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EFFECT OF SPERM EXTENDER AND DILUTION RATIO ON THE SPERM MOTILITY, FERTILITY, AND HATCHING RATES OF DEPIK FISH *RASBORA TAWARENSIS* (PISCES: CYPRINIDAE) EGGS

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ABSTRACT

There is no known artificial breeding method for depik fish *Rasbora tawarensis*. Sperm extender and the dilution ratio are crucial factors in the artificial breeding of fish. The current study aimed to assess the effect of several common fish sperm extenders and its dilution ratio on sperm characteristics and fertilization of depik fish eggs. Two series of experiments were performed to find the best extender and its dilution ratio. In the first experiment, five types of extenders were tested: Ringer's solution, physiological solution, coconut water, sugarcane water, and roomie water. The second experiment studied four dilution levels of sperm and extender (v/v): 1:20, 1:30, 1:40, and 1:50. Every treatment was conducted in four replicates and the experiments were conducted consequently so that the findings in the first experiment were applied in the second. The first experiment showed that Ringer's is the best extender for depik sperm with sperm motility, fertilization and hatching rates of 71.00%, 69.30%, and 53.66%, respectively; and the second experiment revealed that Ringer's at dilution ratio 1:40 gave higher sperm motility, fertilization, and hatching rates of 74.66, 70.33, and 59.00%, respectively. In conclusion, Ringer's solution was the most favorable extender for sperm which yielded best fertilization results at 1:40 dilution ratio (sperm:extender, v/v) in depik fish.

Key words: depik fish, sperm, fertilization, Ringer's solution, physiological solution

INTRODUCTION

Depik fish, *Rasbora tawarensis*, is endemic to Lake Laut Tawar, Takengon City, Indonesia. The population has dropped drastically in the last 20 years (1, 2) and that is why it has been listed on the IUCN critically endangered species of fish. The decline in the depik population is thought to be caused by overfishing, introduced fish species, habitat pertubation, and climate change (3, 4, 5). Therefore, the conservation program of the depik is mainly needed. Basic information on bioecology of this species have been well decomented. Muchlisin et al. (6, 7, 8) has studied the genetic and morphometric differences among the Rasbora group in the Lake Laut Tawar. According to Muchlisin (9), depik fish are widely distributed in Lake Laut Tawar, where adult fish are found in deep water far from the coast, while small fish are found in shallow water close to

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the coast. Depik is a synchronous group that spawns several times a year, with its peak spawning season in September (10, 11). The fish is planktivorous and they feed on zooplankton and phytoplankton (12). Depik fish migrate from the lake to small tributaries around lakes (anadromous) and they lay eggs on the bottom of gravelly waters, clear water, and slow currents (13).

The breeding of depik fish has been initiated by several researchers. Muchlisin et al. (14) studied the application of intracellular cryoprotectant DMSO for cryopreservation of depik sperm. Eriani et al. (15) and Muchlisin et al. (16) have researched antioxidants for cryopreservation of depik sperm reporting the glutathione as most suitable for depik sperm. However, these studies did not research extenders for sperm dilution of depik fish *R. tawarensis*.

In artificial breeding, the extender is very crucial and determines the success of the spawning program (17, 18) along with the dilution ratio (19, 20). The extender is a medium used to dilute sperm achieving larger volumes for artificial breeding (21). This medium can maintain and prevent the initiation of sperm activation and motility during the collection, handling, and storage (22, 23, 24). Ringer and physiological solutions are the most common extenders in the fish breeding program. Besides, several natural materials such as coconut water, sugarcane, and roomie water have been explored for diluting fish sperm (19, 23). The extender must be non-toxic to sperm and the suitability of extender is species-dependent. In addition, the extender provides nutrients and energy to the sperm during storage (22). The electrolyte composition of the extender must be similar to that of the seminal plasma (25) in order to have the same pH and to be isotonic.

The current study aimed to determine the best sperm extender and the most suitable dilution ratio for depik fish sperm.

