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MOTILITY OF RAINBOW TROUT SPERM
IN OVARIAN FLUIDS
AND SALINE SOLUTIONS

by
Carmen Olito and Irvin Brock

Number 99



Alaska Department of Fish & Game
Division of Fisheries Rehabilitation,
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ABSTRACT

Rainbow trout, *Salmo gairdneri*, eggs at the Fort Richardson Hatchery suffered up to 52% mortality in 1986. Sperm motility was monitored throughout several different egg fertilization methods in an attempt to identify the cause of this mortality. Milt and ovarian fluid were taken, mixed, and allowed to sit for 10 min. Milt and a saline solution were also mixed and allowed to sit for 10 min. At one-minute intervals, sperm motility was evaluated. Ovarian fluid alone activated some sperm, but at least 50% of the sperm were motile up to eight minutes after mixing with water. Results were inconsistent for saline. A saline solution should only be used as a diluent if absolutely necessary and with the lowest possible dilution ratio.

KEY WORDS: Rainbow trout, *Salmo gairdneri*, broodstock, sperm motility, fertilization, ovarian fluid, sperm extender, Fort Richardson Hatchery, Broodstock Development Center.

INTRODUCTION

Only 48% of the rainbow trout, *Salmo gairdneri*, eggs taken in 1986 at the Alaska Department of Fish and Game's Broodstock Development Center (BDC), Fort Richardson, Alaska survived from fertilization to the eyed stage. We believe this high level of mortality was the result of unfertilized eggs rather than subsequent physical damage. The method used to add sperm to green eggs may have affected sperm motility. The eggs were stripped into a net, examined for obvious anomalies, then placed in a bucket. After the eggs from 20 to 30 females were placed in the bucket, milt from several males was individually added to the eggs. The addition of milt took from 2 to 10 min. After milt from the last male had been added, the milt and eggs were gently mixed by hand. Water was added and the eggs and milt were again mixed to activate the sperm. If sperm motility was not activated until all gametes were mixed,

then all the males should have contributed to fertilization. If sperm motility was activated by ovarian fluid and lasted for less than a minute, sperm from the first few males may have only fertilized those eggs that were contacted prior to mixing.

Projects conducted during egg takes at the BDC sometimes required a greater volume of milt from one fish than was usually available. In these cases a 0.8% NaCl solution, which may also affect sperm motility, was used as a diluent. This saline solution is simple to prepare and was reported to be as effective as other buffered diluents (Scott and Baynes 1980).

Scott and Baynes (1980) and Stoss (1983) have reviewed and summarized many reports of salmonid sperm biology and sperm preservation. It appears that experimental results are sensitive to subtle differences and may even be site-specific. Dave Erdahl¹ (personal communication), who has worked extensively with preservation of rainbow trout sperm, suggested that some experimentation be done under extant conditions to determine the most appropriate spawning and sperm dilution methods to use. Consequently, this study was designed to approximate the time sperm may be exposed to ovarian fluid or diluent before being activated by the addition of water under normal spawning conditions at the BDC.

MATERIALS AND METHODS

Milt and ovarian fluid were taken from fish in the spawning shed at the BDC during the 1987 rainbow trout egg take. A portion of the milt was set aside as a control to determine if the sperm from a given sample remained motile throughout the time of the experiment. One drop of this milt was mixed with 1/3-ml water and the duration of sperm motility was then timed at the beginning and end of each experiment. This was done to verify that any change observed in sperm motility resulted from test conditions rather than from sperm degeneration. In addition to a sample for the control, three test

¹ Iron River National Fish Hatchery, Wisconsin.

solutions of milt were evaluated to determine sperm motility: in Treatment 1, sperm and ovarian fluid were mixed at a ratio of 1:1; in Treatment 2, sperm and 0.8% NaCl solution were mixed at a ratio of 1:1; and in Treatment 3, sperm and 0.8% NaCl solution were mixed at a ratio of 1:10. Each of these test solutions was evaluated both before and after addition of water. Evaluations were repeated each minute for 10 min and consisted of observing one drop of solution to estimate the percentage of motile sperm. At each sample time immediately after examining the test solution, a second drop mixed with 1/3-ml water to activate sperm was added. A drop of this new solution was then evaluated for motility. A sperm was considered progressively motile (i.e., forward motion) if it was actively swimming rather than simply vibrating. All samples were exposed to air at ambient temperature (9°C-12°C) for the 10 to 12 min of the experiment. Each of the treatments was replicated three times during the spawning season to determine the average value.

RESULTS AND DISCUSSION

After the addition of water, the duration of sperm motility in control samples varied from 50 sec to 25 sec in all controls. These differences were not considered important as evaluations for the percentage of motile sperm were completed within 15 sec. It is assumed that the most effective sperm show progressive motility when activated. Vibratory motion may indicate that sperm have not been completely activated, or swimming motion has ceased (Scott and Baynes 1980). A sperm with only vibratory motion has little chance of fertilizing an egg. Therefore, vibratory motion was not considered motility in this work.

Ovarian Fluid

If motility of rainbow trout sperm is activated by the addition of water rather than ovarian fluid, we would not expect to see motile sperm after the addition of ovarian fluid alone. There were few motile sperm after the addition of ovarian fluid (Figure 1).

