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Effect of Sperm Preservation Solution of Pseudobagrus Hwanghoensis in Artificial Reproduction Based on SCSA

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ABSTRACT

The quality of fish sperm is an important factor affecting artificial reproduction, and the sexual maturation of male and female is not synchronized or for the fish that need to kill the male fish for sperm, in order to meet the needs of large-scale reproduction, sperm preservation solution is used to preserve the sperm temporarily stored and taken as needed during reproduction, so the preservation effect of sperm preservation solution directly affects the artificial reproduction. How to determine the quality of sperm in the preservation solution can be judged macroscopically by microscopic examination, but it is not accurate, SCSA can quantitatively judge the quality of sperm in the preservation solution, thus providing guarantee for reproduction. The zhaos pseudobagrus Hwanghoensis by ourselves has been verified by artificial breeding and SCSA analysis for many times. The artificial sperm preservation solution is better than Hanks sperm preservation solution. Test result at ≤0.15 the sperm is vigorous and the effect is obvious.

Keywords: SCSA; Sperm Preservation Solution; DNA Fragmentation Index; Sperm Motility

Introduction

Fish feed on chironomid larvae. The fish is distributed in Yellow River water systems in Henan, but the overall resources are limited and it is a rare species. The third instar reached sexual maturity, and the spawning period was from the middle of May to the end of July. The natural spawning amount was very small. Artificial breeding needs to kill the male fish for sperm, and large-scale breeding needs to be stored with preservation solution in advance to improve efficiency and effect. Sperm chromatin structure analysis (SCSA) is a method to detect the integrity of sperm DNA. Its principle is that the normal sperm DNA is double stranded, while the damaged sperm chromatin is loose, and the DNA is denatured into single stranded under the action of acid (Table 1). According to the characteristics that acridine orange (AO) combines with double stranded DNA to emit green fluorescence, while it combines with single stranded DNA to emit red fluorescence, the sperm suspension after Ao staining can be analyzed by flow cytometry to determine the quality of sperm [1].

Table 1: Two practical effects of sperm preservation solution.

Sperm preservation time (H)	4	96	144
Fertilization rate of zhaos (%)	98.2	87.6	81.4
Fertilization rate of hanks (%)	83.0	13.3	11.5

Materials and Methods

Reagents

Acridine orange (AO) staining solution, hydrochloric acid, Hanks sperm preservation solution (commercially available), Zhaos sperm preservation solution (self prepared).

Sample

Take the sperm for grinding [2], take the Zhaos and Hanks sperm preservation solutions for 8mi respectively, dilute the semen after microscope observation. According to the experimental design time period, dilute the semen for 4h, 96h and 144H in advance, and then place the test tube label in the refrigerator at 4 ℃ for storage [3-5].

The sperm stored in zhaos and Hanks for 4h, 96h and 144H were taken, 30 for each μl;+ Acid aspirate 80 μl (reaction for 30s)+ Ao staining solution 100 μl (reaction for 3min); On machine (cyto flex flow cytometer) detection. In artificial breeding, the mature parent fish are taken for oxytocin [6-10]. The water temperature for oxytocin is above 20 ℃, and it is best to be stable in the range of 22-24 ℃. Two injections were taken into the thoracic cavity, with a depth of about 1cm. The injection was carried out in two times. The first injection was 17:00-18:00 in the afternoon. The dose was LRH-A2 15-20μg+ HCG 600 IU per kilogram of female parent fish, and the male fish was reduced by half; The needle distance is 12 hours, and the second needle is injected at 5:00-6:00 the next day. The dose is LRH-A2 15 μg + HCG 500 IU, and the male fish is reduced by half. When the water temperature is 20-24 ℃, the effect time is about 24 hours. After the injection, the parent fish shall be stimulated by flowing water (Table 2).

Table 2: Number of seedlings obtained in propagation test.

Breeding batch	1	2	3	4	5
Number of seedlings (tail)	300	5000	10000	50000	300000

The pool shall be kept with slight flowing water, with a flow rate of 10-30 cubic meters per hour, so as to better promote the success of labor. During the water injection, attention shall be paid to prevent water from overflowing the pool. The dissolved oxygen in the tank shall be more than 5mg / L. Keep the environment quiet and away from light. Since the estrous behavior of the pseudo parent fish is not obvious, artificial insemination is started after about 24 hours of labor induction, and the extruded eggs (the eggs are orange, and the average diameter of the eggs is 2.0 mm) of the mature female fish are artificially inseminated by semi dry method. When squeezing eggs, attention should be paid not to bring the pool water into the basin to avoid affecting the fertilization rate. Sprinkle a proper amount of the sperm stored in advance on the extruded eggs, and apply hard chicken feathers to continuously stir, so that the sperm and eggs can be better combined and fertilized [11-15]. The actual artificial insemination verification is to inseminate the sperm with different storage time and the Pseudobagrus Hwanghoensis eggs extruded after artificial induction [8] put the inseminated eggs into the incubation box according to the label, and incubate in the incubation tank for 5h to calculate the fertilization rate [9].

