ESTABLISHING THE EMBRYO VIABILITY FROM FERTILIZED EGGS AND DETERMINATION OF GENETIC SEX TO DANIO RERIO JUVENILE

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Abstract

Establishing the viability of embryos inside the fertilized eggs and the determination of genetic sex to the zebra fish (Danio rerio) was based on the principle of emphasizing the electromagnetic bio-field generated by embryonic cells. The generated electromagnetic bio-field can move a pendulum showing the embryo viability and according to its trajectory (circular or transversal) the genetic sex of embryo or of fry can be established. The studied embryos or fry that generated the transversal movement of pendulum were considered to have male sex, and the studied embryos or fry that generated the circular movement of pendulum were considered to have female sex. The method allows to establish the sex ratio among sexes which was 49.06:50.94. The precision of predicting the genetic sex at 2 weeks at the XY group was 75.38% and to the XX group was of 78.47%. The precision of predicting the genetic sex by emphasizing the electromagnetic biofiled confirmed by establishing the phenotypic sex to the XY group was of 69.66% and to the XX group was 71.05%.

Key words: embryos, sex prediction, electromagnetic bio-field

INTRODUCTION

Genetic sex is determined by the interaction of gonosomes XY (male) and XX (female) in *Drosophila* sex type. Nowadays different methods are used to predetermine the sex of the offspring. Some methods employ sex determination in gametes other in preimplantational embryos.

One of the highly used methods in gametes is based on the flow-cytometric separation of X- and Y-chromosome-bearing sperm based on X/Y DNA content difference (2,4). This method was used with success in cattle, swine, sheep and laboratory animals. Skewed sex ratios of 85 to 95% of one sex or the other were achieved in most species (6). Some of the disadvantages of this method are that it implies the use of expensive equipment and the viability of the gametes is also affected by flow cytometry.

Embryo sexing has been attempted by a variety of methods including cytogenetic analysis, X-linked enzyme activity assays, detection of male specific antigens, use of Y-specific DNA probes and PCR based assays.

Cytogenetic analysis is pointing out the sex chromosomes from embryonic cells. Methods like karyotiping (1), Barr body staining (3) can be used to predetermine the genetic sex. These methods rely on embryo biopsy, which affects the integrity and the viability of embryos.

Measurement of X-linked enzyme activity implies micro-surgical drawing of embryonic cells, their development in special media and the assessment of the quality of enzymes coded by the X chromosome (7). Female embryos having double set of X chromosomes are producing doubled quantity of enzymes. Sex prediction accuracy of 60-70% was reported with this method.

The use of antibodies (H-Y) against a Y chromosome specific surface antigen leads to elimination of male preimplatational embryos whereas the female embryos are surviving. (9) Using this non-invasive method sex prediction accuracy of 80,9% was reported in mice and bovine (8).

Detection of genes known to be presents either on the Y chromosome or on the X chromosomes were adapted for sexing

embryo by polymerase chain reaction (5). Although the procedure seems to be accurate, sensitive and precise, this technique relies on embryo biopsy that affects the viability of the embryos.

MATERIALS AND METHODS

Establishing the viability of embryos inside the fertilized eggs and the determination of genetic sex to the zebra fish (*Danio rerio*) was based on the principle of emphasizing the electromagnetic bio-field generated by embryonic cells. The generated electromagnetic bio-field can move a pendulum showing the embryo viability and according to its trajectory (circular or transversal) the genetic sex of embryo or of fry can be established. The studied embryos or fry that generated the transversal movement of pendulum were considered to have male sex, and the studied embryos or fry that generated the circular movement of pendulum were considered to have female sex. In the case when embryos from the eggs are inactive, are not producing an electromagnet bio-field, tested with the pendulum, are considered dead (10).

In our experiment, to demonstrate practically this phenomenon, we induced spawning to a group of females and males of zebra fish, by the cycle of light and darkness and controlled temperature in an aquarium equipped with a net. The net had a protective role, not allowing the fish to eat its own offspring. The eggs were harvested in the morning after the mating ritual stopped, and the parents were moved to a different aquarium. The viability determination of embryos from the obtained roes was made individually by putting the egg in a Petri dish with the help of the pendulum. If the roe was fertilized and the embryo was alive, the electromagnetic bio-field started to move the pendulum. On the contrary, if the pendulum was not moving, the egg and its embryo were considered dead. From the determined live embryos we established two groups, according to the way of pendulum movement. Therefore, a group was made from embryos that moved in a transversal trajectory the pendulum (considered to be males, on *Drosophyla* sex type with XY chromosomes), and another group was made from embryos that moved in a circular trajectory the pendulum (considered to be females, also on *Drosophyla* sex type with XX chromosomes). The eggs from these two groups were incubated separately.

