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Endemic Fish Conservation: Utilization of Cryopreservation Technology with Fructose in Red Bader Fish (*Puntius bramoides*) Sperm

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Abstract

Cryopreservation in red Bader fish is needed for the conservation and development of gamete cell storage. It is a chemical compound that can prevent cell or tissue damage due to freezing. In addition, dimethyl sulfoxide can penetrate cells quickly during equilibration. This research aimed to study reproductive biology and analyze fructose's ability as an extender in the Red Bader fish sperm cryopreservation process. The cryopreservation process was conducted at the Artificial Insemination Center, Singosari. The test fish were obtained from the Freshwater Cultivation Development Center, Umbulan then they were reared for 2 months to get the level of gonad maturity. This study was designed using a completely randomized design (CRD) which consisted of three treatments and three replications. The treatment given was the use of fructose extenders with different percentages i.e., 0.2%, 0.4%, and 0.6%. The results showed that the sperm characteristics of the red Bader fish (*P. bramoides*) had a volume of 3.18 mL, a pH value of 7.39, a milky white sperm color, a sperm concentration of 3.5x109 cells/mL, a motility value of 81.67%, and a viability of 85 %. The best type of fructose extender with 0,6% dimethyl sulfoxide concentration has a motility value of 38,33% post-cryopreservation and 36,67% post-cryopreservation viability. The type of extender affects the sperm quality of angry Bader fish during the cryopreservation process, the type of extender obtained was fructose with the best concentration of 0,6% dimethyl sulfoxide with the highest motility and viability values.

INTRODUCTION

Indonesia is known as one of the countries with the highest biodiversity in the world, including fish. Based on data recorded in Fishbase, Indonesia has 4,629 native fish species, 133 species of which are endemic (Froese and Pauly, 2018). It is estimated that 1,275 fish species in Indonesia have been included in the list of endangered fish categories (IUCN, 2020), including red Bader fish (*P. bramoides*). Red Bader fish is a freshwater fish and is an endemic fish species from Indonesia and is widely distributed in Kalimantan,

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Java, Cambodia, Malaysia, Thailand, and Vietnam (Murdy *et al*., 1994). The diversity of freshwater fish species in Indonesia ranks third richest in the world with 120 endemic fish species. Endemic fish are fish that only exist in one particular place. This sets goals in conservation activities (Syafei, 2017).

Conservation is essential to protect species-rich habitats and threatened endemic species (Arthington *et al*., 2016). The use of cryopreservation technology in fish has enormous value because populations can be protected from extinction due to disease, natural disasters, or overexploitation (Afriani *et al*., 2021). Conservation efforts to prevent the extinction of the red Bader fish are cryopreservation. Cryopreservation is a biological activity to store biological materials unchanged for centuries with the ability to restore cell function after the thawing process (Bozkurt, 2018). Research on cryopreservation of fish oocytes and embryos is still for renewal and is still in the early stages (Torres *et al*., 2016). The cryopreservation process requires a cryoprotectant that serves as a protector of sperm or embryo during the freezing process, and glucose-type extenders are very efficient in protecting the plasma membrane of sperm during the clotting process (Dietrich *et al*., 2014; Muthmainnah *et al*., 2019) Cryopreservation is used to preserve sperm for later use, to reduce the damage to cells can be used additional extenders that contain sugar (Ciereszko *et al*., 2014), one of which the use of fructose showed higher sperm motility (Arifiantini *et al*., 2009).

Cryoprotectants are chemical compounds that can prevent damage to a cell or tissue due to the freezing process (Bhattacharya, 2018). In the cryopreservation process, the cryoprotectant is needed to protect sperm cells from cold and hot temperatures (Muchlisin *et al*., 2015). The cryoprotectant material that can be used is Dimethyl sulfoxide (DMSO). DMSO solution is effective for sperm preservation as a cryopreservation agent (Sarder *et al*., 2012), DMSO as a cryoprotectant can significantly increase sperm motility of preserved fish (Abinawanto *et al*., 2011), DMSO is successful in sperm cryopreservation in other freshwater species (Irawan *et al*., 2010). The success of sperm cryopreservation is highly dependent on the cryopreservation method or protocol applied, including the suitability of the cryoprotectant concentration (Murgas *et al*., 2014). Each type of fish has a different concentration of dimethyl sulfoxide as a cryoprotectant, as in the study by Danang *et al*. (2012), the best concentration of dimethyl sulfoxide as a cryoprotectant in belida fish sperm at 20%, research by Lutfi (2010), in African catfish at 15% and in tor soro fish at 5% (Zairin *et al*., 2005).

