234.5 ± 67	12012.7 ± 6295	54225.5 ± 25539
	Motile	0.0 ± 0	0.0 ± 0	1.4 ± 1	0.7 ± 0.7	11.4 ± 11	1499.5 ± 1033	8447.7 ±5400
6	Immotile	313.8 ± 78	15.6 ± 4	13.7 ± 7	518.0 ± 439	1567.2 ± 1044	25237.2 ± 19761	27780.7 ± 12008
	Motile	2.2 ± 2	7.1 ± 7	6.4 ± 6	18.5 ± 14	27.2 ± 27	126.6 ± 64	864.7 ± 813
12	Immotile	340.5±322	63.0 ± 55	128.0 ± 125	268.7 ± 225	88.0 ± 30	1405.5 ± 611	24712.5 ± 19091
	Motile	25.7 ± 25	0.0 ± 0	0.0 ±0	0.0 ± 0	0.0 ± 0	41.0 ± 17	597.0 ± 474
15	Immotile	45.2 ± 9	45.7 ± 43	0.7 ± 0.7	1.5 ± 1	452.0 ± 452	1314.7 ± 868	18011.0 ± 5702
	Motile	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ± 0	5.7 ± 5	157.5 ± 156	657.7 ± 165
18	Immotile	23.2 ± 16	16.5 ± 16	4.2 ± 4	15.0 ± 12	37.5 ± 10	650.0 ± 227	4865.5 ± 3414
	Motile	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ±0	1.5 ± 1	32.2 ± 15	472.7 ± 248
24	Immotile	93.0 ± 38	3.0 ± 1	0.0 ± 0	0.0 ± 0	44.5 ± 27	2078.0 ± 1446	11428.0 ± 6010
	Motile	5.0 ± 5	0.0 ±0	0.0 ± 0	0.0 ± 0	0.0 ± 0	109.5 ± 49	776.0 ± 476
28	Immotile	186.0 ±161	8.5 ± 5	0.7 ± 0.7	12.2 ± 12	15.0 ± 11	123.2 ± 89	2580.0 ± 1804
	Motile	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ±0	0.7 ± 0.7	218.7 ± 210
32	Immotile	27.2 ± 15	8.0 ± 7	0.7 ± 0.7	0.7 ± 0.7	1.5 ± 1	312. 5 ± 218	11933.0 ± 2797
	Motile	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ± 0	20.0 ± 7	758.2 ± 207
40	Immotile	27.2 ±15	3.5 ± 1	6.5 ± 5	2.2 ± 2	16.0 ± 11	159.0 ± 75	4435.2 ± 3672

	Motile	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ±0	0.0 ± 0	0.0 ± 0	0.0 ±0
48	Immotile	0.7 ± 0.7	0.0 ± 0	2.1 ± 1	6.4 ± 4	0.0 ± 0	150.1 ± 98	837.9 ± 647
	Motile	0.0 ± 0	0. 0 ±0	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ± 0	0.0 ± 0
56	Immotile	4.2 ± 1	0.7 ± 0.7	0.0 ±0	137.2 ± 135	2.1 ± 2	27.1 ± 27	417.5 ± 298
	Motile	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0
62	Immotile	0.0 ±0	0.0±0	0.0 ± 0	11.4 ± 7	2.8 ± 2	7.1 ± 3	255.2 ± 112
	Motile	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ±0
66	Immotile	2.8 ± 0	2.1±1	0.0 ± 0	0.0 ± 0	0.0 ± 0	4.2 ± 4	143.7 ± 141
	Motile	0.0 ±0	0.0 ±0	0.0 ± 0	0.0 ± 0	0.0 ± 0	0.0 ±0	0.0 ±0
72	Immotile	2.1± 2	6.4 ± 2	0.7± 0.7	2.8 ± 2	0.0 ± 0	10.0 ± 6	84.0 ± 45
	Motile	0.0 ±0	0.0 ±0	0.0 ± 0	0.0 ±0	0.0 ± 0	0.0 ±0	0.0 ±0
78	Immotile	10.7 ±3	22.8 ± 21	8.5± 7	2.8 ± 2	6.4 ± 6	30.7 ± 25	223.6 ± 213
	Motile	0.0 ±0	0.0 ±0	0.0 ±0	0.0 ±0	0.7 ± 0.7	0.7 ± 0.7	0.7 ± 0.7
Control	Immotile	11005.5 ± 3414	11460 ± 5028	8911.2 ± 2330	9310.2 ± 3703	9301.0 ± 4722	90416.0 ± 30792	211753.0 ± 80736
	Motile	0.0 ± 0	57.2 ± 50	14.2 ± 14	50.2 ± 41	329.0 ± 283	2634.7 ± 685	9181.2 ± 4670

