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New classification of motility score in fishes to determine the quality of spermatozoa

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ABSTRACT
Knowledge on physiology of reproduction and gametes is important for the production of quality off spring and solving infertility issues in animals and human beings. Spermatozoa are prime entities having a prominent role in the successful fertilization of ova. Spermatological parameters are therefore considered as evaluation factors through which the quality of the spermatozoa can be determined. Motility is the inherent quality of the spermatozoa and the most preferred parameter that helps the spermatozoa in the search and subsequent entry into the ova. In the quality determination of spermatozoa, "motility score" is an essential factor and fixing the score demands visual observations on the motility pattern of the spermatozoa. It has been invariably evolved out from the animal spermatozoa evaluation and scrupulously followed for fishes too. Nevertheless, there is a wide variation in the fish spermatozoa and therefore a new and improved scoring system for the spermatozoa of the fishes was felt necessary. Various parameters that are to be considered for fixing the score have been included after thorough visual observation of the spermatozoa under varying conditions and as many motility patterns as possible have been included and the scoring chart widened. Although this might not be the ultimate in the motility scoring of fish spermatozoa, it can be a beginning for defining and classifying varied spermatozoa motility in fishes.

Keywords: Sperm, Milt, Spermatozoa, Motility, Scoring, Motility pattern, sperm quality).

1. Introduction
Advent of artificial insemination has brought in a revolutionary change in the dairy industry thereby the unlimited supply of milk and meat for the humans has been assured. In this line, cryopreservation or preservation of spermatozoa at sub-zero temperature has been considered as a viable technique for the successful breeding programmes through artificial insemination. Application of the same technology in fish breeding has received due attention in the recent days for the improvement of offspring quality and availability of fish fry for stocking throughout the year, besides helping in the genetic engineering research in fishes. Success and advancement were made with the experiments conducted involving the storage of salmonid sperm at temperature from 0-9 ^{0}C [1,2,3,4]. In fishes the first successful fertilization of eggs with cryopreserved spermatozoa was carried out in the herring *Clupea harengus* [5]. Many scientists followed this and found success in the use of cryopreserved spermatozoa in fishes [6,7]. Following the successful cryopreservation attempts after the standardization of the protocol, a few sperm banks for fin fishes have been created notably for grouper, salmonids and a few commercial and endangered fish species [7].

Spermatology is the study about various spermatological parameters like sperm motility, velocity, direction of movement of spermatozoa, etc. Among the various parameters, sperm motility gives an overall idea about the physiological status of sperm. Sperm motility can be defined as the movement of spermatozoa from one place to another or *in loco* with or without flagella movements. Although the motile character of a sperm does not indicate the fertilizing capacity, it is still used as a tool for evaluating the viability of spermatozoa.

In animals, four types of motility can be observed namely progressive, circular, oscillatory and reverse movements. Sperm motility test involves estimating the percentage of live sperm, the activity of the sperm and grading the sperm motility. Grading of motility or sperm motility rating is a visual gradation which is based on the nature and speed of the movement of spermatozoa. Sperm motility rating gives a fairly accurate estimate of the percentage of live sperm and the vigor of the sperm.

Fish spermatozoa differ from animal spermatozoa in that, it does not possess acrosome and its size ranges from 50-55µm which can be observed only through electron microscope. In animals, spermatozoa have to travel a long distance in the reproductive tract of the female to fertilize the egg. Therefore, semen samples with progressive motility and +4 or +5 grading are selected for fertilization. In fishes, it has been reported that even an immotile spermatozoa can fertilize the egg [8, 9, 10]. Hence there is a marked difference in motility grading between fish and animal spermatozoa. Many authors have suggested various motility scores based on the movement of spermatozoa and percentage of motile sperms for fishes [11,12,13,14,15,16].

2. Motility score for the spermatozoa of fishes

Spermatozoa of fishes spawning in brackish water and marine waters have more long lasting motility duration [17] otherwise the motility of fish spermatozoa ranges from 30 seconds to 300 seconds [18, 19]. This makes the study much complicated and almost all the species have immotile spermatozoa in their testes that are activated when they come in contact with the medium of lower or higher osmolality [20]. Therefore, the motility is a factor that can be observed only for a limited period and all the observations should be completed within that period.