This research aimed to study reproductive biology and analyze fructose's ability as an extender in the Red Bader fish sperm cryopreservation process.

METHODOLOGY

Ethical Approval

The author stated that the red Bader fish used in this study had been killed in the right way and the cessation process was carried out when the fish were completely dead.

Place and Time

The cryopreservation process was carried out at the Reproduction Laboratory of Brawijaya University, Singosari Center for Artificial Insemination. The red Bader fish sperm was obtained from the broodstock of the Umbulan Freshwater Cultivation Development Center. The thawing process and the observation of Bader fish sperm are carried out by the Fish Cultivation Laboratory which was held in March-June 2015.

Research Materials

The materials used in this study were: male and female broodstock of red Bader fish, ovaprim (Syndel, Canada), 0.2% eosin negrosin (Hi-Tech Cyrobank, India) dimethyl sulfoxide (Arkema, LACQ, France), egg yolk, ringer's solution

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aquadest (NaCl, KCl, $CaCl_2$, and $NaHCO_3$ dissolved in aquabides), 0.4% fructose, liquid nitrogen, tissue, aluminum foil, 70% alcohol. The tools used were incubator (IN 55Plus, Memmert Germany) inverted microscope, 0.25 mL mini straw (Minitube Slovakia), canister, liquid nitrogen container, styrofoam, beaker glass, thermos, filter paper (No 42, Whatman England) infusion tube, petri dish, 250 mL Erlenmeyer, surgical instruments, measuring cup 10 mL, dropper, thermometer, volumetric pipette, syringe, bowl, micropipette, object glass, cover glass, hemocytometer, hand tally counter, quill, aerator, and aquarium, tray.

Research Design

The research method was designed using a Completely Randomized Design (CRD) consisting of three treatments and three replications. The treatment given was the use of a fructose extender in the cryopreservation process of red Bader fish sperm with different concentrations consisting of 0.2%, 0.4%, and 0.6 (Condro *et al*., 2012). Parameters observed during the study included motility, viability, and sperm characteristics of red Bader fish.

Work Procedure

The test fish used was red Bader fish (*P. bramoides*) which had matured gonads with a size of 10-15 cm, obtained from the Umbulan Freshwater Cultivation Center. Sperm collection is done by stripping. Each treatment requires 0.25 ml of fresh sperm, with the number of treatments of gonadal-ripe red Bader fish broodstock, and abdominal stripping to obtain sperm. Sperm samples obtained from each male of broodstock are then examined for sperm quality. Macroscopic observation of sperm characteristics included color, pH, concentration, and volume of sperm. Examination of sperm motility and viability of Bader fish sperm are dripped as much as one drop on the object glass and the glass cover is attached. On the edge of the glass cover, distilled water is dripped to see the movement of sperm under a microscope. The motility assessment was based on the motility assessment criteria.

Sperm samples obtained from each male of broodstock showed motility above 75% and were used in the treatment. Sperm was diluted in a ratio of 1:9. A total of 9 mL of sperm was added to 81 mL of the extender solution so that three groups of sperm were found in the extender solution of 90 mL each. The extenders used were 0.4% fructose, Ringer's lactate, and a combination solution (50% fructose and 50% Ringer's lactate). The extender was stored in the refrigerator for 2 - 3 days, and the supernatant was taken. Sperm was put into a mini straw with a volume of 0.25 mL. Mini straws that will be used first are sterilized using a UV ray stabilizer for 15 minutes. Furthermore, the test sperm was put into a 0.5 mL mini straw that had been stored in the refrigerator, by sucking on the tip coated with polyvinyl alcohol (PVA). This work was done in the refrigerator to prevent a drastic increase in temperature (Sunarma *et al*., 2010).

