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Short communication

Evaluation of activation and storage conditions for sperm of yellow drum *Nibea albiflora*

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ABSTRACT

The yellow drum (*Nibea albiflora*) is a commercially important fish species in East Asia and artificial propagation has recently been initiated due to the rapid decrease of wild capture. To facilitate artificial insemination in aquaculture, sperm activation and storage conditions were evaluated in this study. Our findings revealed that yellow drum sperm can be activated with calcium-free Hank s balanced salt solution (C-F HBSS), KCl, NaCl, and glucose at an osmolality range of 300–1200 mOsm/kg with the highest motility observed at 600–800 mOsm/kg. The average motility duration was less than 6 min. For sperm storage, C-F HBSS, fish Ringer solution, KCl, NaCl, and glucose at 150, 270, and 300 mOsm/kg were compared for selection of the optimal extender, and results showed detrimental effects of KCl, NaCl, and glucose. In contrast, C-F HBSS and fish Ringer solution or sperm stored undiluted in seminal plasma could retain motility for up to 48 h when samples were stored at 4 °C. The highest sperm motility was found with treatments at 150 mOsm/kg for both C-F HBSS and fish Ringer solution. Sperm samples stored at 4 °C were also found to retain motility longer than those stored at room temperature (25 °C). Our findings provide the basic information on sperm handling in yellow drum.

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1. Introduction

The yellow drum (*Nibea albiflora*, Sciaenidae), also called Huang Gu Yu in Chinese or Koichi in Japanese, is a commercially important fish species distributed along the coasts of East Asia (Han et al., 2008). Because of its delicate flavor and rich nutritional value, many Chinese admire this fish, and its cost for food is similar to the popular large yellow croaker (*Pseudosciaena crocea*) in local fish markets. In addition, its swimming bladder has long been used as a traditional Chinese medicine for curing abdominal pain of postpartum women (Xu et al., 1996). In the early 1990s, fishery production of yellow drum mainly relied on wild capture. However, overfishing and ocean pollution in the past decade have led to a dramatic decrease of fish in the wild fisheries of China. Thus, aquaculture has become the main route for satisfying the needs of domestic markets. More importantly, yellow drum also possesses features of fast growing, strong adaptability to environment, high resistance against diseases, and short culture cycle for reaching marketable size, and thus is an ideal aquaculture species.

Despite its importance as a valuable aquaculture fish, studies on yellow drum are limited. Research has just recently been initiated on its artificial breeding, fry production, and holding culture in net cage (Liu, 2009; Sun et al., 2005; Yu et al., 2005). Fry production is relatively low compared with other marine fish cultured in China (Hong and Zhang, 2003). In particular, the fish stock along the Wenzhou coast in East China Sea has a short peak spawning time window of approximately 2 weeks (late May to the early of June) (our observation).

Sperm activation and storage consist of the prerequisite for sperm handling in artificial spawning. Yellow drum are known to breed and spawn in shallow coastal waters during warm seasons while moving back to deeper waters in the cooler seasons (Takita, 1974). In summer, there is a great fluctuation of salinity and temperature in the coastal waters of the East China Sea due to its rainy season. Thus, it is difficult to adapt the existing sperm activation and storage methods that have been developed for freshwater or marine species in the literature. This study was therefore intended to develop the optimal sperm activation and storage conditions for yellow drum and our findings will provide the basis for future studies involve gamete handling of this species.

2. Materials and methods

2.1. Sperm collection

Mature yellow drum *N. albiflora* (2-year old, ~0.5 kg in wet weight) were cultivated in Zhejiang Mariculture Research Institute, Wenzhou,

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China during the peak spawning season (from the end of May to the early of June) in 2009. Males were separated from females and maintained in two tanks at 24 °C with 7 to 8 fish per tank and fed once daily with oysters. Sperm were collected by gently hand-stripping without anesthesia. One ejaculate was collected from each fish throughout the spawning season, and repeated stripping was not possible in our case because these fish were very vulnerable to handling. Sperm samples were stored on crushed ice and experiments were initiated within 30 min of the collection. Semen volume was determined by their weight based on the assumption of the specific gravity of 1.0. Sperm numbers were obtained from the average of duplicate counts using a hemocytometer. Blood samples were collected in micro-hematocrit tubes (VWR Scientific, Niles, IL) by severing the tail, and blood plasma were collected after centrifugation at 15,000 rpm for 10 min. Seminal plasma from undiluted semen samples were collected by centrifugation at 15,000 rpm for 20 min. The osmolality was measured with the Osmomat 030 freezing point depression osmometer (Genotec GmbH, Berlin, Germany).

2.2. Motility estimation

Motility was estimated subjectively using darkfield microscopy with a 200× magnification within 30 s after sperm activation. Samples were activated in room temperature (25 °C) by suspending 1 μl of sperm suspension into 19 μl activation solution (specified below) on a glass slide without a coverslip. Percent motility was defined as the percentage of progressively motile sperm; sperm that vibrated in place without forward movement were not considered to be motile. Estimates were made within 30 s after the addition of activating solutions. The time duration was recorded as the period between the beginning of sperm activation and when all sperm ceased to be motile.

