

A brief Exposure to low pH prior to refrigerated storage reduces the motility and viability of goldfish sperm

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Summary

Goldfish sperm was diluted (1:3) and incubated for one hour in acid (pH 6.51) or alkaline (pH 8.5) conditions. Subsequently the acid and alkaline exposed sperm, was further diluted (1:3) in the alkaline or a neutral pH immobilizing solution with the final preparations having pH 7.51(\pm 0.02) and 7.56 (\pm 0.07) in the acid and alkaline group respectively. The brief exposure of sperm to acid acidic conditions prior to storage resulted in reduced sperm viability and motility in the next days of storage. Compared to the control group, the acid exposed spermatozoa exhibited lower viability and motility after the first 24 hours of chilled storage. The results indicate that even a brief exposure of sperm to low pH, for example by the contamination of sperm sample with urine can compromise motility and viability of goldfish spermatozoa.

Introduction

There are several published protocols for chilled storage of fish sperm (Babiak et al., 2006; Mansour et al., 2004; Penaranda et al., 2010). The dilution of stored sperm in an immobilizing medium can control the ionic environment and overcomes the problems of oxygenation and dehydration during chilled storage (Saad et al., 1983; Babiak et al., 2006). Sperm viability and motility are important parameters for evaluating the quality of sperm. Several factors can influence the viability and motility of sperm treated for short term refrigeration storage and for long term cryopreservation. Exposure of sperm to acidic conditions, for example by the contamination of sperm with urine during sampling, can result in low motility, thus worsened fertilization and hatching rate (Nynca et al., 2012). The exact influence of pH on sperm motility and viability may vary with species and the acidic or alkaline range of the pH that the sperm is exposed (Dziewulska and Domagała, 2013; Gonzalez-Bernat et al., 2013). Even a brief exposure of sperm to acidic conditions may result in lethal or sub lethal cellular damage (Santiso et al. 2012). The purpose of this work was to evaluate goldfish sperm motility and viability after a brief exposure to acidic conditions prior to refrigerated storage.

Materials and Methods

Sperm was collected (using capillary pipette tips as catheters) from 11 goldfish. Selected samples were pooled and subdivided in two equal portions for either acid or alkaline treatment group. For the dilution of spermatozoa, the phosphate buffer which have been used in previous works (Nathanailides et al 2011) was replaced with Tris buffer, as in a pilot experiment we observed that phosphate interfered

with the fluorescent microscopy used for the viability assay. Each group of spermatozoa was diluted (1:3 v/v) in an acid (125 mM NaCl, 0.1 mM CaCl, 20 mM Tris pH 6.5, titrated with 1M HCl) or an alkaline (125 mM NaCl, 0.1 mM CaCl, 20 mM Tris pH 8.5) immobilizing solution (Saad et al. 1983). These final preparations had pH 7.51(\pm 0.02) and pH 7.56 (\pm 0.07) in the acid and alkaline group respectively. Diluted samples were supplemented with 200 mU/mL penicillin+streptomycin and kept in 1.0 ml Ziploc bags (1.9 x 1.9 cm) which were inflated with air and gently rotated daily (Babiak et al., 2003) and stored at 4°C. Viability and motility was estimated in samples obtained daily for a period of five days. The number of dead spermatozoa was assessed by eosin Y according to the methodology described by O'Connell et al. (2002). Motility was estimated after dilution in activating solution as the percentage of spermatozoa exhibiting forward motion (Nathanailides et al., 2011). The data present means \pm SD for triplicate measurements. Statistical analysis was performed by paired Student's t-test. Probability values $p < 0.05$ were considered statistical significant.

Results

In the alkaline treated sperm, total motility decreased from $79.01 \pm 4.75\%$ to $62.79 \pm 2.90\%$ and viability decreased from $96.02 \pm 1.36\%$ to $69.01 \pm 5.90\%$, after four days of chilled storage. The acid pre-incubated sperm exhibited a much faster rate of decline in both sperm viability and motility (Figure 1). As a result, after three days of chilled storage, the acid pre-incubation sperm was not viable.

Discussion and Conclusions

A reduction in sperm quality over a period of chilled storage is a result of sperm activation, oxidative damage, compromised integrity of cell structures and DNA. A reduction in sperm viability over a period of chilled storage indicates the lethal damages whereas sperm motility indicates the level of sublethal damage (Ciereszko et al., 2005). The results indicate that even a brief exposure to acid conditions can affect sperm viability and motility during short term refrigerated storage. There was a good correlation between sperm viability and motility in both the acid and alkaline conditions but sperm incubated in alkaline conditions exhibited higher motility and for longer period of time. The acid group exhibited a dramatic drop in viability and motility after the third day of chilled storage. These results illustrate the importance of avoiding exposure of sperm to unfavourable chemical conditions. For example, even a brief exposure of sperm to urine can compromise gamete quality and the success of sperm cryopreservation (Nynca et al., 2012). In the same manner, a brief exposure of sperm to acidic conditions can compromise both sperm DNA integrity and motility (Santiso et al. 2012).

In the present work, even the control group of spermatozoa exhibited a loss in viability and motility on the fifth day of storage. A reduction in sperm viability and motility is expected during chilled storage, but results may vary with temperature, species, immobilizing solution and the method of sperm aeration.

In agreement with recently published works on other species, the results indicate that even a brief exposure of sperm to low pH, for example by the contamination of sperm sample with urine can compromise the quality of sperm samples. Furthermore, during the fertilisation of some fish species, acidic conditions may compromise sperm motility and fertilising ability of some fish

species (Alavi and Cosson, 2005; Dadras et al., 2013; Gonzalez-Bernat et al., 2013).

In conclusion, motility and fertilising ability of goldfish sperm may be reduced after a brief exposure to acidic conditions.

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LEGEND TO FIGURE

Figure 1. Percentages (%) of viability (squares) and motility (triangles) of goldfish chilled sperm kept at 4 °C. Spermatozoa were either briefly exposed to acidic conditions (Acid exposed sperm, empty triangles and squares) or constantly maintained under alkaline conditions (Control group, filled triangles and squares). Compared to the control group, the acid exposed spermatozoa exhibited lower viability and motility after the first 24 hours of chilled storage ($P < 0.05$).

FIGURE