In the first stage, the mini straws were placed in liquid nitrogen vapor until they reached a temperature of -140 °C for seven minutes. In the second stage, the mini straws were put into the canister and stored in a container filled with liquid nitrogen at a temperature of -196 °C, the mini straws were stored for 14 days. Thawing was carried out to remove the remaining cryoprotectant by soaking the mini straws at 39-40 °C for 10-15 seconds (Sunarma *et al*., 2010). The examination was carried out under an inverted microscope to observe motility and viability.

The test parameters observed were motility and viability. Spermatozoa motility of red Bader fish was determined from the movement of sperm from a field of view. Good sperm movement was tending to move forward which was also called progressive motion. Motility assessment was based on subjective observations under a microscope. Sperm was observed by performing activation by mixing one drop of cement with two drops of water on a

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glass slide (Boonthai *et al*., 2016). The viability of live sperm was based on differences in the permeability or absorption of cells to fluids in spermatozoa treated with eosin fluid on a smear slide to distinguish between live and dead spermatozoa (Sukendi *et al*., 2011).

Data Analysis

Data on sperm motility and viability values of red Bader fish were analyzed using Analysis of Variance (ANOVA). If the results show a significant effect, continue with the Least Significant Difference (LSD) test at the 95% confidence interval. Meanwhile, sperm analysis would be analyzed descriptively supported by the literature.

RESULTS AND DISCUSSION

Sperm Characteristics of Red Bader Fish

Observation of sperm characteristics aims to determine whether sperm was feasible or not to be given further treatment and used as a reference (control). Macroscopic observations include pH, volume, and color. Meanwhile, microscopic observations included the concentration, motility, and viability of spermatozoa. The results of the observation of the quality of control sperm are in (Table 1).

Table 1. Red Bader fish sperm quality control.

Parameter	Score
Volume (mL)	3.18±0.49
Color	milky white
pH	7.39±0.20
Concentration (cell/mL)	$3.5x10^9$ cell/mL
Weight (g)	242.9±19.35
Length (cm)	18.52±1.78
Motility (%)	81.67±2.89
Viability (%)	85.00±5.00

Based on the observation of the quality of the control sperm, it showed that for each brood of red Bader fish, fresh sperm were obtained as much as 3.18±0.49 mL with a concentration of $3.5x10^9$ cells/mL (Babiak *et al*., 1997). The sperm was milky white and had a pH value of 7.39±0.20. The fresh sperm of red Bader fish was categorized as normal and can be used for further process, the sperm volume of Bader fish was not different from the volume of sperm that can be accommodated each time striping was around 2.7 mL, the color was milky white and thick like coconut milk. The value of the volume of sperm produced from each fish species depends on the size of the fish's gonads, where the larger the gonads of a fish species, the greater the volume of sperm produced (Sukendi *et al*., 2011). Sperm with white color as produced by red Bader fish in this study was classified as good because it has a larger number of cells per volume when compared to gray-white sperm.

The pH value of the sperm of the control red Bader fish was in the normal range, this indicated that macroscopically the sperm had good quality. The pH of good fish sperm according to Setyono (2009), ranged from 6.8 to 7.8. Meanwhile for the observation results the concentration of fresh red Bader fish sperm was $3.5x10^9$ cells/mL. The results of calculating the concentration of fresh sperm at each storage using a spectrophotometer were 3.00×10^9 cells/mL so the sperm concentration of Bader fish before dilution was still in the normal range. The motility of spermatozoa was one of the factors that determine sperm quality. In this study, the motility of the control sperm was observed using a DIC microscope with a magnification of 400×. Observations were made on the number of progressive motile sperm (moving straight ahead) from one field of

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view and expressed in percent. Based on the results of observations, the average percentage motility value was 81.67±2.89%. The results of the observation of the viability of the control sperm in this study obtained an average of 85±5.00 %. Fresh fish sperm to be used for storage and freezing must have a viability value of at least 70%.

Sperm Motility of Red Bader Fish After Cryopreservation

The results of observing the average percentage of Bader sperm motility after cryopreservation using fructose extender and dimethyl sulfoxide cryoprotectant during storage for 14 days (Table 2).

Table 2. Results of observation of post-cryopreservation motility.

