

Correlation between the sperm quality and selection for higher growth rate of Nile tilapia (*Oreochromis niloticus*)

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Abstract

Random sample consisted of fifty males' brood stocks fish from the fifth generation of mass selection for higher growth rate and fifty males from non selected group of Nile tilapia (*Oreochromis niloticus*) were used to study the sperm quality. No significant differences were recorded between the selected and non selected fish for length, weight and width. Significant differences were recorded for motility time of sperm between the selected and non selected fish. The percentage of live sperm was higher significantly in non selected group than the selected group.

Both head and tail abnormality percentage were higher significantly in selected fish than non selected fish sperm. The correlation coefficient between tail abnormality of sperm and dead sperm was strong significant for non selected fish, while it was weak for selected fish. Strong correlation coefficient was observed for selected fish and moderate for non selected fish between tail and head abnormalities.

Negative correlation coefficient between pH and head abnormality of sperm was recorded for selected fish whereas, it was positive and very weak for non selected fish sperm. Moderate correlation was revealed for non selected fish for pH and tail abnormal, in the same time it was negative for selected fish. Negative correlation coefficient was observed between pH and sperm motility for both selected and non selected fish.

Negative correlation coefficient was noted for selected and non selected fish for motility time and both live and dead sperm. Motility time and head abnormality of sperm showed positive correlation coefficient moderate for selected and weak for non selected. Motility time and tail abnormality of sperm revealed positive correlation for selected fish and negative for non selected fish.

Keywords: Selection, Motility time, sperm, tail and head abnormalities, correlation

Introduction

The studies on the fish sperm in general, are few comparing to the studies

on eggs. The fish farming industry has been more focused towards the quality of eggs and larvae rather than that of sperm, even though the sperm quality of male broodstock also affects the production of

healthy larvae. Nevertheless, in commercial hatcheries, milt is often inadequate both in terms of quantity and quality and does not always give successful fertilization in the artificial insemination procedures commonly used for aquaculture species (Rurangwa *et al.*, 2004).

Most aquaculture fish species are external fertilizers; sperms are released into the water and their spermatozoa reach and fertilize the eggs (Cabrita *et al.*, 2009). External environmental factors may affect the quality and motility during the activation process (Rurangwa *et al.*, 2004). Fish spermatozoa are immotile in the testis, but gain potentiality for activation during transfer to the sperm duct (Alavi *et al.*, 2009). Teleost sperm is characterized by the absence of an acrosome (Rurangwa *et al.*, 2004). Seminal plasma produced by the sperm duct provides an ionic environment that maintains the viability of spermatozoa after their release from the testes (Ciereszko, 2008). In fish farms and hatcheries, the biotic and abiotic factors that affect sperm quality are diverse and are dependent on complex interactions between genetic, physiological and environmental factors (Rurangwa, *et al.*, 2004).

There are several factors that affect sperm motility such as pH, temperature, ions and osmolality (Cosson *et al.*, 1999; Morisawa *et al.*, 1999; Alavi and Cosson, 2006). Effects of environmental factors including pH, cations and osmolality as well as the role of dilution rate on sperm motility parameters in *Acipenser persicus* were studied (Alavi, *et al.*, 2004). Effect of temperature on sperm beat frequency was recorded for salmonid and cyprinid (Cosson *et al.*, 1985). Higher temperature increased the beat frequency and

decreased the duration of forward movement in trout (Billard and Cosson, 1992), while the lower temperature during natural spawning (4–10°C) increases the duration of sperm movement (Van Look, 2001). In African catfish, low temperature (4°C) also prolonged motility and viability of spermatozoa compared to the culture temperature (25°C) (Mansour *et al.*, 2002).

Using small number of males will affect the genetic pool, if the pool is too small, there is a risk of severe genetic bottlenecks and inbreeding that would ultimately produce homozygous strains of low fitness (Rurangwa, *et al.*, 2004). Also, the differences in sperm quality may be important in reducing the apparent population size and genotype diversity. Although, the important of sperm quality which affect the techniques used to assess sperm quality in fish include monitoring sperm density and motility, consequently fertilization success (Billard, 1978; Aas *et al.*, 1991; Methven and Crim, 1991).

Evaluation the motility was reported as the total period of sperm motility (Stoss, 1983) or the percentage of motile sperm observed visually (Billard, 1978 and Cosson *et al.*, 1999) or quantitative approaches to studying sperm motility use the computer-assisted semen analysis (CASA) system (Cosson *et al.*, 1997; Kime *et al.*, 2001).

