

## Abstract

Most of the scientific researches, regarding the epididymal functions of epididymis deal with epididymal sperm maturation but not sperm storage. Therefore, the present experiments were carried out to determine sperm transfer time and sperm retention times of different regions of hamster epididymis, sperm storage time in the cauda epididymis and functional ability of cauda sperm after placing ligations. To study the sperm transfer times and retention times in different regions of the hamster epididymis, the initial segment was blocked by placing a ligation at the junction between the initial segment and the caput region. The study was focused on sperm motility, total sperm count, sperm morphology and sperm emptying times and sperm transfer times in different regions in different post ligation days up to 78 days. Sperm emptying time was approximately 18 days in the caput alone, 14 days in the corpus region and 46 days in the cauda. By 15 days, sperm motility was decreased rapidly in all regions of the epididymis and the majority of sperms were immotile by the 24<sup>th</sup> day of post ligation. Higher numbers of abnormal sperm were seen from day 36 to day 78 post ligation. According to histological studies, epididymal epithelial structure showed marked alterations compared to the control. Epithelium of the epididymis became stratified and made clusters after 12 days of post ligation in proximal regions of the epididymis and the cauda epithelium became more flattened.

Ligation of epididymal tubule at the junction between the distal corpus and the proximal cauda was carried out to determine the storage time; the hamster sperms are able to stay viable in cauda epididymis. Motility, viability and morphology of cauda sperm were also studied during the storage time. On day 40, the total number of cauda sperms was reduced remarkably ( $5 \times 10^6 \pm 2227/\text{ml}$ ). That amount is more than fifty times reduction when compared to that of day 3.

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In the experimental groups and control, 3% to 6% of sperm motility was maintained until day 40. Even by 40 days post ligation,  $11.6 \pm 4.2\%$  of live spermatozoa were found (Dead spermatozoa,  $88.3\% \pm 19.8$ ). When the sperm morphology of cauda was considered the normal sperm percentage was about 24% in treatment groups and it varied significantly (**P < 0.001**). By the day 32 post ligation, 76% of cauda spermatozoa were abnormal with head defects, mid piece and neck defects, tail defects, headless, tailless and multiple defects.

Fertilization ability of hamster sperm after cauda ligation, was investigated using *in vitro* fertilization technique. For *in vitro* fertilization, the sperm concentration for insemination was kept between  $5 \times 10^5$  and  $1 \times 10^6$  sperms/ml per 20 - 30 eggs. Even though high percentage of oocytes was fertilized in the control group (90% - 100%), it was found that the fertilizing ability of spermatozoa was decreased to 70% in 24 day of post ligation.  $\text{Ca}^{2+}$  ionophore (A23187) was used to induce capacitation and acrosome reaction of hamster sperm in different post ligation days and the Chlortetracycline (CTC) assay was used to assess the capacitation and acrosome reaction of the induced hamster spermatozoa. The CTC results revealed that  $\text{Ca}^{2+}$  ionophore A 23187 was effective to promote sperm capacitation and acrosome reaction even at 48 days after ligation of hamster cauda epididymis.