Fructose Concentration	Post-Cryopreservation Motility	
	Before	After
0.2%	56.67±2.36	33.33±2.36
0.4%	61.67±2.36	36.67±2.36
0.6%	63.33±2.36	38.33±2.36

The results of the analysis of variance that has been carried out show that fructose as an extender has no significant effect on sperm motility before and after the cryopreservation process. The level of motility resulting from fructose treatment was still considered good because according to the requirements of good sperm and can proceed to the freezing process or cryopreservation process sperm that has an individual motility value above 50% (Toelihere, 1993). Fructose acts as an energy-producing substrate in the form of ATP, thus causing spermatozoa to move. The motility (movement) of spermatozoa is highly dependent on the supply of energy in the form of ATP as a result of metabolism (Tambing *et al*., 2000). Although the results of the analysis of variance showed no significant effect.

Concentration at 0.6% (P3) obtained the highest results on sperm motility. Fructose is the fastest chemical compound that can be converted into heat energy in the form of movement energy for spermatozoa. Based on the results of the study, increasing the percentage of fructose used also increased the motility and viability of spermatozoa from red Bader fish. Therefore, it was suggested to increase the percentage of fructose used in the cryopreservation process.

The use of different types of extenders has an effect on sperm motility both before cryopreservation and after cryopreservation. This difference is possible because the composition of the extender fluid is different from the composition of the seminal plasma fluid. Spermatozoa diluent containing fructose because it can increase motility and longevity and maintain pH (Kurniawan *et al*., 2013). The main ingredient used by spermatozoa as an energy source from outside the testes is fructose which can reduce the speed of damage to the permeability of spermatozoa so that the need for nutrients and energy in the form of ATP is not inhibited so that spermatozoa can last a long time (Hidayaturrahmah, 2007).

The Spermatozoa motility of Bader fish was determined from the number of spermatozoa that move from a field of view. Sperm motility can be determined by observing diluted sperm under a microscope with 400x magnification. Sperm movement is active, fast, and zig-zag. There are also sperm whose movements are passive, slow, and irregular. Sperm motility observations were carried out to see and analyze the effect of the process before and after cryopreservation on sperm quality (Suharyati and Hartono, 2011).

Sperm movement for all treatments ranged from 30-40%. Observation of motility seen from active and fast movement. The sperm will move actively forward in a

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zig-zag motion and fast. If you only look at the movement of sperm for all treatments, including active, it's just that some move rather slowly and quickly. In the analysis of fructose variety, the effect was not significant, but the result at P3 (concentration 0.6%) was the highest compared to other treatments. In the treatment with a fructose concentration of 0.6%, sperm movement was active and faster than sperm movement in other treatments. This is because the motility of spermatozoa is influenced by the physiological conditions and morphology of the spermatozoa themselves. The more mature the sperm, the more active the movement and the more complete and perfect morphology, with the head and tail controlling its movement. This is by Setiadi *et al*. (2006), motility is influenced by several factors, and one of them is spermatozoa morphology.

Sperm Viability of Red Bader Fish After Cryopreservation

The percentage of viability was calculated based on the number of live and dead spermatozoa from the total sperm count multiplied by 100%. Observation of live spermatozoa using eosin staining method. Sperm that live will not be stained by dye (transparent), while spermatozoa that have died will be colored red. This is because the membrane on the dead spermatozoa is not permeable (non-selective) to dyes, causing the dead spermatozoa to turn red (Winarto and Isnaini, 2008). Viability is one of the assessments to determine sperm quality in the storage process. The number of live spermatozoa can be used as an indicator in determining sperm quality.

Table 3. Results of observation of post-cryopreservation viability.

Fructose Concentration	Post-Cryopreservation Viability	
	Before	After
0.2%	58.33±2.36[a]	31.67±2.36
0.4%	63.33±2.36[ab]	35.00±7.07
0.6%	66.67±2.36[b]	36.67±2.36

The results of the analysis of variance showed that fructose as an extender had a significant effect on sperm viability of Bader fish before cryopreservation but had no significant effect after cryopreservation. The LSD test results showed that before cryopreservation P3 was significantly higher than P1 but not significantly different from P2. Meanwhile, P1 was significantly lower than P3 but not significantly different from P2. Fujaya (2004) stated that the viability of spermatozoa is highly dependent on the availability of energy contained in the sperm body.

