Evaluation of Intracytoplasmic Sperm Injection (ICSI) in three different genera of finfish

by

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ABSTRACT. - In this work Intracytoplamic Sperm Injection (ICSI) was evaluated in three genera of finfish. The different characteristics that must be considered when performing ICSI include: spawning period and conditions, gamete size and activation characteristics, adhesive properties of eggs, transparency of the chorion, size and localization of the micropyle, and the value of the species studied.

Key words. - ICSI - Micropyle - Fish eggs - Spawning.

Introduction

Results have demonstrated the feasibility of using ICSI in finfishes (Poleo *et al*., 2001, 2005), however the diversity of physiological and morphological characteristics among fish gametes suggests that techniques will need to be modified according to taxa. In this work, gametes from fishes representing three families of teleosts; Cyprinidae (zebrafish, *Danio rerio*), Ictaluridae (channel catfish, *Ictalurus punctatus*) and Cichlidae (Nile tilapia, *Oreochromis niloticus*) were compared for ICSI suitability. The objectives were to evaluate: 1) manipulation of gametes, 2) differences in spawning characteristics, 3) micropyle localization, 4) time required for sperm injection, and 5) to compare fertilization rates after ICSI among the three fishes.

Methods

Sperm injections were performed following the basic method for ICSI modified according to species (Poleo *et al*., 2001, 2005).

Results and discussion

The size of the tools required for the injection of sperm and holding of eggs was dictated by the size of the eggs. The injection pipette was 10 to 15 μm in diameter for all the fish studied. However, the diameter of the holding pipette changed depending on the diameter of the egg: being 100 and 200 μm for zebrafish (~0.8 mm egg diameter), 100 µm to 300 µm for Nile tilapia (eggs varied from 1.5 mm to 2.5 mm) and 2 mm for channel catfish (~4 mm egg diameter). Zebrafish and Nile tilapia gametes can be obtained year-round. Channel catfish spawn seasonally, and eggs are available for only a few months. Some fish eggs have a coat of adhesive material, this adhesiveness is an obstacle for ICSI because it hinders manipulation. Of the three species of fish described here, only channel catfish eggs were adhesive. However, this was overcome by coating the tools used to manipulate the eggs with a silicon and vacuum grease. Localization of the micropyle is essential because it is used to guide the pipette into the cytoplasm of the egg. The homogeneous texture and shape of eggs from zebrafish together with the small size of the micropyle made localization difficult. The morphological structure of the egg and the large size of the micropyle facilitated the localization of the micropyle in Nile tilapia and channel catfish. The average time required for each injection in Nile tilapia eggs was 5 min in comparison to 8 min for zebrafish and 10 min for channel catfish. The number of injections per session performed in Nile tilapia were ~36, as the eggs can be held for at least 3 h in Hanks' without losing their fertilization capability. In zebrafish, approximately 8 injections were administrated in 1 hour per batch of eggs. From a total of 233 zebrafish eggs injected, 4 (2%) developed normally and hatched. Of the 160 Nile tilapia eggs injected, 5 (3%) developed normally and hatched. Injections of 188 channel catfish eggs yielded no fertilization. The potential value of the fish will dictate the amount of time and funding available for ICSI research. Zebrafish is extensively used as a vertebrate model for basic studies in fertilization, development and genetics in many laboratories around the world. Nile tilapia and channel catfish are important food fish which makes them the subject of research in terms of improvement of production, disease resistance and flesh quality.

Conclusion

Each species of fish has evolved intrinsic fertilization characteristics, which require the development of specific ICSI techniques.

References

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