2.3. Sperm activation

There were three trials in this experiment for selection of optimal osmolality and solution for sperm activation. In the first trial, two-way factorial design was employed to evaluate the effects of activation solution and osmolality. Specifically, undiluted sperm were activated with Calcium-free Hanks Balanced Salt Solution (C-F HBSS) (recipe see Dong, 2005), KCl, NaCl, and glucose each at 12 osmolalities ranging from 150 to 1200 mOsm/kg. Sperm samples from five males (one ejaculate per male) were used, and motility and time duration were recorded. We used 2-factor randomized block design with male serving as the block to further refine the optimal activation solution and osmolality in the subsequent two trials. Thus, in the second trial, undiluted sperm samples from four males were further tested with C-F HBSS, KCl, NaCl, glucose each at 700 mOsm/kg and seawater used for raising these fish (710 mOsm/kg). In the third trial, glucose with an osmolality range of 625 to 775 mOsm/kg was compared with seawater for activation of undiluted sperm samples collected from three males.

2.4. Sperm storage

To identify the appropriate solution (extender) and its osmolality, and temperature for sperm storage, this experiment employed a two-way factorial design with repeated measure. Specifically, C-F HBSS, fish Ringer solution (Ginsburg, 1963), KCl, NaCl, and glucose each at 150, 270, 300 mOsm/kg were used as extenders for sample storage at room temperature (25 °C) or 4 °C in a refrigerator. Sperm samples from three males were used. For each ejaculate, samples were divided into 16 subsamples including 15 treatment groups (5 extenders × 3 osmolalities) and one control of undiluted sperm samples. After suspended in appropriate media, these subsamples were further divided into two sets with one set stored at room temperature and the other set stored at 4 °C. Except for the controls with undiluted sperm, all samples for storage were adjusted to a sperm density of ~8×10^8/ml and kept at 100 μl per tube. For motility estimation, sperm were activated with glucose at 700 mOsm/kg and seawater. Samples were checked for motility at 2 h, 4 h, 8 h, 12 h, 24, 48 h, and 72 h after storage.

2.5. Statistical analysis

Data for sperm activation were analyzed using one-way or two-way ANOVA with Bonferroni post-tests for comparisons among different treatment groups. Data for sperm storage were analyzed with a repeated measure two-way ANOVA (SPSS 16.0). Results were presented as means ± SE, and probability values of $P<0.05$ were considered to be significant. Data for sperm motility were arcsine transformed prior to analysis.

3. Results and discussion

3.1. Sperm activation

The average sperm volume was 210 ± 42 μL per ejaculate with a sperm density of $2.37\pm0.64\times10^{10}$/ml. The osmolality of blood plasma was 336 ± 3 mOsm/kg ($n=3$), and of seminal plasma was 465 ± 10 mOsm/kg ($n=4$).

In the first trial, sperm motility increased with the osmolality of activating solutions to the highest level at 700 mOsm/kg and declined thereafter at 800 mOsm/kg and above (Fig. 1). An osmolality range of 600 to 800 mOsm/kg usually yielded significantly higher motility than those at either lower (≤400 mOsm/kg) or higher (≥1100 mOsm/kg) osmolalities ($P<0.05$). However, there was no difference among the four solutions at 700 mOsm/kg ($P=0.803$). Sperm samples at high motility (58%–69%) on average last for 2.8 min when activated with solutions at 700 mOsm/kg, while longer time duration (7–7.5 min) was found in samples activated with C-F HBSS and KCl at 400 mOsm/kg yielding low motility (15%–26%) (Fig. 1), which could be due to the incomplete activation at lower osmolalities. Motility duration did not exhibit any significant difference among activation solutions of 600 mOsm/kg and above ($P>0.05$). In the second trial, both motility and time duration in samples activated with glucose 700 and seawater were higher than samples activated by C-F HBSS, KCl, and NaCl at 700 mOsm/kg though they were not different from each other

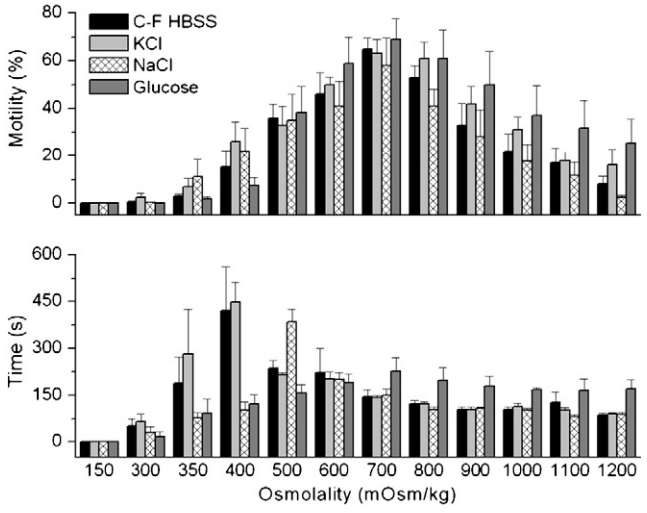

Fig. 1. Motility (%) and time duration (second) of undiluted yellow drum sperm after being activated with C-F HBSS , KCl, NaCl and glucose at different osmolalities ranging from 150 to 1200 mOsm/kg. Data presented were mean ± SE from five males with one ejaculate per male.

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