The main ingredient used by spermatozoa as an energy source from outside the testes is fructose which is converted into lactic acid and energy with the help of the fructolysin enzyme in the glycolysis process. The decrease in the percentage of life in the storage process can also be caused by the metabolism of spermatozoa which produces by-products in the form of lactic acid or CO_2. Lactic acid itself can inhibit the metabolic activity of spermatozoa. Sperm that are alkaline or acidic will decrease metabolism (Hardjopranjoto, 1995). Post-cryopreservation sperm viability (Figure 1).

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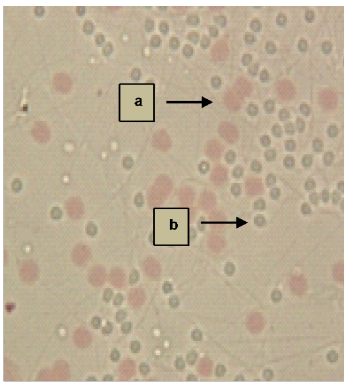

Figure 1. Observation of Fresh Sperm Viability (Dick) Red Bader Fish 400 x magnification. Description: (a) Dead Sperm; (b) Live Sperm.

Figure 1 shows the difference between live and dead sperm. Staining using eosin will make it easier to observe dead sperm and live sperm. Dead sperm will absorb the color of eosin so the color is purple. Solihati *et al*. (2006) added that the decrease in food reserves, and fluid-electrolyte imbalance due to spermatozoa metabolism can cause damage to spermatozoa cell membranes. Damage occurs due to changes between solutions extracellular and intracellular with diluent spermatozoa due to different percentages (Danang *et al*., 2012).

The results of this study indicate that the use of a type of fructose extender affects sperm viability before the cryopreservation process. Viability examination is useful to find out how long the spermatozoa live (viable). Immotile spermatozoa are not necessarily dead spermatozoa. An inappropriate environment will cause the spermatozoa to be unable to move but if the spermatozoa are in a supportive environment, then the spermatozoa will move back. Based on preliminary research, the results showed that the type of extender fructose 0.4% with a concentration of DMSO 10% was the best compared to other types of extenders. Next, the fructose concentration was determined with a 10% DMSO concentration. Sperm motility and viability are strongly influenced by the availability of energy in the cell (Salisbury *et al*., 1985). The cell plasma membrane consists of carbohydrates bound to lipids (glycolipids) and proteins (glycoproteins) or what is called the cell envelope. The cell envelope or glycocalyx is an extracellular carbohydrate to protect the cell membrane from damage during storage at low temperatures (Subowo, 1995). A good condition of the plasma membrane will also have a good effect on sperm motility and viability, and vice versa if the condition of the plasma membrane of the cell's plasma membrane is damaged it will have an impact on decreasing the value of sperm motility and viability (Danang *et al*., 2012).

Staining using eosin will make it easier to observe dead sperm and live sperm. Dead sperm will absorb the color of eosin so that the color is purple. Decreased food reserves, and fluid-electrolyte imbalance due to spermatozoa metabolism can cause damage to spermatozoa cell membranes. This damage can occur due to the exchange of extracellular and intracellular solutions with diluents and spermatozoa caused by differences in concentration (Danang *et al*., 2012). The cement dilution process can cause damage to the plasma membrane and reduce motility. Damage to the spermatozoa cell membrane will have an impact on the membrane which initially has semipermeable properties and is no longer able to select the entry and exit of substances so that when the eosin-negrosine color test is carried out, the substance enters the plasma. This increase in spermatozoa that absorb the dye solution of eosin negrosin as a sign that the spermatozoa have died due to the increased permeability of the cell membrane. Cells that have absorbed the dye will swell (Toelihere, 1993).

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CONCLUSION

The characteristics of red Bader fish sperm are a volume of 3.18 mL, a pH value of 7.39, a milky white sperm color, sperm concentration is 3.5x109 cells/mL. P3 was the best treatment which resulted in sperm motility before and after cryopreservation at 63.33±2.36% and 38.33±2.36% and sperm viability before and after at 66.67±2.36% and 36.67±2.36%.

CONFLICT OF INTEREST

There is no conflict between the authors or other parties in this manuscript.

AUTHOR CONTRIBUTION

The two authors of this manuscript contributed to the implementation of the research and also contributed to the writing of this manuscript.

