Dept. of Theriogenology,
Fac. of Vet. Med., Kafr El-Sheikh, Egypt.

INFLUENCE OF SEMINAL VESICLE SECRETION ON SPERM MOTILITY AND GAMETE ERTILIZABILITY IN AFRICAN CATFISH, *CLARIAS GARIEPINUS*

(With 4 Tables)

By

N. MANSOUR; F. LAHNSTEINER* and B. HELIEL

*Institute for Zoology, Hellbrunnerstrasse 34, 5020 Salzburg, Austria

(Received at 21/8/2004)

تأثير إفرازات الحوصلة المنوية على حركة الحيوانات المنوية وخصوبة الجاميتات فى سمكة القرموط ، *Clarias garipinus*

نبيل منصور، فرانز لانشتينر، بسيونى هليل

إن إفرازات الحوصلة المنوية فى ذكور سمكة القرموط ، *Clarias garipinus* ذات لزوجة عالية. وبخلط هذه الإفرازات بالحيوانات المنوية فقد تم إعاقة حركة هذه الحيوانات إعاقة تامة. وعند تخفيف هذه الإفرازات بالماء فقد قلت لزوجتها وتأثيرها السلبى على حركة الحيوانات المنوية. عند خلط السائل المنوى بإفرازات الحوصلة المنوية فان قدرتها الحيوية انعدمت فى خلال ١٠ دقائق. أيضا كان لهذه الإفرازات تأثيرا سلبيا على عملية الإخصاب من خلال: أ- تأثيرا سلبيا على خصوبة الحيوانات المنوية: بعد خلط السائل المنوى بهذه الإفرازات ثم إجراء عملية الإخصاب، كانت معدلات الإخصاب منخفضة جدا. ب- تأثيرا سلبيا على خصوبة البيض: بعد خلط البيض بهذه الإفرازات ثم إجراء عملية الإخصاب، فقد انعدم إخصاب هذا البيض. ج- تأثيرا سلبيا على تفاعل الحيوانات المنوية مع البيض أثناء عملية الإخصاب: عند خلط السائل المنوى والبيض وإفرازات الحوصلة المنوية معا، فقد انعدمت الخصوبة. وأخيرا تم مناقشة وظيفة إفرازات الحوصلة المنوية فى السلوك والعلاقة بين الذكر والأنثى أثناء التزاوج.

SUMMARY

Pure seminal vesicle secretion (SVS) completely inhibited the sperm motility as the sticky secretion hindered spermatozoa in free swimming. When the seminal vesicle secretion is diluted in water the viscosity decreases and the motility suppressing effect is neutralized. When semen is mixed with seminal vesicle secretion the sperm viability decreases to zero within 10 min. Seminal vesicle secretion had an inhibitory effect on fertilization through: (a) inhibition effect on sperm fertility as when

semen prediluted in seminal vesicle secretion the fertilization rates were very low. (b) Negative effect on eggs as mixing of SVS to the eggs completely inhibit their fertilizability, probably as the sticky secretion plugged the micropyle and inhibited egg activation. (c) Bad effect on the sperm egg contact as mixing of semen, eggs, and SVS simultaneously, the fertility was also prevented. A function of SVS in the male and female communication during the prenuptial spawning behaviour is discussed.

Key words: *Catfish, clarias gariepinus, spermatozoa, gamete viability, vesicle secretion.*

INTRODUCTION

In teleost fish the male gonads of the Clariidae (Van den Hurk *et al.*, 1987), Gobiidae (Lahnsteiner *et al.*, 1992), Heteropneustidae (Chowdhury and Joy, 2000), Ictauridae (Sneed and Clemens, 1963) and Siluridae (Van Tienhoven, 1983) have paired accessory genital glands, the seminal vesicles, which empty into the spermatic duct. These glands consist of one or multiple lobes and produce a highly viscous secretion (Van den Hurk *et al.*, 1987). This secretion increases gradually to reach the peak level during spawning season (Van den Hurk *et al.*, 1987; Singh and Joy, 1999). The secretion has been investigated in *Heteropneustes fossilis* (Heteropneustidae) (Chowdhury and Joy, 2000), *Clarias batrachus* (Clariidae) (Singh and Joy, 1999) and in *Zosterisessor ophiocephalus* (Gobiidae) (Lahnsteiner *et al.* 1992) and consists of mucoprotein and mucopolysaccharid.