3. Subjective methods for motility estimation in fishes

The assessment of motility of fish sperm has essentially relied for a long time on subjective estimates of motility characteristics [11, 21, 12] by the percentage of motile sperm cells, [22] by the total duration of movement, [23] or by a combination of both parameters [24]. The percentage of motile spermatozoa and the swimming vigor have usually been given a motility score corresponding to an arbitrary scale of criteria from 0 (immotile) to 5 (all spermatozoa vigorously motile) [11].

The motility rating has been defined in terms of percent moving spermatozoa in the field of view [12]. This method was also used to evaluate the percentage of moving cells [13]. In sea bass, motility classes were also used according to the percentage of rapid, vigorous and forward-moving motile spermatozoa [25]. Since all of these use arbitrary, nonlinear scales, they cannot be used for any statistical analysis. In the above context, some of the authors have proposed the various motility scores.

In our lab, we observed motility pattern in different semen samples. More than 500 semen samples of *Cyprinus carpio*, *Cirrihinus mrigala* and *Labeo rohita* were visually observed for the motility pattern to describe different motility patterns. We assigned and compared motility scores suggested by various authors [11, 16, 15] and found that some of the movements or motility pattern were found non-described. The criteria under which the motility score was assigned by different authors were not always observed in the samples. For example, a motility score of '1' was assigned for the spermatozoa vibrating *in loco* with few progressive motion [11]. But there was also a condition existed where all the spermatozoa were vibrating *in loco* without any progressive motion. Based on the above observation, an attempt has been made in the present paper to provide a comprehensive scoring for the fish spermatozoa motility and is presented in Table 1.

Table 1: New scoring method

Criteria	Motility score
All spermatozoa (95 – 99%) progressively motile with various flagella movements.	10
Most spermatozoa (90 – 95%) progressively motile, while others exhibit strong vibration with forward movement.	9
Most spermatozoa (85 – 90%) progressively motile, while others exhibit weak vibration with forward movement.	8
Most spermatozoa (80 – 85%) exhibit strong vibration with forward movement, while others vibrate *in loco*.	7
Most spermatozoa (75 – 80%) exhibit weak vibration with forward movement, while others vibrate *in loco*.	6
All spermatozoa (90- 95%) exhibit strong vibration *in loco*.	5
All spermatozoa (90 – 95%) exhibit weak vibration *in loco*.	4
Most spermatozoa (85 – 90%) exhibit strong vibration *in loco* while others oscillate.	3
Most spermatozoa (85 – 90%) exhibit weak vibration *in loco* while others oscillate.	2
Most spermatozoa (75 – 85%) oscillate while others vibrate.	1
Most spermatozoa (60 – 75%) vibrate while others are immotile.	0.75
All spermatozoa (90 – 95%) oscillate.	0.5
Most spermatozoa oscillate while others are immotile.	0.25
All spermatozoa immotile.	0

4. Definition of motility terminologies in the scoring

- **Progressive movement**- Spermatozoa exhibiting various strong flagella movements with unidirectional cell movement; unable to follow the direction and pattern.
- **Forward movement**- Spermatozoa exhibiting various flagella movements with unidirectional cell movement that can be tracked with normal vision in the microscope.
- **Strong *in loco* vibration**- Spermatozoa vibrating in a fixed place; with 5-7 movements per sec with stationary position.
- **Weak *in loco* vibration**- Spermatozoa vibrating in a fixed place; with 1-3 movements per sec without any movement from the place.

  - **Strong oscillation** - Spermatozoa oscillating with 2-3 swings per sec.
  - **Mild oscillation** - Spermatozoa oscillating with 1 swing per sec.
  - **Immotile** - No movement and carried by the medium added.

5. Conclusion

The above description is an attempt to quantify the motility pattern in an empirical manner so as to use the parameter in the evaluation of the quality of spermatozoa. Every effort is made to make it comprehensive covering all types of movements and patterns. But there can be variations with the spermatozoa of other species that might require further investigation and description. This description is provided as a supportive tool in the spermatological research in fish gametology.

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