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REFERENCES

Abinawanto, Bayu, M.D., Lestari, R. and Sunarma, A., 2011. Peranan dimetil sulfoksida sebagai krioprotektan dalam mempertahankan kualitas spermatozoa ikan gurami, *Osphronemus goramy* Lacepede, 1801, dua puluh empat jam pascakriopreservasi. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, *16*(1), pp.10–15. https://doi.org/10.24002/biota.v16i1.54

Afriani, D., Eriani, K., Muchlisin, Z.A. and Hasri, I., 2021. A short review of discovery and development of fish sperm cryopreservation. *DEPIK Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, *10*(1), pp.11–16. https://doi.org/10.13170/depik.10.1.18794

Arifiantini, R.I., Purwantara, B., Yusuf, T.L. and Sajuthi, D., 2009. Peranan fruktosa, rafinosa, dan trehalosa pada kriopreservasi semen kuda. *Media Peternakan*, *32*(3), pp.171–178. https://journal.ipb.ac.id/index.php/mediapeternakan/article/view/8798

Arthington, A.H., Dulvy, N.K., Gladstone, W. and Winfield, I.J., 2016. Fish conservation in freshwater and marine realms: Status, threats and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(5), pp.838–857. https://doi.org/10.1002/aqc.2712

Babiak, I., Glogowski, J., Brzuska, E., Szumiec, J. and Adamek, J., 1997. Cryopreservation of sperm of common carp, *Cyprinus carpio* L. *Aquaculture Research*, *28*(7), pp.567–571. https://doi.org/10.1046/J.1365-2109.1997.00897.X

Bhattacharya, S., 2018. *Cryoprotectants and their usage in cryopreservation process*. In *Cryopreservation Biotechnology in Biomedical and Biological Sciences*. IntechOpen. https://doi.org/10.5772/intechopen.80477

Boonthai, T., Khaopong, W., Sangsong, J., Sooksawat, T., Nimrat, S. and Vuthiphandchai, V., 2016. Semen collection methods affect the bacterial composition of post-thawed semen of silver barb (*Barbodes gonionotus*). *Animal Reproduction Science*, *166*, pp.90–98. https://doi.org/10.1016/J.ANIREPROSCI.2016.01.007

Bozkurt, Y., 2018. Introductory chapter: *Application fields of cryopreservation biotechnology*. *Cryopreservation Biotechnology in Biomedical and Biological Sciences*. IntechOpen, pp.1–4. https://doi.org/10.5772/intechopen.82229

Ciereszko, A., Dietrich, G.J., Nynca, J., Dobosz, S. and Zalewski, T., 2014. Cryopreservation of rainbow trout semen using a glucose-methanol extender. *Aquaculture*, *420–421*, pp.275–281. https://doi.org/10.1016/j.aquaculture.2013.11.014

Cite this document as Yodarta, D. and Faqih, A.R., 2023. Endemic Fish Conservation: Utilization of Cryopreservation Technology with Fructose in Red Bader Fish (*Puntius Bramoides*) Sperm. *Journal of Aquaculture and Fish Health*, *12*(3), pp.432-442.

Condro, H.S., Mubarak, A.S. and Sulmartiwi, L., 2012. Pengaruh penambahan madu pada media pengencer NaCl fisiologis dalam proses penyimpanan sperma terhadap kualitas sperma ikan komet (*Carassius auratus*). *Journal of Marine and Coastal Science*, *1*(1), pp.1-12. http://journal.unair.ac.id/filerPDF/marine4b1241673bfull.pdf

Danang, D.R., Isnaini, N. and Trisunuwati, P., 2012. Pengaruh lama simpan semen terhadap kualitas spermatozoa ayam kampung dalam pengencer ringer's pada suhu 4 C. *TERNAK TROPIKA: Journal of Tropical Animal Production*, *13*(1), pp.47-57. https://ternaktropika.ub.ac.id/index.php/tropika/article/view/163

Dietrich, G.J., Nynca, J., Dobosz, S., Zalewski, T. and Ciereszko, A., 2014. Application of glucose-methanol extender to cryopreservation of semen of sex-reversed females rainbow trout results in high post-thaw sperm motility and fertilizing ability. *Aquaculture*, *434*, pp.27–32. https://doi.org/10.1016/j.aquaculture.2014.07.015

Froese, R. and Pauly, D., 2018. *FishBase (version Feb 2018). In: Species 2000 & ITIS Catalogue of Life, 2018 Annual Checklist* (Roskov Y., Abucay L., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt R.E., Decock W., De Wever A., Nieukerken E. van, Zarucchi J., Penev L., eds.). www.catalogueoflife.org/annual-checklist/2018

Fujaya, Y., 2004. *Fisiologi ikan: Dasar pengembangan teknologi perikanan*. Rineka Cipta, Jakarta.