Results and Analysis

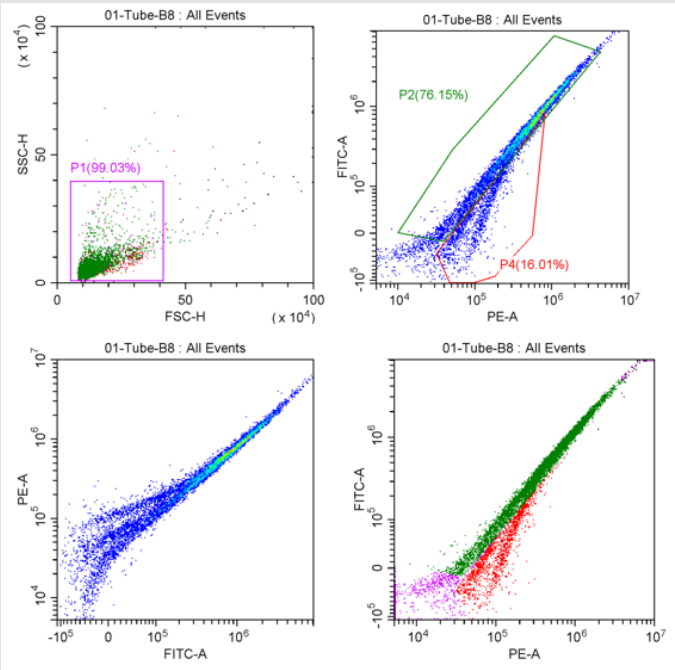
Results

The results of the on-line test of the sperm stored in zhaos are as follows:

(Graph 1).

The test results of sperm stored by Hanks are as follows:

(Graph 2).



试管名称： 01-Tube-B8
样本ID：

群体	颗粒数	%父群	Mean FITC-A	Median FITC-A	Mean PE-A	Median PE-A
All Events	10000	100.0...	606386.5	345408.8	532885.9	330489.3
P1	9903	99.03%	572015.3	339914.2	500383.4	326515.3
P4	1601	16.01%	78474.3	31598.4	160210.4	119594.8
P2	7615	76.15%	731553.9	547137.8	617882.8	491705.9

Graph 1.

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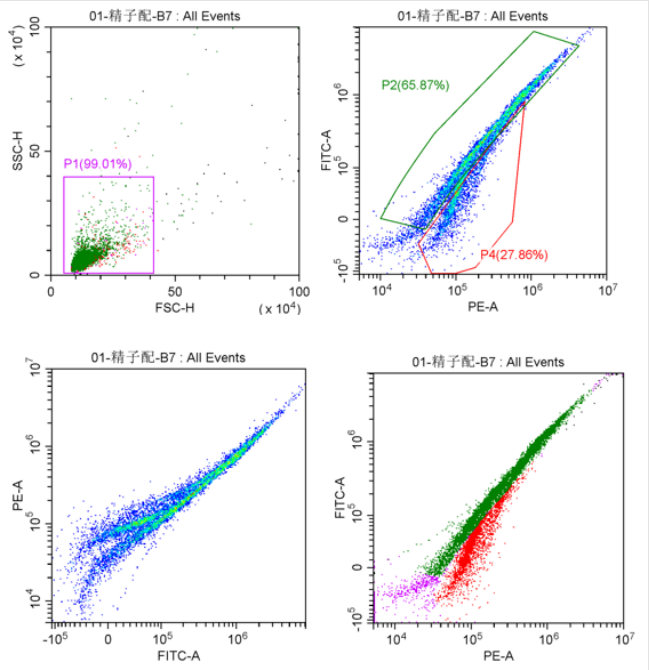

试管名称： 01-精子配-B7
样本ID：

群体	颗粒数	%父群	Mean FITC-A	Median FITC-A	Mean PE-A	Median PE-A
All Events	10000	100.0...	441229.1	119661.5	405492.2	153798.8
P1	9901	99.01%	367040.9	117431.8	328854.0	151999.9
P4	2786	27.86%	54770.3	35689.9	132396.0	104448.8
P2	6587	65.87%	527570.9	264338.1	435435.3	259487.9

Graph 2.

