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In vitro Study of the Spermatozoa Motility in the Lizard *Eutropis carinata*

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ABSTRACT

Reptilian epididymis is considered as an important excurrent duct system required for the sperm maturation. Reptilian epididymis synthesizes and secretes proteins which vary in different regions of the epididymis. Hence, to investigate the effect of the secretions of different regions of the epididymis on spermatozoa motility, an *in vitro* study was undertaken to observe the changes in the patterns of motility of the testicular spermatozoa incubated with luminal contents of different regions of the epididymis in the lizard *Eutropis carinata* for the first time. The non motile testicular spermatozoa from the testis exhibited different patterns of motility, when incubated with the luminal contents of different regions of the epididymis. The spermatozoa from the testis, different regions of the epididymis exhibited 8 different patterns of motility (a-h). Testicular spermatozoa incubated with the anterior and middle epididymal luminal contents showed the motility patterns almost similar to that of the spermatozoa of the anterior and middle epididymis respectively. In contrast to the spermatozoa of the posterior epididymis, none of the testicular spermatozoa showed any movement when incubated with the posterior epididymal luminal contents. This study throws light on the importance of each region of epididymis in the physiological maturation of spermatozoa.

Key words: *Eutropis carinata*, spermatozoa, lizards, motility, epididymis

INTRODUCTION

In reptiles testicular spermatozoa are immotile and infertile. The spermatozoa released from the testis of reptiles, develop motility, which is a sign of maturation, during their transit through the excurrent duct system (Licht, 1984; Nirmal and Rai, 1997). The epididymis is the site of sperm maturation and additionally functions in sperm transport, protection and storage. The epididymal sperm maturation (morphological, biochemical and physiological) is the result of sequential events which occur in the epididymis and complete maturation of spermatozoa results in the ability to move, recognize and bind to the zona pellucida and to fuse with the plasma membrane of the oocyte and the acrosome reaction.

Reptilian epididymis synthesizes and secretes proteins (Depeiges and Dufaure, 1980, 1981; Depeiges *et al*., 1985, 1987, 1988; Ravet *et al*., 1987; Nirmal and Rai, 1997, 2000; Mesure *et al*., 1991) which are major constituents of luminal secretory material. There is no regional difference in secretory proteins of epididymal luminal fluid in *Hemiductylus flaviviridis* (Nirmal and Rai, 2000) whereas, it exists in *Lacerta vivipara* (Depeiges and Dacheux, 1985) and *Mabuya carinata* (Aranha *et al*., 2006). In *M. carinata*, the whole epididymal and the vas deferens

spermatozoa showed highest percentage of motile spermatozoa in contrast to very low percentage of motile spermatozoa in the testicular lumen. Hence, new proteins (compared to the testis) of the epididymal and the vas deferens secretions might be involved in spermatozoa maturation (Aranha *et al*., 2006).

The purpose of the present study was to determine the maturational changes taking place in each region of the epididymis as well as the role of each region in the maturational process, as there is regional difference in the secretions. Therefore, the immotile testicular free spermatozoa were incubated with luminal contents of different regions of the epididymis. Here the spermatozoa motility is considered as the index of postulated maturation.

MATERIALS AND METHODS

Sexually mature male lizard, *Eutropis carinata* were collected in and around Mysore University campus during the breeding season (October to December). Guidelines of Institutional Animal Ethics Committee (IACE) of University of Mysore and "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA) Ministry of Statistics and Programme Implementation, Government of India were followed in handling and sacrifice of animals. The protocols were approved by IACE. They were sacrificed and the testis and the epididymis were removed free of blood and connective tissue. The testis and three regions of the epididymis (anterior, middle and posterior regions) were transferred to separate culture dishes containing 5 mL of the Tyrode's buffer. The testis was transferred into a culture dish containing 5 mL of the Tyrode's buffer. The testis was punctured, the tunica albugenia was removed and the tubules were cut into small pieces and kept for 20 min at 25°C for the release of testicular free spermatozoa. Three regions of the epididymis were gently squeezed to release the luminal contents of each separately into the Tyrode's buffer. The suspension was centrifuged and spermatozoa were isolated. The luminal contents of the each region were transferred into 12 well sterile cell culture dish. Isolated testicular (free) spermatozoa were transferred into culture dish containing luminal contents of the different epididymal regions as follows:

- Luminal content of anterior epididymis+testicular spermatozoa
- Luminal content of middle epididymis+testicular spermatozoa
- Luminal content of posterior epididymis+testicular spermatozoa

The culture dish was incubated at 25°C and was observed at regular time intervals to record the different patterns of spermatozoa movement. The first observation was made after 15 min and further observations were made at regular interval of 30 min. The observations were carried out for 2 h. There were no further changes observed in the motility pattern after initial 15 min incubation. The motility was recorded with the camera attached to the inverted microscope using the software Cellsense Standard. The different patterns of the motility were already established by the previous studies on the animal based on the movement developed in different parts of the spermatozoa and the time taken by the spermatozoa to travel from one end to other in the visible field. The number of spermatozoa in five different visible fields was taken in each slide. Each experiment was repeated thrice using different animals. The data was pooled to calculate the percentage of each pattern of spermatozoa motility.

RESULTS

In the lizard *E. carinata*, the testicular spermatozoa exhibited different types of motility patterns, when incubated with luminal contents of different regions of the epididymis as

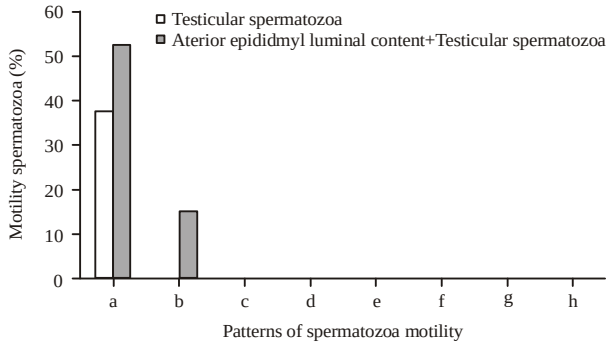

Fig. 1: Graph comparing the spermatozoa motility in testicular lumen and testicular spermatozoa incubated with anterior epididymal luminal content, (a) Slow head movement, (b) Medium fast head movement, (c) Wavy movement in the tail principal piece, (d) Head zig-zag movement, (e) Rotating head, (f) Rotating head with slow forward movement, (g) Rotating forward and (h) Rotating very fast forward

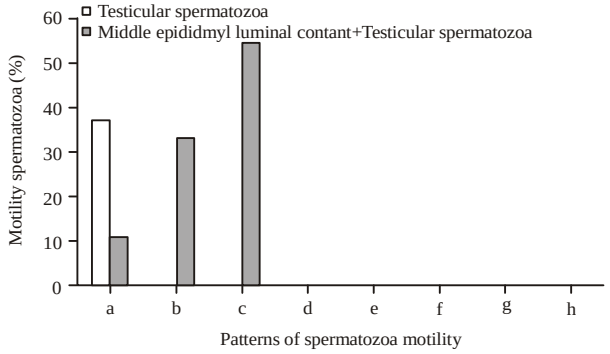

Fig. 2: Graph comparing the spermatozoa motility in testicular lumen and testicular spermatozoa incubated with middle epididymal luminal content, (a) Slow head movement, (b) Medium fast head movement, (c) Wavy movement in the tail principal piece, (d) Head zig-zag movement, (e) Rotating head, (f) Rotating head with slow forward movement, (g) Rotating forward and (h) Rotating very fast forward

follows: (a) slow head movement, (b) medium fast head movement, (c) wavy movement in the tail principal piece, (d) head zig-zag movement, (e) rotating head, (f) rotating head with slow forward movement, (g) rotating forward and (h) rotating very fast forward. As shown in Fig. 1, 52.77% of the testicular spermatozoa incubated with anterior epididymis luminal contents showed (a) slow head movement and 15.27% showed (b) medium fast head movement. Among the testicular spermatozoa incubated with middle epididymal luminal content (Fig. 2), 11.18% showed (a) slow head movement, 33.56% showed (b) medium fast head movement and 55.23% showed (c) wavy movement in the tail principal piece. The testicular spermatozoa with posterior epididymal luminal contents (Fig. 3) did not show any movement. In all the experiments the observations were made at every 30 min interval and there was no further changes seen in the motility pattern, after initial 15 min. The testicular spermatozoa showed no movement.