MATERIAL AND METHODS

Experimental design

The completely randomized design was used in this study. The study was divided into two series of experiments. The first experiment was designed to assess the effect of different semen extenders on the motility, fertility, and hatching rate of depik eggs. Two chemical base extenders (Ringer and physiological solutions), and three natural base extenders (coconut water, sugarcane waters, and roomie water) were used in this experiment. The second experiment was designed to determine the effect of different sperm dilution rates (1:20, 1:30, 1:40, and 1:50, sperm: extender, v/v) with the most suitable sperm extender from the first experiment. Every dilution rate was tested in three replicates. The fresh sperm was used as a control group in each experiment. The experiment was conducted at the Lukop Badak Hatchery, Takengon City, Aceh Province, Indonesia. The study was conducted complying to the standard ethical procedure for animals used in scientific research, approved by the University Syiah Kuala (Ethic Code Number 958/2015).

Extender preparation

The Ringer solution contained 7.5 g NaCl L^{-1}, 0.2 g KCl L^{-1}, 0.2 g $CaCl_2$ L^{-1}, 0.2 g $NaHCO_3$ L^{-1}, and 7 g L^{-1} glucose. The physiological solution is an intravenous solution with a concentration of 0.9% NaCl. The coconut water, sugarcane water, and roomie water were purchased from the local market in Aceh Besar district, Indonesia. These solutions were freshly prepared and were kept and transported to the laboratory at 4 °C on crushed-ice in an icebox. The water solutions were filtered using a whatman paper before being used in the experiment.

Broodstock

The broodfish was caught during spawning migration in Lake Laut Tawar, Takengon City, Indonesia. Dedeuseun traps were used to bar the lake tributaries with framed nets. A total of 25 males and 5 females matured broodfish were collected and transported to Lukop Badak Hatchery in Takengon City. They were domesticated for two weeks. The length and body weight range of the male broodfish were 75.98-113.31 mm and 2.98-7.32 g, respectively. In female broodstock they were 121.32-150.33 mm and 3.52-11.83 g, respectively. The broodfish was domesticated in concrete tanks with continuously flowing water. They were fed twice per day (8 a.m. and 6 p.m.) with a commercial pelleted feed produced by Central Protein Prima (CCP) with 35% crude protein.

Sperm collection and initial evaluation of sperm quality

A total of 25 matured males were intramuscularly injected with 0.30 ml kg^{-1} of Ovaprim to induce spermiation process. The sperm was collected 8 hours after injection by gentle abdominal pressure. The genital pore was cleaned with cloth to avoid contamination by water and fish urine. The sperm

was taken with a sterile syringe which was then transfered into a glass tube and was kept in an icebox (4 °C). The sperm was analyzed for initial quality including pH, color, and motility. Only good quality sperm with motility higher than 60% was used for the experiment (26).

The sperm was diluted with the extender at dilution rate (1:20, sperm:extender, v/v) according to Muchlisin et al. (14) and was then kept in an icebox (4 °C) for six hours before being analyzed for motility, fertility, and hatching rates. One drop of diluted sperm and two drops of tap water were placed on a glass slide. They were gently mixed to activate the sperm and were covered with a cover-glass. Sperm motility was observed with a stereo microscope (Zeiss Primo, Switzerland) at 100-400x magnifications.

Fertilization and egg incubation processes

A total of five late mature female broodstock were taken from the broodstock tank. The genital pore was cleaned using a cloth to avoid contamination by water and urine. The eggs were collected by applying finger pressure from the abdominal to the genital pore, and the eggs were placed in a plastic jar which was kept in an icebox (4 °C). Then, 1 ml of eggs were taken randomly and mixed with 0.25 ml of diluted sperm in a plastic jar (4:1, egg:sperm). Two mililiters of tap water were added to the basin and mixed homogeneously by using soft feather. They were in contact for 5 min (23, 24). A total of 100 eggs were taken randomly for the basin then incubated in the aquarium glass tank at a room temperature of 28-29 °C. The aquarium was equipped with a portable aerator (Jebo P-30, China). The success of the fertilization was recorded 6 hours after incubation. The experiment was performed in three replications.

