Original Research Article

Variation amount of sperm cells in the first generation of nontransgenic and transgenic Mutiara Catfish hybrid

ABSTRACT

One of the factors that influence the success of catfish spawning, including male broodstock gonad fertility determine differences in the number of the sperm produced. The use of transgenesis technology (insertion of the exogenous growth hormone gene) in catfish shows an influence especially multiplication of the sperm counts. The purpose of this study was to compare the variations in the number of sperm cells of the male transgenic Mutiara catfish (comprising the African catfish growth hormone gene) with nontransgenic Mutiara. These research was conducted at the Fish Hatchery, Fisheries Biotechnology Laboratory, and Central Laboratory of Padjadjaran University. The experimental method was used completely randomized design and analyzed quantitatively and qualitatively. The male catfish broodstock were used as treatments consists of the first generation transgenic Mutiara hybrids with nontransgenic Mutiara (G1_MT-MNT), transgenic Mutiara hybrids with Sangkuriang (G1_MTS), and Sangkuriang (G1_S). The sperm from testes of each broodstock were used as replications for sperm count test parameters and motility (n = 5). Qualitative analysis was used for the identification parameters of the transgenic male broodstock using the Polymerase Chain Reaction (PCR) and sperm volume. Quantitative analysis uses the Sigma Plot 12.0 program for parameters for sperm cell count and sperm motility. The results showed that the highest number of sperm (6.06×10^6 cells) and the highest motility (score 4.2) were found in the broodstock of the G1_MT-MNT male catfish.

Keywords: sperm cells, transgenesis, transgenic Mutiara catfish,

1. INTRODUCTION

The success of catfish fingerling mass production involves the contribution of quality male broodstocks in spawning activities. The abundance of the sperm production and sperm motility is one of the important factors in spawning success (Viveiros *et al*., 2001). One pair of African catfish (*Clarias gariepinus*) can produce 50,000 to 60,000 catfish larvae and further rearing was only obtained 32,500-38,000 juveniles catfish aged 5-6 weeks (mortality ranges from 35-45%) (Steyn and van Vuren, 2011).

The frequency of using the same male broodstock causes the quality of male fertility to decreased. Broodstock quality improvement can be done by the transgenesis (insertion of African catfish growth hormone gene, *C. gariepinus* Growth Hormone, *Cg*GH) on early generation catfish (G0) and first generation (G1) of the Mutiara catfish (Buwono *et al.,* 2016). The study of transfer of catfish growth hormone gene (*Pangasionodon hypopthalmus* Growth Hormone, *Ph*GH) in Dumbo catfish was also carried out by Marnis *et al.* (2014) to produce transgenic catfish.

The male transgenic Mutiara catfish has a fast growth compared to normal catfish (including testicular growth). GH receptors work on gonadal cells of male fish to induce sperm multiplication in the testes (Maestro *et al.,* 1997). The exogenous GH expression in the male catfish can increase sperm count and trigger fish spawning (Devlin *et al*., 1995). So far it is not known whether of the male transgenic Mutiara catfish has a different number of sperm cells compared to non-transgenic Mutiara catfish (Buwono *et al*., 2019).

2. MATERIALS AND METHODS

The study began in December 2018 with a processing time of approximately two months. The parameters observed included the identification of the hybrid transgenic male catfish, sperm volume, sperm cell count,

and sperm motility (G1_MTS, G1_ MT-MNT, and G1_S). Identification of the transgenic male catfish using the RNeasy mini kit (QIAGEN, USA) for RNA isolation and MyTaq *OneStep* RT-PCR kit (Bioline, UK) for RT-PCR reactions. Physiological NaCl (0.9%) and immersion oil are used in observing sperm cell count and sperm motility.