Hardjopranjoto, H.S., 1995. *Ilmu kemajiran pada ternak*. Airlangga University Press, Surabaya. https://opac.perpusnas.go.id/DetailOpac.aspx?id=451992

Hidayaturrahmah, 2007. Waktu motilitas dan viabilitas spermatozoa ikan mas (*Cyprinus carpio* L) pada beberapa konsentrasi larutan fruktosa. *Bioscientiae*, *4*(1), pp.9–18. https://ppjp.ulm.ac.id/journals/index.php/bioscientiae/article/view/158

[IUCN] International Union for Conservation of Nature and Natural Resources., 2020. *The IUCN red list of threatened species version 2020-1*. https://www.iucnredlist.org

Irawan, H., Vuthiphandchai, V. and Nimrat, S., 2010. The effect of extenders, cryoprotectants and cryopreservation methods on common carp (*Cyprinus carpio*) sperm. *Animal Reproduction Science*, *122*(3-4), pp.236–243. https://doi.org/10.1016/j.anireprosci.2010.08.017

Kurniawan, I.Y., Basuki, F. and Susilowati, T., 2013. Penambahan air kelapa dan gliserol pada penyimpanan sperma terhadap motilitas dan fertilitas spermatozoa ikan mas (*Cyprinus Carpio* L.). *Journal of Aquaculture Management and Technology*, *2*(1), pp.51–65. https://ejournal3.undip.ac.id/index.php/jamt/article/view/1831

Lutfi, 2010. Pembekuan semen lele dumbo (*Clarias gariepinus* Burchell 1822) sebagai model kriopreservasi semen ikan. *Jurnal Natural*, *9*(1), pp.48-53. http://jurnalnatural.unipa.ac.id/index.php/jn/article/view/38

Muchlisin, Z.A., Nadiah, W.N., Nadiya, N., Fadli, N., Hendri, A., Khalil, M. and Siti-Azizah, M.N., 2015. Exploration of natural cryoprotectants for cryopreservation of African catfish, *Clarias gariepinus*, Burchell 1822 (Pisces: Clariidae) spermatozoa. *Czech Journal of Animal Science*, *60*(1), pp.10–15. https://doi.org/10.17221/7906-cjas

Murdy, E.O., Kottelat, M., Whitten, A.J., Kartikasari, N. and Wirjoatmodjo, S., 1994. Freshwater fishes of Western Indonesia and Sulawesi. *Copeia*, *1994*(3), p.830. https://doi.org/10.2307/1447208

Murgas, L.D.S., de Oliveira Felizardo, V., de Souza Andrade, E., Ferreira,

M.R., de Jesus Paula, D.A. and de Carvalho, A.F.S., 2014. Cryopreservation of sperm in Brazilian migratory freshwater fish. *Recent Advances in Cryopreservation*, *16*. https://doi.org/10.5772/58685

Muthmainnah, C.R., Muchlisin, Z.A., Eriani, K., Hasri, I., Fadli, N. and Muhammadar, A.A., 2019. Kriopreservasi sperma ikan kawan *Poropontius tawarensis* menggunakan dimetil sulfoxida (DMSO). *DEPIK Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, *8*(3), pp.158–166. https://doi.org/10.13170/depik.8.3.15072

Salisbury, G.W, van Demark, N.L. and Djanuar, R., 1985. *Fisiologi reproduksi dan inseminasi buatan pada sapi*. Gadjah Mada University Press, Yogyakarta. p.869. https://opac.perpusnas.go.id/DetailOpac.aspx?id=92314

Sarder, M.R.I., Sarker, M.F.M. and Saha, S.K., 2012. Cryopreservation of sperm of an indigenous endangered fish species *Nandus nandus* (Hamilton, 1822) for ex-situ conservation. *Cryobiology*, *65*(3), pp.202–209. https://doi.org/10.1016/j.cryobiol.2012.06.004

Setiadi, M.A., Suprayogi, A. and Yulnawati., 2006. Viabilitas dan integritas membran plasma spermatozoa epididymis anjing selama penyimpanan pada pengencer yang berbeda metode penelitian. *Media Kedokteran Hewan*, *22*(2), pp.118–123.