The unfertilized eggs were identified by their opacity and were removed from the aquarium to avoid fungus contamination (27, 28). The hatching eggs were monitored in 2-hour intervals for 72 hours. The best extender which was found in the first experiment was used to dilute the sperm. The fertilization and hatching rate rates were calculated based on Muchlisin et al. (14) as follows:

Fertilization rate (%) = (total of fertilized eggs/ total of incubation eggs) x 100

Hatching rate (%) = (total hatched eggs/total of incubation eggs) x 100

Data analysis

The data on sperm motility, fertility, and hatching rates were calculated for mean values and standard deviations, then were subjected to one-way Analysis of Variant (one-way ANOVA) followed by Duncan's multiple range test using the SPSS software ver. 20.0.

RESULTS

The fresh sperm had a milky white color with an average pH of 6.55 and high viscosity. The fresh sperm had a good motility of 75.00% which was deemed suitable for the experiment. The results showed that sperm motility, egg fertility, and hatching rates decreased after storage for six hours in all extenders. The first experiment showed that different extenders have significant effect on sperm motility, fertility, and hatching rates of eggs ($p<0.05$). The highest sperm motility and egg fertility were recorded in sperm diluted with Ringer's solution. However, these values were not significantly different from those obtained in the physiological solution and control (the fresh sperm). While the higher hatching rates were recorded in sperm diluted with the physiological solution, it was significantly different only with the roomie water group. The lowest fertilization and hatching rates were recorded in sperm diluted with roomie water (Table 1).

Table 1. Sperm motility, fertility, and hatching rates of depik *Rasbora tawarensis* eggs based on the type of extender

Extender	Motility (%)	Fertility (%)	Hatching (%)
Control (fresh sperm)	73.00±1.00^{c}	73.00±1.00^{c}	63.33±0.31^{b}
Ringer's	71.00±1.00^{c}	69.30±0.57^{c}	53.66±4.72^{b}
Physiological	67.33±2.08^{bc}	64.66±13.80^{bc}	63.66±13.80^{b}
Coconut water	64.33±4.04^{ab}	51.00±8.54^{b}	51.00±8.54^{b}
Sugarcane water	60.33±1.52^{a}	51.00 ±8.54^{b}	51.00±8.54^{b}
Roomie water	60.00±2.64^{a}	32.33±2.50^{a}	32.33±2.51^{a}

The mean values (±SD) with different superscript in the same column are significantly different ($p<0.05$)
The values are derived from three replicates

Table 2. Sperm motility, fertility and hatching rates of depik *Rasbora tawarensis* eggs based on dilution ratio levels in Ringer's. Treatment was conducted in three replicates

Dilution ratio (v/v)	Motility (%)	Fertility (%)	Hatching (%)
1:20	64.00±3.60^{a}	63.00±7.02^{c}	47.33±2.64^{bc}
1:30	67.66±5.85^{ab}	52.33±5.13^{b}	46.33±7.50^{b}
1:40	74.66±3.50^{b}	70.33±8.54^{c}	59.00±1.52^{c}
1:50	70.00±1.00^{ab}	36.67±3.20^{a}	31.33±4.16^{a}

The mean values (±SD) with different superscript in the same column are significantly different ($p<0.05$)

The second experiment showed that different dilution rates of Ringer's solution produced a significant effect on sperm motility, fertility, and hatching of eggs ($p<0.05$). The highest values for all parameters were recorded for the dilution ratio of 1:40 (Table 2). Sperm motility in the dilution ratio of 1:40 was not significantly different with that in 1:30 and 1:50. Fertility and hatching values achieved in the 1:40 dilution ratio were not significantly different from those observed in the 1:20 ratio. The significantly lowest values were achieved at the dilution ratio of 1:50.

DISCUSSION

In the first experiment, we found that Ringer's solution gave the best results compared to other extenders. This finding indicates that Ringer's is a suitable extender for depik sperm. This is probably because Ringer's solution could maintain the viability of the sperm and less energy was expended by spermatozoa during preparation and storage. This yielded higher post-activation motility resulting in higher fertilization and hatching rates. The least positive results were recorded in sperm samples diluted with coconut water, sugarcane water, and roomie water probably due to low pH (pH 5.0-5.6) during the fertilization process. The pH dropped from 5.0 to 4.9 with the addition of coconut water, from 5.6 to 5.1 for sugarcane water, and 5.0 to 4.8 for roomie water. Ringer's and physiological solution had pH of 6.8 and 6.9, respectively, without alterations in the pH level. These extenders yielded better results compared to coconut water, sugarcane water, and roomie water which are natural materials with higher glucose (29, 30) and other microbial contents that can be easily fermented, thus reducing the pH.