Total RNA extracted from fish fin tissue was used as a template for amplification of the exogenous GH using Cg-F (5'-ATGGCTCGAGTTTGGTGCTGCT-3') and Cg-R (5' CTACAGAGTGCAGTTGGAATCCAGGG-3') primers (Zhang *et al.,* 2009) . The RT-PCR reaction mixture consists of 2x MyTaq *OneStep* RT-PCR mix (25 µl), Cg-F primers (10 µM) and Cg-R (10 µM) each of 1.25 µl, reverse transcriptase (0.5 µl), ribosafe RNase inhibitors (1.0 µl), DEPC-H2O (14.5 µl), and RNA templates (5 µl). The RT-PCR reaction was carried out using a thermal cycler in 1 cycle for RT reaction at 48ºC for 20 minutes. The PCR stage consisted of pre-denaturation at 95ºC for 1 min, denaturation at 95ºC for 10s, annealing at 60ºC for 20s. Extensions at 72ºC for 30s, and replication 40 times. Final extension at 72ºC for 5 min, hold at 4ºC for 1 min. The amplification product was separated with 1% agarose gel and the transgenic broodstock was indicated by a 600 bp GH fragment (parallel to the pCMV-*Cg*GH plasmid fragment as a positive control).

The research method used a completely randomized design consisting of the three types of the G1 fish sperm treatments (G1_MT-MNT, G1_MTS, G1_S) and five replications. Sperm was taken as much as 1µl from the right testis or left testis in each test fish and repeated five times.

Data analysis were used in this study consisted of qualitative and quantitative. The transgenic identification and sperm volume using qualitative analysis descriptively. Parameters of sperm cell count and sperm motility were analyzed by Duncan Multiple Range Test at 0.05 level with the help of Sigma Plot 12 software.

3. RESULTS AND DISCUSSION

3.1 Identification of transgenic male catfish broodstock

3.2 Sperm Volume

Based on the results of the test, it was found that the catfish broodstock of the G1_MT-MNT and G1_MTS were transgenic fish (containing the African catfish GH). These was shown from the fragments that appear on the catfish broodstock PCR products G1_MT-MNT and G1_MTS was 600 bp (Fig 1).

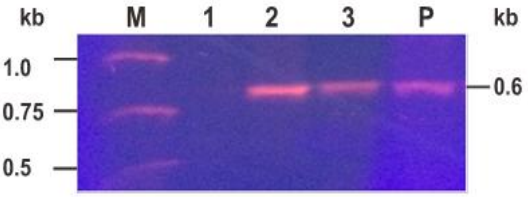

Fig 1. Identificaton of the transgenic male catfish broodstock. M= marker DNA ladder 1 kb (Promega); 1= G1_S ; 2= G1_MT-MNT; 3= G1_MTS; P= plasmid (pCMV-*Cg*GH, 600 bp)

Verification of the African catfish GH fragment size was carried out by Buwono *et al*. (2016) where the presence of the African catfish GH gene was detected at 600 bp. The primers used were Cg-F and Cg-R primers (Zhang *et al*., 2009) specifically designed to detect the African catfish GH gene found from Mutiara catfish. The DNA fragment that appears is a visual representation of African catfish GH detected in test fish. The GH African catfish gene insert (600 bp) was also obtained in transgenic catfish from fish fin tissue samples (Iskandar *et al*., 2018).

3.3 Amount of sperm cells

Observation of the sperm volume was carried out objectively or directly, by looking at the size scale on the 1.5 ml eppendorf tube. Based on observations, the highest volume of the sperm was produced by G1_MT-MNT catfish (2.2 ml), then followed by the G1_ MTS (1.6 m), and G1_S (1.2 ml). The results obtained were not different with the Papadaki *et al*. (2008) study which stated that the volume of sperm produced by the male brooders is generally more than 1 ml in the range 2-4 ml or an average of 3 ml.

Increased sperm volume in the male transgenic hybrid catfish broodstock, is associated with the expression of exogenous GH which induces an increase in testosterone production which stimulate

spermatogenesis (Dubey nee Pathak *et al*., 2015). This activity of spermatogenesis can increase sperm count so that the volume of sperm also increases. The transgenic fish have rapid growth so that the process of producing sperm is also more, because of the increase in the growth of the testicular organs.

This was also indicated by the research of Iskandar *et al*. (2018) that the growth of transgenic Mutiara catfish was 2-3 times faster than nontransgenic catfish.

The reproductive organs of transgenic Mutiara catfish have a larger size than nontransgenic catfish, can be positively correlated with the amount of sperm volume (cement). The advantages of transgenic Mutiara catfish can provide benefits in accelerating the maturation of the reproductive organs for spawning activities. The broodstock sperm volume of catfish G1_MT-MNT, G1_MTS, and G1_S was shown in Fig 2.