The hatched fry was reared for two weeks, when the sex was determined again by the same method. Finally, the fish were reared to allow phenotypic macroscopic sex determination.

RESULTS AND DISCUSSIONS

Table 1 is presenting the results regarding the number of obtained eggs and the viability test of embryos from the eggs.

Table 1
Viability and genetic sex of zebra-fish embryos

Total number of obtained eggs	Total number of viable embryos		Number of embryos with genetic sex XY		Number of embryos with genetic sex XX	
	Total	%	Total	%	Total	%
680	640	94,12	314	49,06	326	50,94

From the above table, we observe that from 680 eggs, a number of 640 had an electromagnetic bio-field, being alive, which represents 94.12%. From these 640, 314 (49,06%) had showed a specific field to the male genetic sex (XY), and 326 (50,94%) manifested a female sex specific electromagnetic bio-field. In this way we can appreciate the sex ratio between sexes of 49,06:50,94, being very close to 1:1.

The following table presents data referring the electromagnetic bio-field determination to 2 weeks old zebra fish fry.

Table 2
Obtained results regarding the electromagnetic bio-field determination to 2 weeks old zebra fish fry

Specification	Total number of embryonated eggs by sexes	Number of obtained fry		Number of fry with determined sex at the age of 2 weeks				Total number of fry at the age of 2 weeks	
		Total	%	XX	%	XY	%	Total	%
XY	314	226	71,97	49	24,62	150	75,38	199	88,05
XX	326	230	70,55	164	78,47	45	21,53	209	90,86

The above table shows that from the two groups of embryonated eggs XY and XX were obtained cca 70% of live fry. At the group with the genetic sex considered male XY from 314 eggs, 226 fry were obtained (71.97%), and from the group with the considered female genetic sex XX the 326 eggs produced 230 live fry (70.55%). In order to handle the live fry without producing any injuries, the determination of the electromagnetic bio-field was made at the age of 2 weeks. In these 2 weeks, the losses recorded to both groups were of cca 10%. From the live fry in XY group (199) only 150 (75.38%) showed the specific electromagnetic bio-field for the male sex and the other 49 (26.64%) showed a female specific electromagnetic bio-field. From the live fry in group XX (209) only 164 (78.74%) manifested the electromagnetic bio-field specific for female and the rest (21.54%) manifested a male specific electromagnetic bio-field. The precision in predicting the genetic sex at the age of 2 weeks for XY group is of 75.38% and for XX group is 78.47%.

In table 3 we present the results regarding the determination of phenotypic sex at 3 months old fingerlings.

Table 3.
Obtained results regarding the determination of phenotypic sex at 3 months old fingerlings

Specification	Total number of fry at the age of 2 weeks	Total number of fry at the age of 4 months		Number of fingerlings with phenotypic sex at 3 months			
		Total	%	XX	%	XY	%
XY	199	178	89,45	54	30,34	124	69,66
XX	209	190	90,91	135	71,05	55	28,95

From the 3 months fingerlings in group XY (178) only 124 (69.66%) manifested specific male phenotypic sexand the other 54 (30.34%) manifested specific female phenotypic sex. From the 3 months fingerlings in group XX (190) only 135 (71.05%) manifested specific male phenotypic sexand the other 55 (28.95%) manifested specific female phenotypic sex.

Recorded losses from different causes at both groups were around 10%. The precision in predicting the genetic sex by the emphasize of electromagnetic bio-field confirmed at the age of 3 months by the presence of phenotypic sex at group XY is 69.66% and for females is 71.05%.

CONCLUSIONS

- Electromagnetic bio-field generated by the live embryo in zebra fish egg can move the pendulum;
- The method allows the appreciation of sex ratio which is 49.06 : 50.94, which is very close to 1:1 ratio;
- The precision in predicting the genetic sex at the age of 2 weeks for XY group is of 75.38% and for XX group is 78.47%;
- The precision in predicting the genetic sex by the emphasize of electromagnetic bio-field confirmed at the age of 3 months by the presence of phenotypic sex at group XY is 69.66% and for females is 71.05%.

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