Zhou et al. (31) and Dhumal et al. (32) reported that sperm motility is strongly depending on the pH of the medium. Gao et al. (33) stated that declines in fertility are probably caused by low pH caused by the extender. In general, sperm is inactive in the seminal plasma which has a pH range between 7.2 to 8.2 (34). Therefore, pH of the diluent should be similar or close to the pH of the seminal plasma (35). This condition makes the sperm preserve their energy and results in higher motility in post-activated sperm. Besides, the presence of ions such as K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+} also plays an important role in the diluent (36). The concentration of ions influences the osmolarity and osmotic pressure of the diluent (37). Therefore, the extender should have similar composition and osmolarity with the seminar plasma (38). In this study, Ringer's solution contained Na^{+}, K^{+}, and Ca^{2+}, and the physiological solution contained only Na^{+}. The ionic composition of the natural extenders was not examined. According to Barlina et al. (30), the coconut water contains sucrose, fructose, magnesium, and potassium. Sodium plays a vital role as a buffer in stabilizing pH, osmotic pressure, and electrolytes of the diluent. However, the presence of higher potassium concentrations in the diluent leads to reduced sperm motility (39).

The second experiment determined that the dilution ratio produced a significant effect on the success of sperm motility, fertilization, and hatching rates. In general, sperm motility, fertilization and hatching rates increased from a dilution ratio of 1:20 to 1:40, then slightly decreased when the dilution ratio increased to 1:50. Therefore, the optimum level of dilution ratio for depik fish was 1:40 (sperm: extender, v/v). Aside from being affected by the extender, the success of fertilization of fish eggs is also affected by sperm concentration or dilution ratio (40). The optimal sperm dilution ratio is species-dependent. The optimal dilution rate of 1:20 was reported for grouper (25), bagrid catfish *Mystus nemurus* (27), and for African catfish *Clarias gariepinus* (24). According to Zhou et al. (41), there is a strong correlation between dilution ratio and sperm motility. Higher dilution ratio results in lower sperm density but higher sperm motility. However, the higher dilution ratio may result in lower fertilization rate as it was recorded in the current study.

CONCLUSION

It was concluded that Ringer's solution at 1:40 dilution rate (sperm:extender) was most effective for artificial breeding of depik fish *R. tawarensis* which is endemic and threatened fish species in Lake Laut Tawar, Indonesia.

CONFLICT OF INTERESTS

The authors declare that they have no known conflict of interest in the conduction of the current study.

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AUTHORS' CONTRIBUTION

ZAM conceived the research idea, research methodology and proposal development, financial acquisition, and gave final approval of the manuscript. DFA, PIS, LSH, SM, IH, KE managed the broodstock, conducted the experiments, gathered the data, prepared the research reports and processed data. NF, AAM, MNS, FKK, and MK were involved in data analysis, manuscript writing, and proofreading.