The function of the seminal vesicle secretion (SVS) is still unclear. For *Clarias gariepinus* it was suggested that the secretion may enhance sperm motility and fertility (Van den Hurk *et al.*, 1987). In Clariidae, the interstitial cells of the seminal vesicle produce pheromones (Resink *et al.*, 1989; Singh and Joy, 1998) and suggested to produce energy sources for the sperm cell (Lehri, 1967; Van den Hurk *et al.*, 1987). In Gobiidae, SVS is suggested to improve sperm egg contact during fertilization as the eggs were placed onto a semen and SVS layer (Ota *et al.*, 1996).

From the above mentioned studies, the function of the seminal vesicle secretion in teleosts is still unclear as studies on effect of this secretion on gamete physiology and interaction during natural spawning is lacking. Therefore, this work was conducted to study the effect of

SVS on spermatozoa, eggs, and sperm egg contact to find out its influence on gamete viability.

MATERIALS and METHODS

Fishes and collection of semen, seminal plasma, eggs and seminal vesicle secretion

Mature African catfish, *Clarias gariepinus*, with a weight of 750 - 1200 g were bought from the commercial hatchery IBAU in Potsdam (Germany) and transported to the University of Salzburg. The catfish were kept in plastic tanks of 2.5 - 3 m^3 volume. Water supply was ground water warmed to 28 $\pm$ 1°C, the stocking density was 10 fishes per tank. Approximately 10% of the water volume was renewed daily. The photo-period was a 12 hour light to 12 hours dark cycle and the catfish were fed on a commercial pellet diet (2 - 3% of the body weight/day). The fish were acclimatized 5 - 7 days before they were used for the experiments.

For collection of semen male fishes were anaesthetized with 200 mg/l MS-222 and portions of the testis removed surgically. Alternatively the fish were killed by an overdose of MS 222 and the whole gonads were removed. Testes were cleaned from the blood by surgical towels. Then the testes were incised and the out-flowing semen droplets were collected into small glass vessels. Semen was stored at 4°C before the onset of the experiments. Seminal plasma was obtained by centrifugation of testicular semen for 10 min at 1000 rpm.

For collection of eggs ovulation was artificially induced. Females were intra-peritoneally injected with Ovopel pellets (gonadotropin-releasing hormone analogue [D-Ala^6,Pro^9NEt] containing 2.5-3 mg of water soluble dopamine antagonist metoclopramide - Interfish Ltd, Hungary) dissolved in 0.7% NaCl. One pellet was used per kg body weight. Twelve hours after injection the fish were stripped and the eggs were collected in clean dry plastic containers.

For collection of seminal vesicle secretion (SVS) the seminal vesicles were excised and cleaned using surgical towels. Then the seminal vesicle lobes were cut open at their distal end (the end opposite to the spermatic duct) and the SVS was stripped out by gentle finger pressure and collected directly into reaction vessels. Care was taken to avoid contamination of the secretion with cell debris and blood. One - 2 ml secretion was collected per seminal vesicle pair and stored at –70 °C.

Effect of SVS on the sperm motility

All motility experiments were performed at 28 ± 1°C. The influence of SVS on sperm motility was investigated by trying to activate sperm motility in pure SVS and in SVS diluted at a rate of 1: 50, 1: 100, 1: 200, 1: 400 and 1: 800 in distilled water. The influence of SVS on immotile spermatozoa (=on sperm viability) was investigated by incubation of semen together with SVS (secretion: semen = 1: 1 or 1: 2, final volume 100 µl) for 5, 10 or 20 min. Thereafter motility was activated with distilled water as described below.