Setyono, B., 2009. Pengaruh perbedaan konsentrasi bahan pada pengencer sperma ikan “skim kuning telur “terhadap laju fertilisasi, laju penetasan dan sintasan ikan mas (*Cyprinus carpio* L.). *Jurnal Gamma*, *5*(1), pp.1-10. https://ejournal.umm.ac.id/index.php/gamma/article/view/749

Solihati, N., Idi, R., Setiawan, R., Asmara, I.Y. and Sujana, B., 2006. The Storage Time Effect of The Local Chicken Chilled Semen at 5 °C on Fertility and Fertile Period of Sperm. *Jurnal Ilmu Ternak*, *6*(1), pp.11–17. http://jurnal.unpad.ac.id/jurnalilmuternak/article/view/2258

Subowo, 1995. *Biologi sel*. Angkasa, Bandung.

Suharyati, S. and Hartono, M., 2011. Preservasi dan kriopreservasi semen sapi limousin dalam berbagai bahan pengencer. *Jurnal Kedokteran Hewan*, *5*(2), pp.53-58. https://doi.org/10.21157/j.ked.hewan.v5i2.355

Sukendi, R.M., Yurisman, P. and Asiah., N., 2011. Pengaruh kombinasi penyuntikan ovaprim dan prostaglandin F 2 α (PGF 2 α) terhadap volume semen dan kualitas spermatozoa ikan tambakan (*Helostama temmincki* C.V.). *Jurnal Perikanan dan Kelautan*, *16*(2), pp.132–143. http://dx.doi.org/10.31258/jpk.16.02.%25p

Sunarma, A., Budihastuti, D.W. and Sistina, Y., 2010. Penggunaan ekstender madu yang dikombinasikan dengan krioprotektan berbeda pada pengawetan sperma ikan nilem (Indonesian Sharkminnow, *Osteochilus hasseltii* Valenciennes, 1842). *Omni-Akuatika*, *9*(11), pp.51-55. https://doi.org/10.22146/jfs.8931

Syafei, L.S., 2017. Keanekaragaman hayati dan konservasi ikan air tawar. *Jurnal Penyuluhan Kelautan dan Perikanan Indonesia*, *11*(1), pp.48–62. https://doi.org/10.33378/jppik.v11i1.85

Tambing, S.N., Toelihere, M.R., Yusuf, T.L. and Sutama, I.K., 2000. Pengaruh gliserol dalam pengencer tris terhadap kualitas semen beku kambing peranakan etawah. *Jurnal Ilmu Ternak Dan Veteriner*, *5*(2), pp.1-8. https://repository.pertanian.go.id/server/api/core/bitstreams/2dd9cbab-d2c6-4852-abe1-76640a4d37de/content

Toelihere, M.R., 1993. *Inseminasi buatan pada ternak*. Angkasa, Bandung, p.292.

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Torres, L., Hu, E. and Tiersch, T.R., 2016. Cryopreservation in fish: Current status and pathways to quality assurance and quality control in repository development. *Reproduction, Fertility and Development*, *28*(8), pp.1105–1115. https://doi.org/10.1071/RD15388

Winarto, A. and Isnaini, N., 2008. Pengaruh tingkat pengenceran terhadap kualitas spermatozoa kambing pe setelah penyimpanan pada suhu kamar. *TERNAK TROPIKA Journal of Tropical Animal Production*, *9*(2), pp.72–80. https://ternaktropika.ub.ac.id/index.php/tropika/article/view/150

Zairin, M., Handayani, S. and Supriatna, I., 2005. Quality of sperm from cryopreserved semen of *Tor soro* in Dimethylsulfoxide and Glycerol 5, 10 and 15%. *Jurnal Akuakultur Indonesia*, *4*(2), pp.145–151. https://doi.org/10.19027/jai.4.145-151

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