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A Study of the Spermatozoa Motility Acquisition Patterns in Excurrent Duct System of the Lizard *Eutropiscarinata*

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Abstract:
The invivo study was carried out to reveal the different patterns of motility of the spermatozoa in the lizard Eutropiscarinata for the first time. The spermatozoa from the testis, different regions of the epididymis and the vas deferens exhibited 8 different patterns of motility (a-h). Initially the motility develops from the head region of the spermatozoa and gradually moves to the midpiece and principal piece of the tail and finally to the end piece of the tail, making the spermatozoa move forward. Among testicular spermatozoa only a few showed slow head movement. In the anterior epididymis, majority of spermatozoa showed slow head movement along with a few of them exhibiting faster head movement and also wavy movement in the principal piece of the tail. In the middle epididymis a very low number of spermatozoa gained slow forward movement. In the posterior epididymis and vas deferens all the spermatozoa exhibited forward movement. In the present study, different patterns of motility of spermatozoa reveal the stages of motility development of the spermatozoa while their transit through the epididymis and the vas deferens. The rotating fast forward movement is the ultimate pattern of spermatozoa motility observed. This pattern of spermatozoa movement was attained in the posterior epididymis and remained same in the vas deferens. This type of movement was the highest exhibited pattern in both posterior epididymis and the vas deferens.

Keywords: *spermatozoa, epididymis, vasdeferens, motility, lizard*

1. Introduction

Reptiles, the most primitive amniote vertebrates, which successfully colonized the land in the course of vertebrate evolution, are well adapted for terrestrial mode of life. The major adaptive features seen in their reproductive systems to cope up with terrestrial reproduction were development of accessory reproductive organs, and the advent of internal fertilization and cleidoic eggs. Hence, reptiles are pivotal group in vertebrate phylogeny, to understand the evolution of reproductive mechanisms associated with internal fertilization and viviparity. The testicular spermatozoa in the reptiles are immature and they lack motility like other higher vertebrates. The physiological maturation of the spermatozoa is marked by the gain of motility while their transit through the accessory ducts (Licht, 1984; Nirmal and Rai, 1997). Sperm entering the epididymis gain progressive motility and fertilizability in a process termed maturation. The epididymis is the site of sperm maturation and additionally functions in sperm transport, protection and storage. Maturation of spermatozoa of eutherian mammals depends on a special environment created by androgen-dependent activities in the epithelium of the epididymis (Depieges and Dachaux, 1985). But this process of maturation is poorly studied and understood in most vertebrates especially in reptiles. However, our knowledge gained thus far on spermatozoa maturation phenomenon in reptiles is meagre to develop concepts.In the lizard *Eutropiscarinata* (earlier known as *Mabuyacarinata*), the epididymis is well distinguished into three regions, i.e., anterior, middle and posterior (Aranha *etal*., 2006). The purpose of the present study was determining the different patterns of motility acquisition during their transit through different regions of the epididymis. The motility of spermatozoa is postulated as an index of the physiological maturation of spermatozoa in this study.

2. Materials and Methods

Sexually mature male lizard *Eutropiscarinata* were collected in and around Mysore University campus during the breeding season (October to December). They were sacrificed and the testis, the epididymis and the vas deferens were removed free of blood and connective tissue. The testis, three regions of the epididymis (anterior, middle, posterior regions) and the vas deferens were transferred to separate culture dish containing modified Tyrode's buffer. The tissues from the testis, three regions of the epididymis and the vas deferens were minced. A drop of the sperm suspension (10µl) was taken on a clean glass slide and observed under the light microscope (40x) for the different patterns of movement. The different patterns of spermatozoa movement were observed and recorded with the camera attached to the inverted microscope using the software Cellsense Standard.The different patterns were classified based on the time taken by the spermatozoa to travel across the visible field and number of movements shown in the time interval of 5seconds. The number of spermatozoa for each pattern of movement from the testis, three regions of the epididymis and the vas deferens were recorded. This process was repeated three more times and mean percentage of spermatozoa for different pattern of movement was calculated.