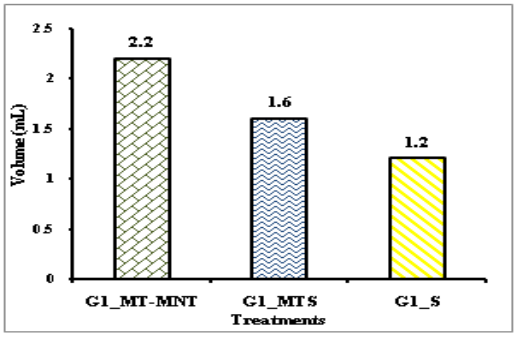

Fig 2. Sperm volume

Visually, the form of the broodstock G1_MT-MNT was large and milky white, the broodstock G1_MTS was medium size and creamy white, while G1_S was small and pale white (Fig 3).

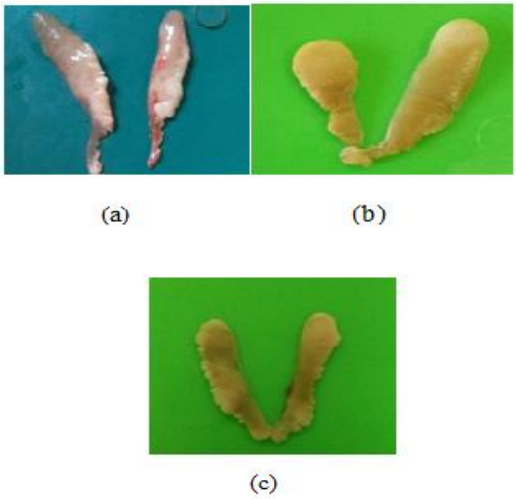

Fig 3. Profile of testes (a).G1_MT-MNT, (b).G1_MTS, dan (c).G1_S

3.4 Amount of sperm cells

Based on the results of the research that has been done, the highest number of the sperm cells was obtained by the catfish broodstock G1_MT-MNT (6.06 x 10^6 sperm/ml), then followed by G1_ MTS (4.92 x 10^6 sperm/ml), and G1_S (1.97 x 10^6 sperm/ml). The results of statistical analysis showed that the number of sperm cells G1_MT-MNT with G1_MTS was not significantly different, but the number of sperm cells G1_MT-MNT with G1_S and the number of sperm cells G1_MTS with G1_S was significantly different.

The calculation of the number of sperm cells in this study is not much different from that obtained by Steyn and van Wren (1986) in African catfish (*C. gariepinus*) of 6.2 x 10^6 sperm/ml and Nayak *et al*. (2016) in *C. batrachus* (6.0 x 10^6 sperm/ml). The MT-MNT male broodstock containing GH African catfish was derived from *C. gariepinus* as Mutiara catfish so the number of sperm cells was not much different.

The number of sperm cells in the study of Hu *et al*. (2011) in European catfish (*Ictalurus punctatus*) is 2.9 x 10^6 sperm/ml. This amount is lower when compared to MT-MNT male catfish (6.06 x 10^6 sperm/ml) and MTS male catfish (4.92 x 10^6 sperm/ml). This shows that transgenesis has an influence on the production of the number of sperm cells.

The G1 male Sangkuriang catfish was used as a control in these study to represent the presence of catfish in the community. The G1_MT-MNT catfish broodstock produces more sperm than G1_S because the catfish broodstock was a hybrid of transgenic Mutiara and nontransgenic Mutiara (Fig. 4).

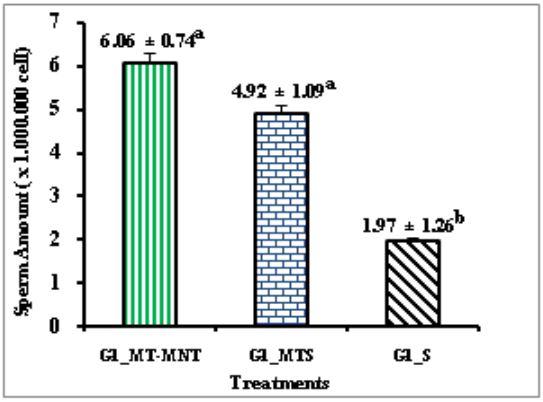

Fig 4. Amount of sperm cells

Spermatogonia cells in transgenic fish can increase in number due to over-expression of exogenous insertion of growth hormone (GH) (Caelers *et al*., 2005). This causes an increase in growth hormone that can induce multiplication of the spermatogonia cells. The production of sperm counts is induced by growth hormone controlled by the GH gene. In nontransgenic fish the production of sperm counts is less than that of transgenic fish (only containing endogenous GH), whereas transgenic fish contain exogenous GH and endogenous GH which causes over-expression which promote the production of sperm of fish broodstocks (Dewi *et al*., 2013).