Motility was measured with computer assisted cell motility analysis (Mansour *et al.* 2003). For motility activation 100 µl sperm motility activating solution was added into the investigation chamber and 2-4 µl of undiluted or prediluted semen (depending on the sperm density) was added and mixed. The chamber was closed with a coverslip where upon excess sperm suspension was drained. Then the sample was quickly transferred into the microscope and the motility was recorded on videotapes.

Sperm motility measurements were made 10, 20, 30 and 40 s after motility activation. The following sperm parameters were measured: % immotile (velocity > 5 µm s^{-1}), % locally motile (velocity of 5-20 µm s^{-1}), and % motile spermatozoa (velocity < 20 µm s^{-1}); average path swimming velocity of the motile sperm (µm s^{-1}).

Effect of the SVS on fertilization

All fertilization assay were performed at 28 °C. The same semen and egg batches were used for each experimental series. Each fertilization assay was repeated three times.

Effect of SVS on semen fertility: In the first experiment 5 µl semen was mixed with 50 mg SVS in a Petri dish. After 30 sec 0.25 g eggs (200 ± 20) were placed on this mixture, 1 ml water was added and all compounds were mixed. In the second experiment 5 µl semen, 50 mg SVS and 1 ml water were mixed. After 1 or 5 min 0.25 g eggs were added.

Effect of SVS on egg fertilizability: In experiment 1, 50 mg SVS was weighed into Petri dishes. Eggs (0.25 g) were added and gently mixed with the secretion. After 30 sec 5 µl semen and 1 ml water were added and all components were mixed. In experiment 2, 50 mg SVS, 0.25 g eggs and 1 ml water were mixed. After 2 min 5 µl semen was added.

Effect of SVS on sperm-egg contact: In the first experiment 5 µl semen, 0.25 g eggs and 50 mg SVS were mixed together in the petri dishes and after 2 min 1 ml water was added. All compounds were re-

mixed. In the second experiment 0.25 g eggs, 5 µl semen and 1 ml of diluted SVS (1:50, 1:400 or 1:800 in water) were mixed.

Control: Eggs (0.25 g) were weighed into petri dishes, 5 µl semen added and mixed with them. Immediately thereafter 1.0 ml water was added and all components were re-mixed.

Determination of fertilization and hatching rate

About 3 min after fertilization the petri dish was completely filled with water and incubated for 90 min at 28 C°. Then the fertilization rate was counted in the 4 cell stage under a stereo-microscope at 10 fold magnification. For hatching the eggs were placed in small round metal tea mesh eggs (diameter of 5 cm) according to a recently published method (Mansour and Lahnsteiner, 2003) and incubated in an aerated water tank for 22 - 24 hours at 28 °C (until they reached the embryo stage shortly before hatching). The rate of embryos was counted in relation to the total number of eggs.

Statistical analysis

Data are represented as mean ± S.D. Multifactorial analysis of variance (ANOVA) with subsequent student *t*-test was used for comparison of mean values of the various treatments at a significance level of $P< 0.05$.

RESULTS

Effect of the SVS on gamete physiology

Effect on sperm motility: Spermatozoa were completely immotile when testicular semen was mixed with pure SVS. The motility rate and velocity were partially suppressed in diluted SVS (1: 50, 1: 200, 1: 400 in water, w/v). The sperm motility duration was only 10 s at a dilution rate of 1: 50 due to the viscosity of SVS. At higher dilution rates of 1: 200, 1: 400 and 1: 800 motility duration was slightly prolonged in comparison to water. At a still higher dilution rate of 1: 800 the motility rate and velocity were similar to that in water (Table 1).