*Values are given as mean ±SEM

of sperm number was marked by 32 days of post-ligation and continued until day 78 (Table 1). In proximal and distal cauda, a large number of sperm could be seen. Similar to other regions sperm number decreased rapidly in the cauda regions and it was highly significant ($p< 0.05$) from the control data. In the proximal cauda rapid decrease of sperms started from day 12 and in the distal cauda, it started by day 24. According to the finding of this experiment, complete emptying could not be seen in the cauda regions and a large number of sperm could be seen even after 78 days of post-ligation in the distal cauda.

Results showed that by day 15, sperm count reduced to extremely low levels in all epididymal regions. Though there were indications on presence of sperm in all regions even after day 78 the number of sperm was extremely low. The complete transfer of sperm in any of the regions was not observed in 78 days after ligation.

Investigations indicated that blockage of sperm at the junction between the initial segment and caput region significantly reduced not only the sperm number but also the sperm motility. This was proved by the absence of fast moving sperms. In the caput region, when taken as a whole, sperm motility was almost 0 by day 15 (table 1). According to the present study, sperm motility retained 15 days even after initial segment blockage and this indicates long lasting independent epididymal functions.

In corpus region motile sperms were not observed after the 18th day. Observations on sperm motility were very much in parallel with earlier studies (Cooper, 1986; Turner, 1995; Cooper, 1998). When blocked and artificially retained in the distal corpus, epididymal sperms showed higher motility without entering to the cauda region. However, when sperms retained longer time in the corpus region that would reduced the sperm motility rapidly.

In cauda region, sperm motility was retained until day 24 in the proximal cauda and until day 32 in the distal cauda. As normal sperm motility patterns in animals, highest number of motile sperms was observed in the cauda region of the control side while there were less numbers of highly motile sperm in all regions of the epididymis, in the ligated side.

Sperm emptying times in the ligated epididymis indicated that sperms took 18 days to leave the caput region completely. In the Corpus region, this was 14 days. In contrast, sperm retained in cauda for 46 days and even on day 78 of post-ligation, at the time of termination of the experiment small number of sperms was present in it (Table 1).

Our findings showed that sperm transit time of proximal segments was quite fast while that of distal regions were comparatively very slow. This might due to the morphology of caput and corpus regions as slender, tube-like proximal corpus region has shorter sperm emptying time than the much wider distal corpus region.

Longer retention time in the distal regions shows that distal parts of the epididymis are important for sperm retention and storage. This was mainly due to large storage capacities in the cauda region of the epididymis compared to the other regions.

In the present study, although sperm transfer was blocked from the testis no action was taken to reduce or block androgen influence. In fact the right testis was left unaltered and smooth flow of androgen from the right testis to the blood system was unhindered. Most probably the left testis might have had some influence due to sperm movement blockage from the seminiferous epithelium.

Finally, sperm transfer time in hamster epididymis was more than 78 days due to the ligation at the junction between the initial segment and caput epididymis. Sperm motility and normal sperm number decreased after the ligation. This reduction was significant in later days of post-ligation. The present study will be provided vital baseline information to study mechanisms of sperm transport and sperm storage in the cauda epididymis in detail in the future.

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