The aim of the present study that study the effect of selection for higher growth rate of Nile tilapia (*Oreochromis niloticus*) on sperm quality (motility, % live, % dead, head and tail abnormalities)

Materials and methods

Broodstock husbandry and sperm collection

This experiment was carried out at World Fish Center at Abbassa, Abou,

Hammad, Sharkia. One hundred random samples of brood stocks fish from the fifth generation of mass selection of tilapia for high growth rate and non selected (control) groups were used. Fish were held in separate cement concrete tanks supplied with aeration for two weeks for acclimatization. The temperature was $28 \pm 1^\circ\text{C}$; dissolved oxygen was 4-6 mg/L; pH was 7.2-8.3; nitrite was less than 0.05 mg/L; ammonia was less than 0.2 mg/L and salinity was 2‰ during the experiment. Ten females were stocked in large hapa in each tank to release from one to enhance and activate the male to produce sperm. To avoid the semen contamination by urine, mucus or blood the external urogenital pore was wiped dry with paper towel before stripping. Semen used in the present study was collected from the male in dry clean Pastier glass pipette. A haemocytometer was used to determine spermatozoa concentration. A drop of diluted semen (10µl) was placed onto a haemocytometer covered with a cover slip and left for 10 min to allow sperm sedimentation before 16 cells (0.1 mm depth and 0.2 length) were counted. Total number of spermatozoa is recounted as spermatozoa according to Rana (2002). Wide range pH paper (Hydrion (93) S/R Insta-Chek pH Paper 0.0-13.0) was used for pH test.

One step eosin-nigrosin staining technique was used to study live and dead sperm according to Björndahl *et al.* (2003). Using a Prosilica EC-650 digital camera mounted on a light microscope (400× magnification), an image was taken of visible spermatozoa (20 sperm images per male). Sperm head, midpiece and tail/flagellum length (end of midpiece to end of tail) were measured to the nearest 0.1 µm using Image J (v. 1.42q, available at <http://rsb.info.nih.gov/ijl>). Measurements were calculated by

drawing a freehand line over each sperm section using an Intuos graphic table (Wacom Co. Ltd., Japan). For each male, an average length of each sperm piece was calculated from the 200 images.

Data Analysis

SPSS program Version 11 statistical software package (SPSS, Inc., Chicago, Illinois, USA) was used to analyze T test and correlation according to Dytham (1999). Correlation is significant at the 0.01 and 0.05 levels.

Results

No significant differences were recorded between the selected and non selected fish for length, weight and width (Table 1). A significant difference was recorded for motility time between the selected and non selected fish. No significant difference was recorded between the selected and non selected fish for head length and tail length / head length ratio.

The percentage of live sperm was higher significantly in non selected group than the selected group (Table 1). Both head abnormality percentage and tail abnormality percentage were significantly higher in selected fish than non selected fish sperm.

The correlation coefficient between tail abnormality of sperm and dead sperm was strongly significant for non selected fish, while it was weak for selected fish (Table 2). Negative correlation coefficient was recorded for selected and non selected fish for head abnormality and live sperm. Strong correlation coefficient was noted for selected fish and moderate for non selected fish between tail and head abnormalities.

Negative correlation coefficient between pH and head abnormality of

sperm was recorded for selected fish whereas, it was positive and very weak for non selected fish sperm.

Moderate correlation was recorded for non selected fish for pH and tail abnormal, in the same time it was negative for selected fish. Negative correlation coefficient was observed between pH and sperm motility for both selected and non selected fish (Table 3).

Negative correlation coefficient between pH & head abnormality of sperm was recorded for selected fish whereas, it was positive and very weak for non selected fish sperm. Moderate correlation was recorded for non selected fish for pH & tail abnormal, in the same time it was negative for selected fish. Negative correlation coefficient was observed between pH and sperm motility for both selected and non selected fish (Table 3).

Negative correlation coefficient was recorded for selected and non selected fish for motility time and both live and dead sperm (Table 4). Motility time & head abnormality of sperm showed a positive correlation coefficient moderate for selected and weak for non selected. Motility time and tail abnormality of sperm revealed positive correlation for selected fish and negative for non selected fish.