When spermatozoa were incubated together with SVS they were viable for 10 min, as sperm motility could be still initiated after this incubation period with distilled water. Due to coagulated protein particles approximately similar size as the spermatozoa, the sperm motility could not be measured by CMA analysis but only estimated. After 5 min incubation the motility rate was about 60% (untreated semen control 75%), after 10 min 35%. After 20 min no motile sperm cells were observed any more.

When semen was mixed with SVS and such a droplet was placed on a slide and overlaid gently with water without mixing, the outer, superficial secretion layers started to coagulate. The single spermatozoa located in this area started to activate and swim. From time to time, further spermatozoa left the secretion and started to swim, motility of single sperm cells being observed for up to 5 min. The sperm motility activation was heterogenous and the estimated motility rate was 10 - 25% (untreated semen 75%). Due to protein remnants it could not be measured by CMA analysis.

Effect of SVS on fertilization: This was studied through its effect on:

(a) Sperm fertility: When semen was pre-mixed with SVS, and after 30 sec water and eggs were added the fertilization rates were zero. Also when the semen - SVS mixture was diluted in water for 1 or 5 min before it was used for fertilization the fertilization rates were zero (Table 2).

(b) Egg fertilizability: When the eggs were mixed with SVS and after 2 min semen and water was added the fertilization rates were zero. When eggs, SVS and water were mixed and semen added after 2 min the fertilization rates were zero, too. When eggs were incubated in water in the absence of SVS for 2 min fertilization rates were about 80% from freshly fertilized eggs (Table 3).

(c) Sperm-egg contact: When semen, eggs and SVS were pre-mixed and after 2 min water was added no fertilization was obtained (Table 4). When semen and eggs were pre-mixed and immediately thereafter diluted SVS (1: 50, 1: 400 or 1: 800 in water) was added the fertilization rates increased with the dilution rates (Table 4).

DISCUSSION

Until now it is unclear if seminal vesicle secretion is released together with semen. In aquaculture, African catfish eggs are fertilized with testicular semen and high fertilization rates up to 100% can be obtained without seminal vesicle secretion (Haylor, 1993; Otémé *et al.*, 1996). Direct observations on the mode of semen release would be the key to elucidate the exact functions of SVS as it would be necessary to know how and when during the spawning process the secretion is released. However, thus is complicated as spawning occurs at night, and in grass containing muddy water which turbidity still increases by the spawning activities (Van der Waal, 1974; Bruton, 1979). In captivity African catfish do not spawn spontaneously and hormone treatment is

necessary for final oocyte maturation (Hogendoorn, 1979). Under histological aspects the seminal vesicle lobes and the spermatic ducts of the African catfish have a prominent layer of smooth muscle cells (Van den Hurk *et al.*, 1987). Contraction of this musculature can lead to simultaneous or separate release of semen and SVS. However, we exclude that semen and the viscous SVS are mixed in the spermatic duct: To mix the semen with the viscous SVS intense contractions of the spermatic duct would be required. In the lumen of the spermatic ducts no mixture of SVS and spermatozoa is visible by histological methods (Van den Hurk *et al.*, 1987).

On base of evidences obtained by our experimental methods the following functions of seminal vesicle secretion can be clearly excluded:
(1) Seminal vesicle secretion has no positive or stabilizing effects on sperm motility, sperm or egg fertility, or the process of sperm egg contact as suggested in other studies (Van den Hurk *et al.*, 1987, Chowdhury and Joy, 2001): (a) Pure seminal vesicle secretion completely inhibited the motility as the sticky secretion hindered spermatozoa in free swimming. When seminal vesicle secretion was diluted with water the rate of inhibition decreased with the concentration of the secretion. (b) Seminal vesicle secretion had not any stimulating or stabilizing effect on sperm fertility as with semen prediluted in seminal vesicle secretion the fertilization rates were very low. (c) Also the effect of seminal vesicle secretion on eggs was negative: When seminal vesicle secretion was mixed with the eggs the fertilization rate was zero, probably as the sticky secretion plugged the micropyle and the pores of the egg shell thus inhibiting the egg activation (cortical reaction, egg water uptake). (d) Seminal vesicle secretion had also a negative effect on the sperm egg contact. When semen, eggs, and seminal vesicle secretion were mixed simultaneously fertility was prevented indicating that the secretion itself did not act as a sperm guidance substance and also did not influence sperm egg interactions.
(2) SVS has no functions in adhering the eggs to substrates or in covering the eggs for mechanical protection or antibacterial defense. Generally, mucopolysacchrids and mucoproteins are responsible for the stickiness of benthic eggs of teleost fish (Riehl & Patzner, 1998). For the mentioned functions high quantities of SVS should be released to cover the eggs and the SVS should be stable in water. However, the volume of SVS released per mating cannot exceed 100 – 200 µl as in *Clarias gariepinus* the pairs mate up to 50 times in 1 - 2 hours (Aboul-Ela, 1973; Van der Waal, 1974) and the total amount of SVS collected per male is