Experimental Analysis

Fracture Index Analysis: Circle the sperm cells on the FSC / SSC diagram and set the gate to the FL1 (FITC) / FL3 (ECD) scatter diagram for analysis. Sperm with good DNA integrity mainly emit green fluorescence (P2 gate); Sperm with poor DNA integrity mainly emit red fluorescence (P4 gate). The sperm DNA quality was evaluated by calculating the DNA fragmentation index at by formula, at = red/(red + green)

The following results are obtained from the experimental test graphs and data:

Zhaos:

$$at1 = 6.37 \div (39.03 + 6.37) = 0.14$$

$$at2 = 11.28 \div (40.65. + 11.28) = 0.21$$

$$at3 = 9.48 \div (23.23 + 9.48) = 0.29$$

Hanks:

$$at1 = 9.44 \div (32.10 + 9.44) = 0.23$$

$$at2 = 8.29 \div (17.18 + 8.29) = 0.32$$

$$at3 = 18.85 \div (38.54 + 18.85) = 0.32$$

From the above breaking index results, the breaking index of the sperm stored in the hnscz sperm preservation solution we configured is smaller than that of the purchased Hanks sperm preservation solution: Zhaos at_1 < Hanks at_1; Zhaos at_2 < Hanks at_2; Zhaos at_3 < Hanks at_3. The results showed that Zhaos was superior to Hanks sperm preservation solution.

Experimental Verification Analysis: The results of artificial insemination showed that the fertilization rate of Zhaos preserved sperm was 81.4% at 144H, which kept a high level, which was consistent with the lower level of DNA fragmentation index zhaos at3 = 0.29 measured by flow cytometry; Similarly, the 144 hour fertilization rate of Hanks preserved sperm is 11.5%, which has reached a very low level and can not be used for actual artificial reproduction, which is consistent with the results of higher level of DNA fragmentation index Hanks at3 = 0.32 measured by flow cytometry.

Microscopic Observation: Microscopic observation [5-6] showed that the sperm stored in Zhaos preservation solution was still vigorous on the 6d, and began to weaken on the 7d, and died on the 9d; The spermatozoa stored in Hanks preservation solution had stronger vitality on 1d and 2d, weaker vitality on 4d and died on 5d.

Actual Verification of Artificial Propagation

When all the larvae are hatched, the newly hatched larvae are gathered in the four corners of the incubation frame, with a total length of only about 6.0mm. They are like tadpoles, and the yolk sac is large and nutritious. They are weak and tender and cannot swim freely; After 5-7 days of temporary feeding, the total length of the larvae can reach 1.0cm, and the yolk sac is greatly reduced. The larvae start

to eat. At this time, the fry can be cultured. However, during temporary feeding, the water quality is required to be clean, the dissolved oxygen is greater than 5mg / L, and the direct sunlight is also required [15]. Judging from the number of seedlings obtained in 5 batches of propagation, the propagation technology is constantly improving and maturing.

Conclusion

- SCSA is a fast and accurate method to determine the quality of sperm, and can be used for quantitative analysis. It is an important means to detect the vitality of artificial preserved sperm before use.
- Through SCSA analysis and practical application test, zhaos, a sperm preservation solution of pseudobagrus Hwanghoensis, can be artificially stored for one week in a 4 °C refrigerator and keep the sperm vigorous, which is the guarantee for large-scale reproduction.
- For the artificially preserved spermatozoa of the Yellow River, according to the analysis of reference data [1] and test results, it is believed that if the DNA fragmentation index at ≤ 0.15, the spermatozoa are vigorous, and the preserved spermatozoa can be used as the source of artificial insemination. If the DNA fragmentation index at ≥0.3, the sperm tends to be exhausted and is no longer used as the semen source of artificial insemination.
- The artificial reproduction of pseudobagrus Hwanghoensis is to prepare "artificial semen" with self-developed sperm preservation solution before insemination. The reproduction effect is very good, the fertilization rate is as high as 98.2%, the average is 93%, and the hatching rate is 75%. After five batches of experiments, the reproduction technology is gradually mature, and the reproduction can be customized according to the number of users; We have independently developed a special sperm preservation solution for human beings and established an evaluation system based on SCSA sperm preservation solution through DNA fragmentation index analysis.

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