Discussion

Mass selection designs are basically much simpler and less expensive than selection designs based on individual tagging and pedigree records, but may only be used to select for traits that can be recorded on live broodstock candidates themselves. The growth rate may easily be selected for mass selection (Bentsen and Olesen, 2002). The present investigation showed no significant differences were recorded between the

selected and non selected fish for length, weight and width. No significant correlations were observed between the total number of spermatozoa, sperm volume, and length as well as weight of males which is in accordance with Alavi *et al.* (2009).

Lower sperm number in the selected fish than the non selected fish pointed to the negative effect of selection on sperm. Even though highly concentrated sperm does not always give the highest motility or the highest fertilization rate (Geffen and Evans, 2000 and Williot *et al.*, 2000).

No significant variation for tail length, head length and their ratio were recorded in the tested fish. Sperm with longer flagellum is expected to swim faster in relative to that smaller shorter flagellum. The ratio between sperm tail length and sperm head length would be the most appropriate metric evidence to use when studying the correlation between sperm morphology and motility in external fertilizers (Humphries *et al.*, 2008).

The rapid decrease ($P < 0.05$) in sperm motility parameters after sperm activation agrees with Alavi *et al.* (2009). The semen showed alkaline pH for selected and non selected fish which enhanced the sperm motility which agrees with Alavi and Cosson (2005). These authors reported that alkaline conditions of diluents enhance the motility parameters of sturgeon sperm. No significant difference was recorded for pH for selected (8.6 ± 1.0) and non selected (8.4 ± 0.9) which was lower than the pH values of individual tilapia milts ranged from 6.2 to 8.2 as recorded by Chao *et al.* (1987). While the results for pH (7.5 to 8.5) was recorded by Alavi and Cosson (2005).

The sperm head houses the cell's genetic material (Kunz, 2004) and sperm cells are particularly susceptible to DNA damage (Lewis and Aitken, 2005).

Negative correlation coefficient was observed between pH and sperm motility for both selected and non selected fish which in contrast with Alavi and Cosson (2005). They reported significant correlation between seminal plasma pH and sperm motility. Meanwhile, Williot *et al.* (2000) found no correlation between sperm motility and pH in *A. baeri*. The initiation of sperm motility, swimming speed and period of forward motility may also be influenced by pH (Wojtczak *et al.*, 2007).

The percentage of live sperm was higher and significantly differences in non selected group than the selected group. On the other hand, head and tail abnormalities as well as dead sperms were higher in selected fish than non selected fish. The results indicated negative effect of selection on sperm quality which in parallel with Dunham (2006) who concluded that correlated responses to selection affected several other traits, both positively and negatively. In the same line, Gjedrem and Baranski (2009) reported that selection for one trait will influence other traits that are genetically correlated.

There were significant correlations between spermatozoa concentration - length ($r=0.7$) and - weight ($r =0.8$) of males. Percentage of motile spermatozoa decreased rapidly as a function of time post activation and depended on the osmolality of activation media (Alavi *et al.*, 2009). Empirical studies demonstrate that high sperm competition selects for more, longer, faster moving sperm (Fitzpatrick *et al.*, 2010).

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Table (1): The differences between selected and non selected groups of *Oreochromis niloticus* broodstocks and its sperm characters.

Parameters	Selected	Non selected	probability
Weight (g)	470.9 ± 115.40	469.4 ± 95.70	0.478
Length (cm)	29.10 ± 3.50	28.80 ± 4.20	0.275
Width (cm)	9.30 ± 1.40	9.60 ± 1.10	0.500
sperm No/ sperms ml⁻¹	6.20 ⁸	1.70 ⁹	0.014
Motility time	28.90 ± 8.40	26.30 ± 11.10	0.012
pH	8.60 ± 1.00	8.40 ± 0.90	0.462
Sperm length	10.21 ± 0.13	10.13 ± 0.16	0.015
Live Sperm	52.0 ± 7.4	70.5 ± 7.4	0.019
Dead Sperm	16.6 ± 7.2	8.1 ± 3.2	0.004
Head abnormality	17.1 ± 9.6	10.4 ± 2.1	0.002
Tail abnormality	14.3 ± 2.6	10.9 ± 4.2	0.006
Head length	1.5 ± 0.05	1.6 ± 0.00	0.279
tail length / head length	6.80 ± 0.02	6.33 ± 0.03	0.849

Significant = P < 0.05

Table (2): Correlation coefficient among abnormality for both head and tail for selected and non selected fish sperm.