only 1 – 2 ml (unpublished data). Therefore, this amount of SVS is not sufficient to cover the eggs.

(3) When semen and seminal vesicle secretion were premixed and the droplet overlaid with water spermatozoa were liberated out of the superficial layers in which the secretion coagulated and started to move one by one leading to delayed and non-homogenous motility activation. Delayed motility activation could lead to a constant supply with spermatozoa over a long time period. As the motility duration of the African catfish spermatozoa is very short (Mansour *et al.*, 2002), this may be an advantage to obtain high fertility. However, this hypothesis is doubtable as in teleost fish spermatozoa reach the micropyle randomly, the efficiency of sperm egg contact is low and therefore high numbers of spermatozoa are necessary to fertilize an egg (Hart, 1990). For a single spermatozoon the chance is very low to fertilize an egg unless additional sperm guidance mechanisms are established. The function of constant sperm supply was proposed for SVS of the grass goby, *Zosterisessor ophiocephalus*. Semen and SVS are supposed to form a layer adhering to the substrate on which the eggs are placed and from which the spermatozoa are liberated to swim into the micropyle (Ota *et al.*, 1996, Giulianini *et al.*, 2001). However, the African catfish does not spawn onto substrates (Bruton 1979), but the already fertilized eggs sink down and stick to the substrate with their microvilli rings (Riehl and Appelbaum, 1991).

It might be possible that seminal vesicle secretion has importance in the male and female communication during the prenuptial spawning behaviour. During these activities (see Bruton, 1979) one fixed sequence is obligatory and remarkable. The males make a U-shaped position around the head of the female (Bruton, 1979). In this position which lasts about 10- 20 sec, the mouth of the female touches the male urogenital papilla (Aboul-Ela *et al.*, 1973; Bruton, 1979). We consider it possible that SVS and also steroid glucuronides of the interstitial cells of the seminal vesicle (Resink *et al.*, 1989) are released during this behaviour and stimulate the egg release which occurs a few seconds later (Bruton, 1979). So it might be speculated that SVS is an indicator for the female to check the maturity stage of the male and its potential to fertilize the eggs. Release of semen was not observed directly but is considered to occur shortly before the egg release (Bruton 1979). Detailed experiments are in progress to test the influence of SVS on the spawning behavior.

REFERENCES

Aboul-Ela, I.; Amer, F.I. and Bolock, A.R. (1973): Studies on spawning and spawning behaviour of *Clarias lazera* in fish farms of the A.R. Egypt. Bull Zool Soc Egypt 25: 25–33.

Bruton, M.N. (1979): The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in lake Sibaya, South Africa, with a review of breeding in species of subgenus Clarias *(Clarias).* Trans Zool Soc Lond 35: 1–45.

Chowdhury, I. and Joy, K.P. (2000): Annual cyclic variations in some biochemical constituents of seminal vesicle and testis of the catfish *Heteropneustes fossilis* (Bloch): a study correlating plasma testosterone level. Acta Biol Hung 51: 53–62.