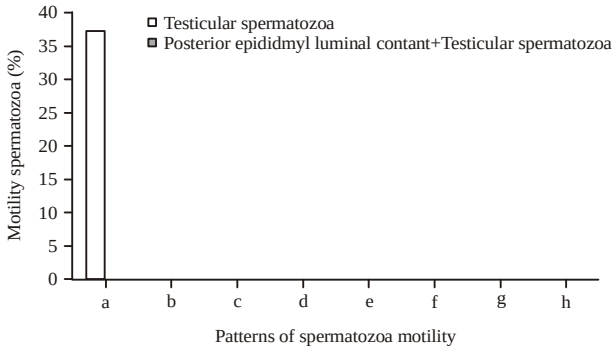

Fig. 3: Graph comparing the spermatozoa motility in testicular lumen and testicular spermatozoa incubated with posterior epididymal luminal content. (a) Slow head movement, (b) Medium fast head movement, (c) Wavy movement in the tail principal piece, (d) Head zig-zag movement, (e) Rotating head, (f) Rotating head with slow forward movement, (g) Rotating forward and (h) Rotating very fast forward

DISCUSSION

When the testicular spermatozoa were incubated with the anterior epididymal luminal contents majority of spermatozoa showed slow movement in the sperm head region which was similar to that of the *in vivo* condition. When incubated with the middle epididymal luminal contents majority of the testicular spermatozoa showed faster movement in the head region and movement in the tail principal piece, which were almost similar to that of the *in vivo* condition. But unlike the *in vivo* condition, here the spermatozoa did not show any slow forward movement. Contradicting to the *in vivo* condition, none of the testicular spermatozoa showed any movement when incubated with the posterior epididymal luminal contents.

Since, the earlier studies (*L. vivipara*, Depeiges and Dacheux, 1985; *H. flaviviridis*, Nirmal and Rai, 1997) and *M. carinata* (Aranha *et al*., 2008) have shown an increase in the percent forward progressive motility of the spermatozoa occurs during their transit from anterior to posterior end of the epididymis, it appears that the epididymal luminal fluid influences the progressive motility on passage through the epididymis. The acquisition of surface proteins by epididymal spermatozoa support the speculation that epididymis influences maturation of spermatozoa in the snake, *N. fasciata* (Esponda and Bedford, 1987). Regional difference in *M. carinata* in the secretion of proteins in the epididymis is shown by the presence of less number of protein bands and low protein content in anterior region compared with middle and posterior regions. The light and electron microscopic observations on the epididymis of *M. carinata* reveals histological differences in different regions of the epididymis (Aranha *et al*., 2006). Hence, earlier studies in *M. carinata* show that regional morphological differences in the epididymal epithelium are accompanied by differences in quality and quantity (number) of protein secretion (Aranha *et al*., 2006). In the present study when the testicular spermatozoa incubated directly with anterior and middle epididymal luminal contents showed the movement patterns almost similar to the *in vivo* conditions. But the testicular spermatozoa when incubated with the posterior region luminal contents directly showed no movements indicating the importance of the anterior and the middle region of the epididymis. The posterior epididymis is the region, where the spermatozoa attain complete motility. This study clearly shows although the posterior region of the epididymis is the site, where the spermatozoa attain complete and final motility, the anterior and middle region play

a key role in the maturation process. In the *in vivo* condition, the testicular spermatozoa passes through anterior and middle epididymis respectively before entering the posterior region. But in the present experiment the testicular spermatozoa was directly incubated with the posterior epididymal luminal contents without passing through anterior and middle epididymal contents. Hence, according to the results of our experiment the motility is gained only, if the spermatozoa pass through anterior and middle region of the epididymis before entering the posterior region. Further, the testicular spermatozoa incubated with middle epididymal luminal contents showed the motility patterns almost similar to the spermatozoa of the middle epididymis, though skipped the anterior epididymis. But when middle epididymal region was skipped the spermatozoa did not show any movement. This study indicates that it might be the middle epididymal secretion which plays the key role in the spermatozoa motility. It is speculated that the secretions of middle epididymis are necessary for the activation or functioning of the posterior epididymal secretions which are responsible for final motility of the spermatozoa.