3. Results

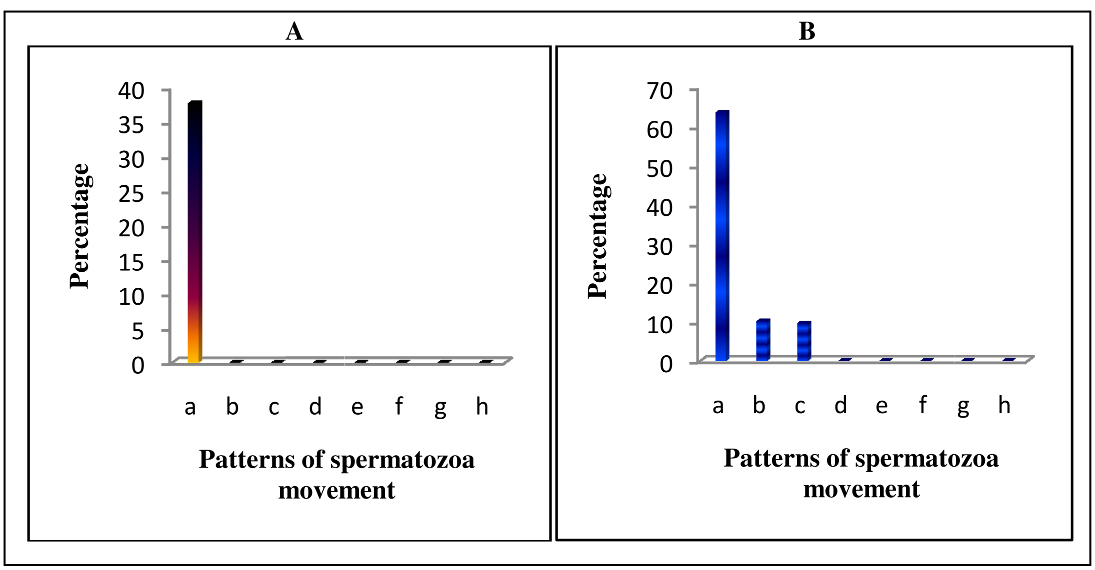
The spermatozoa from the testis, different regions of the epididymis and the vas deferens exhibited 8 different patterns of motility (a-h). The types of motility patterns observed are as follows-

a. Slow head movement: Only the head of the spermatozoa shows slow movement (0-3 times in 5sec).
b. Fast head movement: The head of the spermatozoa exhibits fast movement (4-10 times in 5 sec)
c. Wavy movement in then tail principal piece: The principal piece of the tail of the spermatozoa shows wavy movement along with the fast head movement (head movement more than 10 times in 5secs)
d. Head zig-zag movement: The head showed zig-zag movement along with the tail principal piece (movement more than 10 times in 5sec)
e. Rotating head movement: The head of the spermatozoa exhibited rotating movement in the anticlockwise direction. This movement also seen in the midpiece and tail principal piece.
f. Rotating head with slow forward movement: The head of the spermatozoa along with midpiece and tail principal piece showed rotating movement in the anticlockwise direction with slow forward movement.
g. Rotating fast forward movement: The spermatozoa exhibited fast forward movement. Here even the tail tip of the spermatozoa showed rotating movement in the anticlockwise direction.
h. Rotating very fast forward movement: The whole spermatozoa showed very fast rotating forward movement in the anticlockwise direction. (distance travelled by the spermatozoa was double that of rotating fast forward movement)

The number and percentage of the motile spermatozoa in the different regions are given in the table 1 and figure 1.

Sl. No.		Percentage of motility patterns							
		a	b	c	d	e	f	g	h
1	Testis	37.7	-	-	-	-	-	-	-
2	Anterior Epididymis	63.56	10.17	9.59	-	-	-	-	-
3	Middle epididymis	-	34.70	28.48	3.32	21.31	12.15	-	-
4	Posterior epididymis	-	-	-	-	-	8.86	84.34	2.48
5	Vas deferens	-	-	-	-	-	0.83	94.99	0.83

Table 1: Percentage of different patterns of spermatozoa movement in testis, anterior, middle and posterior epididymis and vasdeferens of Eutropiscarinata