Chowdhury, I. and Joy, K.P. (2001): Seminal vesicle and testis secretions in *Heteropneustes fossilis* (Bloch): composition and effects on sperm motility and fertilization. Aquaculture 193: 355–371.

Giulianini, P.G.; Ota, D.; Marchesan, M. and Ferrero, E.A. (2001): Can goby spermatozoa pass through the filament adhesion apparatus of laid eggs. J Fish Biol 58: 1750–1752.

Hart, N.H. (1990): Fertilization in teleost fishes: Mechanisms of sperm-egg interactions. Internat Rev Cytol 121: 1–66.

Haylor, G.S. (1993): Controlled hatchery production of African catfish, *Clarias gariepinus* (Burchell): an overview. Aquacult Fisher Manag 24:245–252.

Hogendoorn, H. (1979): Controlled propagation of the African catfish, *Clarias lazera* (C & V). I. Reproductive biology and field experiments. Aquaculture 17: 323–333.

Lahnsteiner, F.; Seiwald, M.; Patzner, R.A. and Ferrero, E.A. (1992): The seminal vesicle of the male grass goby, *Zosterisessor ophiocephalus* (Teleostei, Gobiidae): Fine structure and histochemistry. Zoomorph 111: 239–248.

Lehri, G.K. (1967): The annual cycle in the testis of the catfish, *Clarias batrachus* (Linn.). Acta Anat 67: 135–154.

Mansour, N.; Lahnsteiner, F. and Berger, B. (2003): Metabolism of intratesticular spermatozoa of a tropical teleost fish (*Clarias gariepinus*). Comp Biochem Physiol B 135: 285–296.

Mansour, N.; Lahnsteiner, F. and Patzner, R.A. (2002): The spermatozoon of the African catfish: fine structure. motility. viability and its behaviour in seminal vesicle secretion. J. Fish Biol. 60: 545–560.

Mansour, N. and Lahnsteiner, F. (2003): A new technique for incubating small batches of African catfish eggs. North Am J Aquaculture 65: 155–157.

Ota, D.; Marchesant, M. and Ferrero, E.A. (1996): Sperm release behaviour and fertilization in grass goby. J Fish Biol 49: 246–256.

Otémé, Z.J.; Rodriguez, N.J.; Kouassi, C.K.; Hem, S. and Agnese, J.F. (1996): Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis* (Valenciennes, 1840). Aquacult Res 27: 805–813.

Resink, J.W.; Voorthuis, P.K.; Van den Hurk, R.; Peters, R.C. and Van Oordt, PGWJ. (1989): Steroid glucuronides of the seminal vesicle as olfactory stimuli in African catfish, *Clarias gariepinus.* Aquaculture 83: 153–166.

Riehl, R. and Appelbaum, S. (1991): A unique adhesion apparatus on the eggs of the catfish, *Clarias gariepinus* (Teleostei, Clariidae). Jap J Ichthyol 38: 191–197.

Riehl, R. and Patzner, R.A. (1998): Minireview: the modes of egg attachment in teleost fishes. Italian J Zool 65: 415–420.

Singh, M.S. and Joy, K.P. (1998): A comparative study on histochemical distribution of some enzymes related to steroid and glucuronide synthesis in seminal vesicle and testis of the catfish, *Clarias batrachus.* Zool Sci 15: 955–961.

Singh, M.S. and Joy, K.P. (1999): Annual correlative changes in some biochemical contents of seminal vesicle and testis in the catfish, *Clarias batrachus* (L). Zool Sci 16: 345–356.

Sneed, K.E. and Clemens, H.P. (1963): The morphology of the testes and accessory reproductive glands of the catfishes (Ictaluridae). Copeia 1963: 606–611.

Van den Hurk, R.; Resink, J.W. and Peute, J. (1987): The seminal vesicle of the African catfish, *Clarias gariepinus,* A histological, histochemical, enzyme-histochemical, ultrastructural and physiological study. Cell Tissue Res 247: 573–582.