REFERENCES

1. Muchlisin, Z.A., Fadli, N., Rudi, E., Mendo, T., Siti-Azizah, M.N. (2011). Estimation of production trend of the depik, Rasbora tawarensis (Teleostei, Cyprinidae), in Lake Laut Tawar, Indonesia. AACL Bioflux 4(5): 590-597.
2. Muchlisin, Z.A., Hasri, I., Batubara, A.S. (2018). A mini review on endemic and threatened Fish Rasbora tawarensis in Lake Laut Tawar, Indonesia. IOP Conf Ser. Earth Environ Sci. 2016, 012045. https://doi.org/10.1088/1755-1315/216/1/012045
3. Muchlisin, Z.A. (2008). The endangered depik fish. Buletin Leuser 6(17): 9-12. [In Indonesian]
4. Muchlisin, Z.A. (2011). Policy analysis of introducing alien species of fish in inland waters of Aceh province. Jurnal Kebijakan Sosial Ekonomi Kelautan dan Perikanan. 1(1): 79-89. [In Indonesian] https://doi.org/10.15578/jksekp.v1i1.9256
5. Muchlisin, Z.A. (2012). First report on introduced freshwater fishes in the waters of Aceh, Indonesia. Arch Polish Fish. 20(2): 129-135. https://doi.org/10.2478/v10086-012-0015-1
6. Muchlisin, Z.A., Thomy, Z., Fadli, N., Sarong, M.A., Siti-Azizah, M.N. (2013). DNA barcoding of freshwater fishes from Lake Laut Tawar, Aceh Province, Indonesia. Acta Ichthyol Piscat. 43(1): 21-29. https://doi.org/10.3750/AIP2013.43.1.04
7. Muchlisin, Z.A., Fadli, N., Siti-Azizah, M.N. (2012). Genetic variation and taxonomy of Rasbora group (Cyprinidae) from Lake Laut Tawar, Indonesia. J Ichthyol. 52(4): 284-290. https://doi.org/10.1134/S0032945212030034
8. Muchlisin, Z.A. (2013). Morphometric varations of Rasbora group (Pisces: Cyprinidae) in Lake Lawar, Aceh Province, Indonesia. Hayati J Biosci. 20(3): 138-143. https://doi.org/10.4308/hjb.20.3.138
9. Muchlisin, Z.A. (2013). Distributions of the endemic and threatened freshwater fish depik, Rasbora tawarensis Weber & de Beaufort, 1916 in Lake Laut Tawar, Aceh Province, Indonesia. Songklanakarin J Sci Technol. 35(4): 483-488.
10. Muchlisin, Z.A., Musman, M., Siti-Azizah, M.N. (2010). Spawning seasons of Rasbora tawarensis (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh Province, Indonesia. Reprod Biol Endocrinol. 8, 49. https://doi.org/10.1186/1477-7827-8-49 PMid:20482777 PMCid:PMC2887878
11. Muchlisin, Z.A., Musman, M., Fadli, N., Siti-Azizah, M.N. (2011). Fecundity and spawning frequency of Rasbora tawarensis (Pisces: Cyprinidae) an endemic species from Lake Laut Tawar, Aceh, Indonesia. AACL Bioflux 4(3): 273-279.
12. Muchlisin, Z.A., Rinaldi, F., Fadli, N., Adlim, M., Siti-Azizah, M.N. (2015). Food preference and diet overlap of two endemic and threatened freshwater fishes, depik (Rasbora tawarensis) and kawan (Poropuntius tawarensis) in Lake Laut Tawar, Indonesia. AACL Bioflux. 8(1): 40-49.
13. Muchlisin, Z.A. (2011). Spawning sites of depik, Rasbora tawarensis (Teleostei, Cyprinidae) in Lake Laut Tawar, Indonesia. Proceedings of the Ann. Int. Conf. Syiah Kuala Univ.-Life Sci. Engin. Chapt. 1(1): 95-99. https://doi.org/10.1186/1477-7827-8-49 PMid:20482777 PMCid:PMC2887878
14. Muchlisin, Z.A., Sarah, P.I., Aldila, D.F., Eriani, K., Hasri, I., Batubara, A.S., Nur, F.M. et al. (2020). Effect of dimethyl sulfoxide (DMSO) and egg yolk on sperm motility, fertility and hatching rates of depik Rasbora tawarensis (Pisces: Cyprinidae) eggs after short-term cryopreservation. Aquac Res. 51(4): 1700-1705. https://doi.org/10.1111/are.14516

15. Eriani, K., Mustaqim, M., Hasri, I., Amalia, R., Azhar, A., Nur, F.M., Muchlisin., Z.A. (2022). Effect of fetal bovine serum on the sperm quality of depik (Rasbora tawarensis) after short-term cryopreservation. Indian J Anim Res 56(7): 843-847. https://doi.org/10.18805/IJAR.B-1379