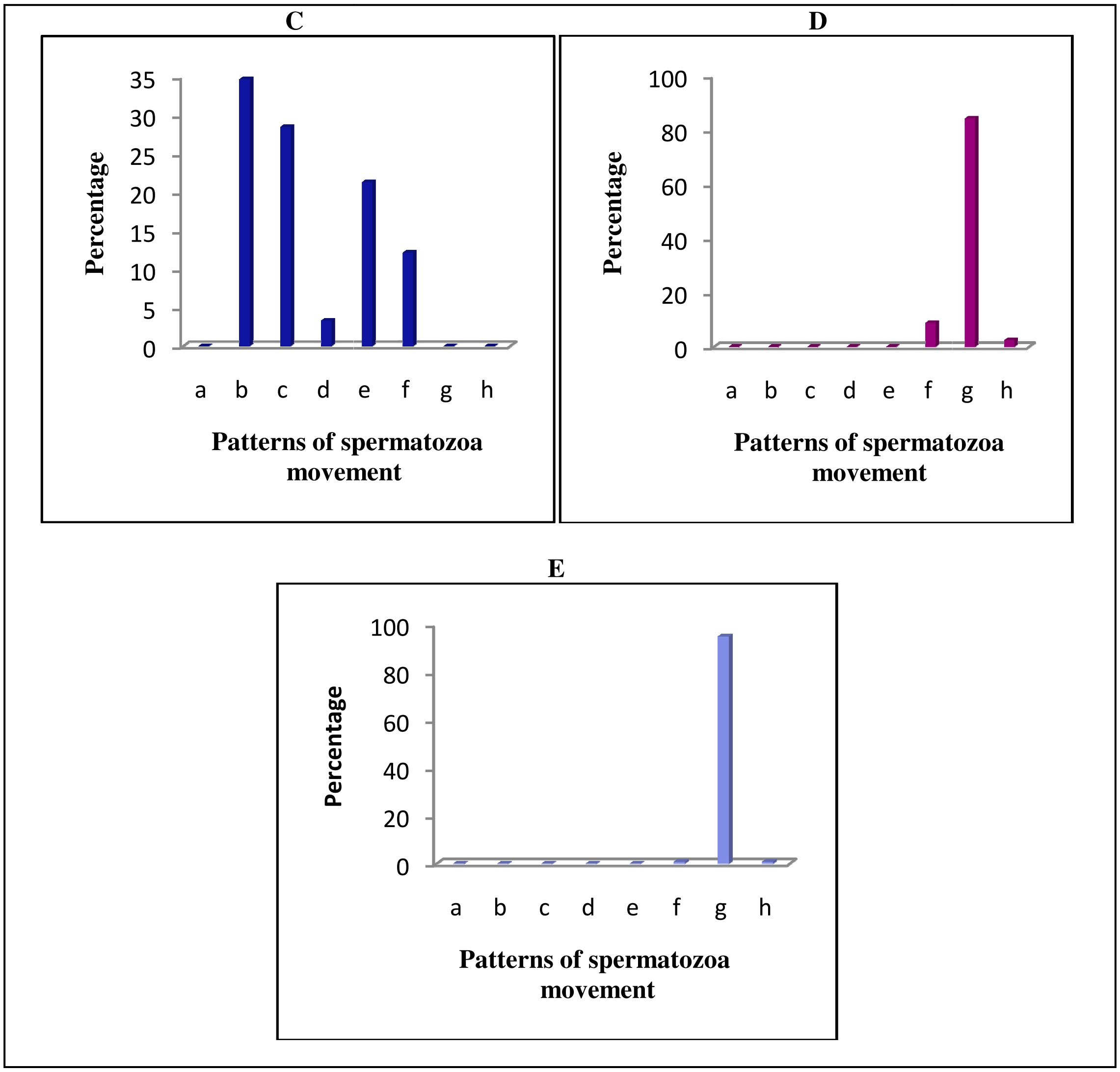


Figure 1: Percentage of different patterns of spermatozoa movement in testis (A), anterior (B), middle (C) and posterior (D) epididymis and vasdeferens (E) of Eutropiscarinata

4. Discussion

Like in other higher vertebrates the testicular spermatozoa are non motile even in case of *E. carinata*. The testicular spermatozoa did not show any forward movement but only some showed slow movement in the head region. Majority of spermatozoa in the anterior region of the epididymis showed the slow movement in the head region, a few showed faster head movement and movement in the tail principal piece. But none of the spermatozoa showed forward movement. This indicates that the initiation of spermatozoa motility acquisition begins in the anterior region. In the middle epididymal region all the spermatozoa had gained the movement and majority of them showing faster head movement and the movement in the tail principal piece, a few showed zig-zag and rotating head movement in the anticlockwise direction and some of them were showing very slow forward movement with rotating head. The movement patterns exhibited by the spermatozoa in the middle region of the epididymis show that the increase in the speed the head movement transfers the movement to tail principle piece along the midpiece and also as the speed of head movement increases it finally gains a rotating movement and causes the forward movement. In the posterior region of the epididymis all most all the spermatozoa had gained forward motility and majority of them showed rotating forward movement. The vas deferens spermatozoa also showed the similar kind of motility as that of the posterior epididymis. But a very small percentage of spermatozoa in the vasdeferens showed very fast rotating movement. This study shows that the spermatozoa gains initial motility at the head region. The motility is then attained by neck midpiece and finally the tail region. This study also reveals the unique pattern of spermatozoa in the lizard *E. carinata* for the first time. Reptilian epididymis synthesizes and secretes proteins (Depeiges and Dufaure, 1980, 1981; Depeiges *et al.,* 1985, 1987; Ravet *et al.,* 1987; Nirmal and Rai, 1997, 2000; Mesure *et al.,* 1991) which are major constituents of luminal secretory material. Since an increase in the percent forward progressive motility of the spermatozoa occurs during their transit from anterior to posterior end of the epididymis (*L. vivipara,* Depeiges and Dacheux, 1985; *H. flaviviridis,* Nirmal and Rai, 1997 and *M. carinata*, Aranha et al., 2008), it appears that the epididymal luminal fluid influences the progressive motility on passage through the epididymis. There is no remarkable difference in the motility patterns of the spermatozoa between posterior epididymis and vasdeferens indicating the fact that the motility is completely gained in the posterior region of epididymis itself. Hence the final pattern of spermatozoa motility is rotating forward movement and is attained in the region of posterior epididymis. The motility gain is only the postulated index of physiological maturation of spermatozoa. But the studies on the proteins secreted by different regions of excurrent duct of *E. carinata* showed a few additional proteins in the vasdeferens than in the posterior region of the epididymis (Aranha et al., 2006). In this study a very few spermatozoa in the vasdeferens showed very fast rotating forward movement, hence further studies on the fertilising capacity of these spermatozoa will throw light on the role of vasdeferens in the lizard *E.carinata*

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