This study was also supported by the ultrastructural study carried out by us. The ultrastructure of the spermatozoa of anterior middle and posterior regions of the epididymis shows the increase in the number of mitochondria. The spermatozoa exhibits 12-13 in the anterior region; 12-14 in the middle region and 14-17 mitochondria in the posterior region of the epididymis (Fig. 4). Mitochondrial volume has also been reported as being an important determinant of

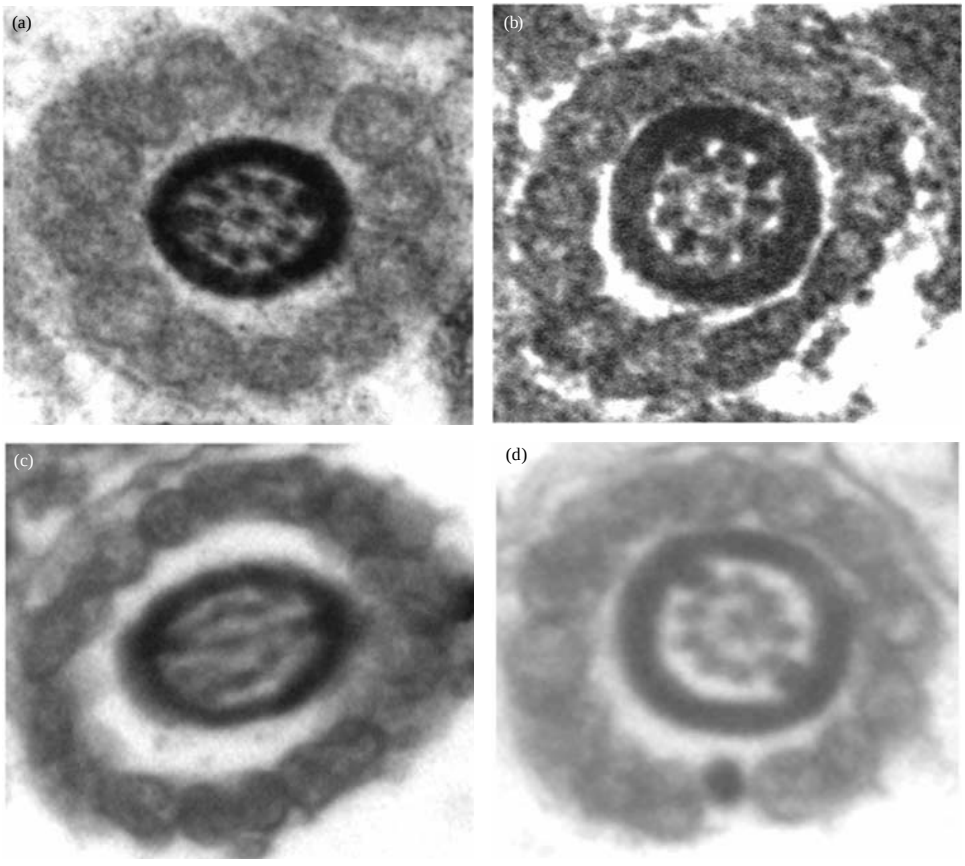

Fig. 4(a-d): Ultrastructure of midpiece of spermatozoa of the (a) Testis, (b) Anterior, (c) Middle and (d) Posterior epididymis showing the increase in the number of mitochondria

flagellar beat frequency (Cardullo and Baltz, 1991). Motility depends largely on mitochondrial volume within the sperm midpiece rather than on specific enrichment of the complexes themselves within each mitochondrion (Cardullo and Baltz, 1991). Hence, the increase in the number of mitochondria in the spermatozoa midpiece can be co-related to the gradual gain of motility while their transit through different regions of the epididymis.

CONCLUSION

The present study revealed that the testicular spermatozoa transit through anterior to posterior regions of the epididymis is necessary to gain complete motility. This study throws light on the importance of each region of the epididymis in physiological maturation of the reptilian spermatozoa, particularly lizards.

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