16. Muchlisin, Z.A., Afriani, D., Eriani, K., Hasri, I., Nur, F.M., Maulida, S., Handayani, L.S., et al. (2023). Improvement of sperm quality of the depik fish, Rasbora tawarensis, after cryopreservation using antioxidant. Cryo Letters 44(1): 13-19. https://doi.org/10.54680/fr23110110312 PMid:36629837

17. Stefanov, R.G., Anev, G., Abadjieva, D.V. (2015). Effect of different extenders and storage periods on motility and fertility of ram sperm. Mac Vet Rev. 38(1): 85-89. https://doi.org/10.14432/j.macvetrev.2014.12.036

18. Maulida, S., Eriani, K., Fadli, N., Nur, F.M., Handayani, L.S., Muchlisin, Z.A. (2022). The effect of extender on the quality of climbing perch (Anabas Testudineus) sperm after refrigerated storage. Aquac Res. 53(12): 4497-4504. https://doi.org/10.1111/are.15947

19. Adami, Y., Fadli, N., Nurfadillah, N., Eriani, K., Jalil, Z., Muchlisin, Z.A. (2016). A preliminary observation on the effect of sperm extenders on the fertilization and hatching rates of seurukan fish (Osteochilus vittatus) eggs. AACL Bioflux. 9(2): 300-304.

20. Muchlisin, Z.A. (2005). Review: Current status of extenders and cryoprotectants on fish spermatozoa cryopreservation. Biodiversitas 6(1): 66-69.

21. Chapman, F.A. (2016). A semen extender for the short-term storage of fish sperm. Program in Fisheries and Aquatic Sciences, School of Forest Resources and Conservation. UF/IFAS Extension, University of Florida, USA https://doi.org/10.32473/edis-fa193-2016

22. Ahn, J.Y., Park, J.Y., Lim, H.K. (2018). Effects of different diluents, cryoprotective agents, and freezing rates on sperm cryopreservation in Epinephelus akaara. Cryobiology 83, 60-64. https://doi.org/10.1016/j.cryobiol.2018.06.003 PMid:29885288

23. Muchlisin, Z.A., Nadiya, N., Nadiah, W.N., Musman, M., Siti-Azizah, M.N. (2010). Preliminary study on the natural extenders for artificial breeding of African catfish Clarias gariepinus (Burchell, 1822). AACL Bioflux 3(2): 119-124.

24. Handayani, L.S., Muchlisin, Z.A., Eriani, K., Maulida, S., Rahayu, S.R., Nur, F.M. (2023). Exploration of the natural extender for dilution of walking catfish Clarias batrachus sperm in refrigerated storage. IOP Conf Ser. Earth Environ Sci. 1221(1): 012010. https://doi.org/10.1088/1755-1315/1221/1/012010

25. Agarwal, N.K. (2011). Cryopreservation of fish semen. In M. Thapliyal, A. Thapliyal (Eds.) Himalayan Aquatic Biodiversity Conservation and New Tools in Biotechnology. JP Bhatt Transmedia Publication. Srinagar, India

26. Maulida, S., Eriani, K., Fadli, N., Siti-Azizah, M.N., Kocabas, F.K., Kocabas, M., Wilkes, M., et al. (2024). Effect of type and concentration of antioxidant on sperm motility, viability, and DNA integrity of climbing perch Anabas testudineus Bloch, 1792 (Pisces: Anabantidae) post-cryopreservation. Cryobiology 114, 104851. https://doi.org/10.1016/j.cryobiol.2024.104851 PMid:38237749

27. Muchlisin, Z.A., Hashim, R., Chong, A.S.C. (2004). Preliminary study on the cryopreservation of bagrid catfish (Mystus nemurus) spermatozoa; the effect of extender and cryoprotectants on the motility after short-term storage. Theriogenology 62(1-2): 25-34. https://doi.org/10.1016/j.theriogenology.2003.05.006 PMid:15159098

28. Muchlisin, Z.A., Nadiah, W.N., Nadiya, N., Fadli, N., Hendri, A., Khalil, M., Siti-Azizah, M.N. (2015). Exploration of natural cryoprotectants for cryopreservation of African catfish, Clarias gariepinus, Burchell 1822 (Pisces: Clariidae) spermatozoa. Czech J Anim Sci. 60(1): 10-15. https://doi.org/10.17221/7906-CJAS