Correlation coefficient	Selected	Non selected
Head abnormality & live sperm	-0.533	-0.278
Head abnormality & dead sperm	0.017	0.562(*)
Tail abnormality & live sperm	-0.345	0.046
Tail abnormality & dead sperm	0.227	0.891(**)
Tail & Head abnormalities	0.831(**)	0.665(*)

* P < 0.05 and ** P < 0.01

Table (3): Correlation coefficient between pH and sperm (live, dead and abnormality for both head and tail) for selected and non selected fish.

Correlation coefficient	Selected	Non selected
pH & live sperm	0.140	0.54
pH & dead sperm	0.299	0.604(*)
pH & head abnormality	-0.441	0.093
pH & tail abnormality	-0.383	0.621(*)
pH & sperm motility	-0.552(*)	-0.613(*)

* P<0.05

Table (4): Correlation coefficient among motility time and live, dead and abnormality for both head and tail of sperm for selected and non selected fish

Correlation coefficient	Non selected	Selected
Motility time & live sperm	-0.754(**)	-0.640(*)
Motility time & dead sperm	-0.656(*)	-0.028
Motility time & head abnormality	0.166	0.734(**)
Motility time & tail abnormality	-0.449	0.744(**)

* P<0.05 and ** P<0.01

الإرتباط بين الانتخاب الوراثى وجودة الحيوانات المنوية فى أسماك البلطى النيلى

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الملخص العربى

اشتملت هذه الدراسة على عينة عشوائية من خمسين ذكرا من المخزونات السمكية للجيل الخامس من الانتخاب الوراثى الجماعى لاسماك البلطى النيلى وكذلك مجموعة مماثلة من اسماك بلطى غير منتخبة استخدمت كمجموعة ضابطة لهذه الدراسة. لم تسجل أي اختلافات معنوية بين الاسماك المنتخبة وغير المنتخبة بالنسبة للوزن والطول والعرض. و لكن قد سجلت اختلافات معنوية بين وقت القدرة على الحركة للاسماك المنتخبة وغير المنتخبة. وكانت نسبة الحيوانات المنوية الحية مختلفة اختلافا معنوي في اسماك البلطى غير المنتخبة التى استخدمت كمجموعة ضابطة عن الاسماك المنتخبة. وكانت نسبة التشوهات فى كل من الرأس والذيل مختلفة اختلافا معنويا في الحيوانات المنوية فى الأسماك المنتخبة عن الاسماك غير المنتخبة. وكان معامل الارتباط بين التشوهات فى ذيل الحيوانات المنوية والحيوانات المنوية الميتة قوية للأسماك غير المنتخبة ، فى حين أنه كان ضعيفا للأسماك المنتخبة. ولوحظ معامل الارتباط قوي للأسماك المنتخبة ومتوسطة للأسماك غير المنتخبة بين الذيل وتشوهات الرأس للحيوانات المنوية.

تم تسجيل معامل ارتباط سلبى بين درجة الحموضة وتشوهات الرأس للحيوانات المنوية للأسماك المنتخبة فى حين أنه كان إيجابيا ولكنه ضعيف للغاية فى الحيوانات المنوية للأسماك غير المنتخبة.

تم الكشف عن علاقة معتدلة للأسماك غير المنتخبة بين درجة الحموضة وتشوهات الذيل ، فى نفس الوقت كان سلبيا بالنسبة للأسماك المنتخبة. وقد لوحظ معامل ارتباط سلبى بين درجة الحموضة و القدرة على الحركة فى الحيوانات المنوية لكل من الأسماك المنتخبة وغير المنتخبة

ولوحظ ان معامل الارتباط كان سلبيا لكل من الاسماك المنتخبة وغير المنتخبة لوقت القدرة على الحركة للحيوانات المنوية وعلى حد سواء لكل من الحيوانات الحية والميتة. وكانت العلاقة لمعامل الارتباط بين وقت القدرة على الحركة للحيوانات المنوية وتشوهات الرأس كانت إيجابية ومعتدلة للاسماك المنتخبة وضعفيفة لغير المنتخبة. كان هناك ارتباط معنوى موجب للعلاقة بين كل من التشوهات للذيل ووقت القدرة على الحركة للاسماك المنتخبة، ب بينما كان ارتباطا سلبيا للاسماك غير المنتخبة