Van der Waal BCW. (1974): Observations on the breeding habits of Clarias gariepinus (Burchell). J Fish Biol 6: 23–27.

Van Tienhoven, A. (1983): Reproductive Physiology of Vertebrates. Second edition, Cornell University Press, Ithaca.

Table 1: Sperm motility and average path velocity of African catfish in diluted seminal vesicle secretion.

Dilution ratio [1]	After 10 sec		After 20 sec		After 30 sec	
	Motility, %		Motility, %		Motility, %	
Dilution 1:50	15.3 ± 3.9^{c}	42.7 ± 9.3^{b}	0.0 ± 0.0^{d}	21.9 ± 4.2^{c}	0.0 ± 0.0^{d}	12.2 ± 3.1^{c}
Dilution 1:200	43.4 ± 3.4^{b}	81.6 ± 11.7^{a}	13.7 ± 2.5^{c}	53.2 ± 8.7^{b}	0.0 ± 0.0^{d}	44.9 ± 6.7^{b}
Dilution 1:400	$57.3 \pm 5.3^{a,b}$	99.5 ± 12.4^{a}	$24.9 \pm 4.5^{b,c}$	$70.3 \pm 7.9^{a,b}$	2.2 ± 0.9^{d}	49.8 ± 5.2^{b}
Dilution 1:800	75.1 ± 5.9^{a}	118.5 ± 16.2^{a}	39.2 ± 4.9^{b}	85.1 ± 10.4^{a}	$5.7 \pm 2.4^{c,d}$	54.3 ± 10.1^{b}
Water control	76.5 ± 6.1^{a}	122.5 ± 19.6^{a}	34.5 ± 3.7^{b}	79.4 ± 6.3^{a}	$4.5 \pm 1.5^{c,d}$	16.4 ± 3.2^{c}

Values are represent mean ± S.D., n = 8. Values with the same superscript letter are not significantly different, p>0.05.

[1] Seminal vesicle secretion diluted in distilled water

Table 2: Effect of seminal vesicle secretion on semen fertility

Treatment of semen	Fertilization process	Hatching, %
Dilution in SVS for 30 sec	Addition of eggs and water	0.0 ± 0.0^{b}
Dilution in SVS and water for 1 min	Addition of eggs	0.0 ± 0.0^{b}
Dilution in SVS and water for 5 min	Addition of eggs	0.0 ± 0.0^{b}
Control, untreated testicular semen	Addition of eggs and water	57.0 ± 8.5^{a}

Values are represent mean ± S.D., n = 6. Values with the same superscript letter are not significantly different, p>0.05.

Table 3: Effect of seminal vesicle secretion on egg fertilizability.

Treatment of eggs	Fertilization process	Hatching, %
Incubation in SVS for 30 sec	Addition of semen and water	0.0 ± 0.0^{b}
Incubation in SVS and water for 2 min	Addition of semen	0.0 ± 0.0^{b}
Incubation in water for 2 min	Addition of semen	46.4 ± 5.6^{a}
Control, untreated eggs	Addition of semen and water	57.0 ± 8.5^{a}

Values are represent mean ± S.D., n = 6. Values with the same superscript letter are not significantly different, p>0.05.

Table 4: Effect of seminal vesicle secretion on sperm egg contact

Fertilization process	Gamete activation	Hatching, %
Eggs, semen and SVS for 2 min	Water	0.0 ± 0.0^{a}
Eggs and semen for 5 sec	SVS diluted 1:50 in water	1.0 ± 1.4^{a}
Eggs and semen for 5 sec	SVS diluted 1:400 in water	16.8 ± 5.4^{b}
Eggs and semen for 5 sec	SVS diluted 1:800 in water	25.0 ± 6.9^{b}
Eggs and semen for 5 sec	Water	57.0 ± 8.5^{c}

Values are represent mean ± S.D., n = 6. Values with the same superscript letter are not significantly different, p>0.05.