The sperm count of G1_MTS was lower compared to the G1_MT-MNT broodstock even though both were transgenics, due to the G1_MT-MNT has better hybrid quality. The low sperm of Sangkuriang catfish (nontransgenic) due to cell multiplication is only caused by endogenous GH itself. In transgenic catfish (MT-MNT and MTS), sperm production is higher due to over-expression of exogenous and endogenous GH (Schulz *et al*., 2012)

3.5 Sperm Motility

Sperm quality can be measured by sperm motility parameters. There are various methods that can be used to measure sperm motility scores, one of which is using a method developed by McMaster (1992). The lowest score is represented by number 1 and the highest score is represented by number 5.

The highest sperm motility was found in the G1_MT-MNT broodstock with a score of 4.2 (progressive moves). Based on its movements, almost 85% of sperm cells move to all sides (Fig 5). These results are close to the range of the Kovacs *et al*., (2010) study on the sperm motility test of the African catfish (*C. gariepinus*) showing maximum sperm movement can reach 94% (80 ± 14%).

Superior transgenic and hybrid factors also determine sperm motility. Transgenic fish have an exogenous GH insertion which results in a large number of spermatogonia cell production so that the number of motile sperm is relatively higher than other sperm which is indicated by a score of 4.2.

The subsequent effects of transgenesis and hybrid effects caused sperm count and sperm motility in the broodstock crossing of MT-MNT to be higher than those of MTS or S broodstock. The broodstock sperm motility of G1_MTS was not significantly different from the broodstock G1_S. Due to, the hybrid broodstock of the G1_MTS is a transgenic Mutiara catfish crossing (medium sperm motility) and Sangkuriang (low sperm motility) which causes G1_MTS motility at a score of 3.4 which is relatively similar to the Sangkuriang motility (score 3) (Fig 5).

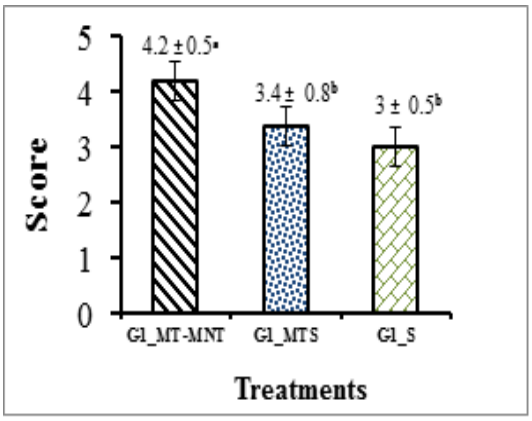

Fig 5. Sperm motility

Based on its movement, the G1_MTS and G1_S sperm motility is quite progressive about 50% moves to all sides. This shows that sperm motility is quite high. The Toelihere study (1981) states that the percentage of motility of spermatozoa is low (40%) causing the ability to fertilize the egg decreases.

The results of the study showed that the number of spermatozoa in G1 transgenic Mutiara catfish (MT-MNT and MTS) was higher than nontransgenic G1 catfish. As compensation, more larvae are produced and the use of the G1 transgenesis-GH Mutiara catfishes is needed to overcome the reduced reproductive performance of catfish in conventional spawning.

This study discovered the *Cg*GH that can be beneficial for fertility of the male catfish. This study will help the researchers to uncover the critical areas of selection of the male broodstock quality that many researchers were not able to explore. Thus a new theory on transgenesis and hybridization may be arrived at.

4. Conclusion

The G1_MT-MNT male catfish broodstock produces higher sperm cell count and sperm motility than other test fish. The sperm volume of G1_MT-MNT male catfish is greater than G1_MTS and G1_S. The results of molecular identification showed that the male catfish G1_MT-MNT and G1_MTS were transgenic fish (containing of 600 bp exogenous GH).

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