29. Muthmainnah, C.R., Eriani, K., Hasri, I., Irham, M., Batubara, A.S., Muchlisin, Z.A. (2018). Effect of glutathione on sperm quality after short-term cryopreservation in seurukan fish Osteochilus vittatus (Cyprinidae). Theriogenology 122, 30-34. https://doi.org/10.1016/j.theriogenology.2018.08.024 PMid:30227301

30. Barlina, R., Karouw, S., Towaha, J., Hutapea, R. (2007). Effect of coconut water and coconut water ratio and storage time on quality of the coconut drink powder. J Littri 13(12): 73-80 [In Indonesian]

31. Zhou, J., Chen, L., Li, J., Li, H., Hong, Z., Xie, M., Chen, S., Yao, B. (2015). The semen pH affects sperm motility and capacitation. Plos One 10(7): e0132974. https://doi.org/10.1371/journal.pone.0132974 PMid:26173069 PMCid:PMC4501804

32. Dhumal, S.S., Naik, P., Dakshinamurtyhy, S., Sullia, K. (2021). Semen pH and its correlation with motility and count - A study in subfertile men. JBRA Assist Reprod. 25(2): 172-175. https://doi.org/10.5935/1518-0557.20200080 PMid:33507718 PMCid:PMC8083857

33. Gao, Y., Kim, S.K., Lee, J.Y. (2011). Effects of pH on fertilization and the hatching rates of far eastern catfish (Silurus asotus). Fish Aquat Sci. 14(4): 417-420. https://doi.org/10.5657/FAS.2011.0417

34. Williot, P., Kopeika, E.F., Goncharov, B.F. (2000). Influence of testis state, temperature and delay in semen collection spermatozoa motility in the cultured siberian sturgeon (Acipenser baeri, Brandt). Aquaculture 189(1-2): 53-61. https://doi.org/10.1016/S0044-8486(00)00358-6

35. Wilson-Leedy, J.G., Kanuga, M.K., Ingermann, R.L. (2009). Influence of osmolality and ions on the activation and characteristics of zebrafish sperm motility. Theriogenology 71(7): 1054-1062. https://doi.org/10.1016/j.theriogenology.2008.11.006 PMid:19185341

36. Alavi, S.M.H., Cosson, J. (2006). Sperm motility in fishes. (II) Effects of ions and osmolality: A review. Cell Biol Int. 30(1): 1-14. https://doi.org/10.1016/j.cellbi.2005.06.004 PMid:16278089

37. Butts, I.A.E., Alavi, S.M.H., Mokdad, A., Pitcher, T.E. (2013). Physiological functions of osmolality and calcium ions on the initiation of sperm motility and swimming performance in redside dace Clinostomus elongatus. Comp Biochem Physiol A Mol Integr Physiol. 166(1): 147-157. https://doi.org/10.1016/j.cbpa.2013.05.011 PMid:23688507

38. Lim, H.K., Le, M.H. (2013). Evaluation of extenders and cryoprotectants on motility and morphology of longtooth grouper (Epinephelus bruneus) sperm. Theriogenology 79(5): 867-871. https://doi.org/10.1016/j.theriogenology.2013.01.004 PMid:23391353

39. Adipu, Y., Sinjal, H., Watung, J. (2011). The effect of extender ratio on the sperms motility, fertilization and hatching rate of catfish, Clarias sp. J Perikanan dan Kelautan Tropis 7(1): 48-55. https://doi.org/10.35800/jpkt.7.1.2011.16

40. Suquet, M., Dreanno, C., Fauvel, C., Cosson, J., Billard, R. (2001). Cryopreservation of sperm in marine fish. Aquac Res. 31, 231-243. https://doi.org/10.1046/j.1365-2109.2000.00445.x

41. Zhou, J., Chen, L., Li, J., Li, H., Hong, Z., Xie, M., Chen, S., Yao, B. (2015). The semen pH affects sperm motility and capacitation. PloS One 10(7): e0132974. https://doi.org/10.1371/journal.pone.0132974 PMid:26173069 PMCid:PMC4501804

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