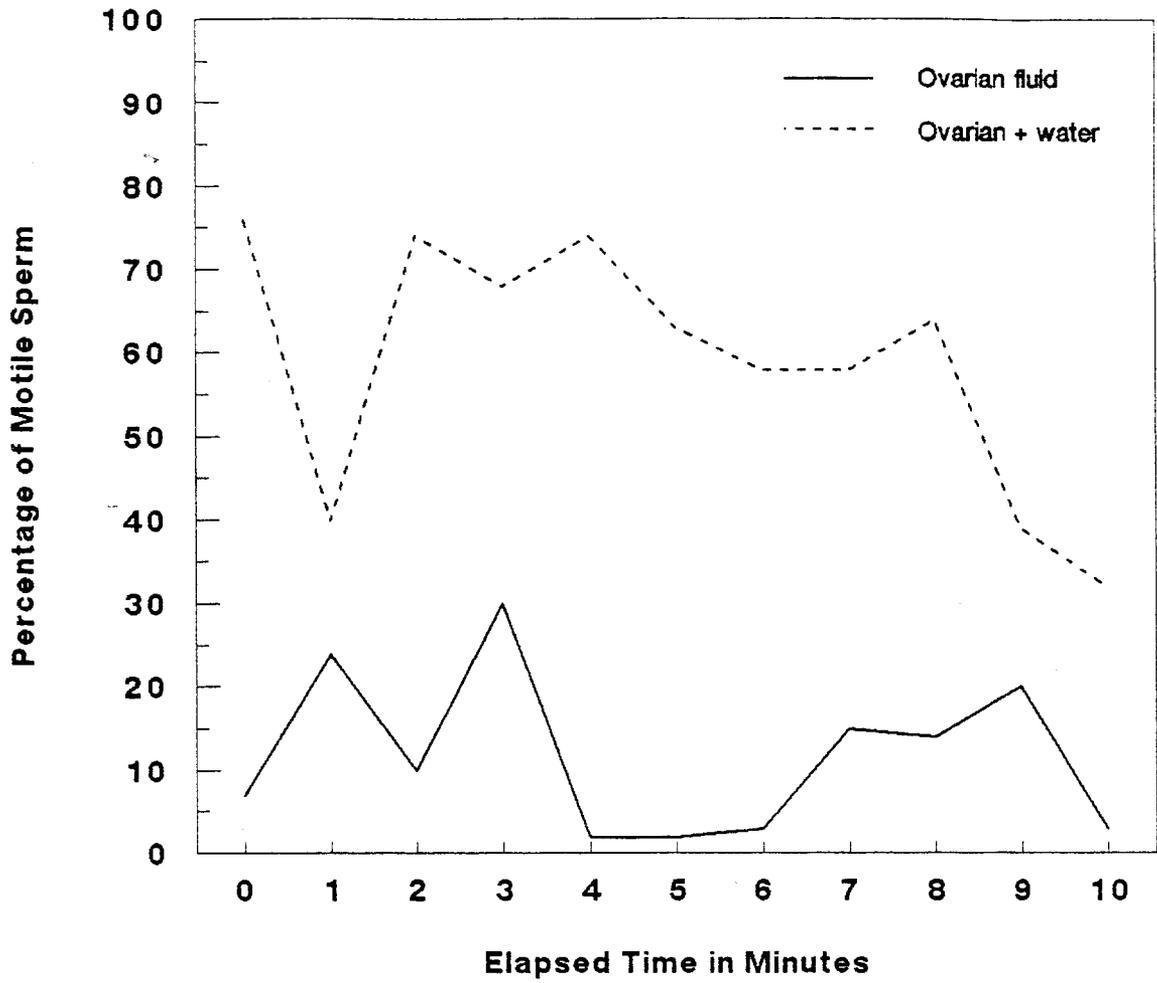


Figure 1. Percentage of progressively motile sperm using milt and ovarian fluid in a 1:1 ratio and milt and ovarian fluid with water.

In several instances, there were more motile sperm than expected. These samples may have inadvertently been contaminated with water.

The addition of water to the ovarian fluid/milt mixture should have increased the number of motile sperm. This was seen in our results. The low percentage of motile sperm at 1 min may have been due to incomplete mixing with water which exaggerated the effect of poor sperm from a male in one of the replicates. After 8 min, the percentage of progressively motile sperm dropped to less than 50%. We considered this to be only marginally acceptable.

Saline Solution, 1:1 Ratio

If the 0.8% NaCl solution functioned as a diluent for milt without activating sperm motility, we would have seen results similar to those observed after the addition of ovarian fluid. This did not prove to be the case as there was sperm activity after the addition of saline (Figure 2). The level of motility increased only slightly after water was added. It is possible that the concentration or pH of the saline solution may not have been appropriate for the conditions. An inappropriate saline solution may have activated sperm over time rather than the immediate or delayed activation of all sperm.

Saline Solution, 1:10 Ratio

There was a dramatic drop in the percentage of sperm motility after the first minute when milt was diluted with 10 parts saline (Figure 3). Erdahl (personal communication) pointed out that higher dilution ratios of saline to milt reduced the time that sperm can be stored prior to water activation. This was apparent in our results which showed that most of the sperm were activated within 2 min. The small increase in sperm motility observed at 3 and 7 min may have resulted from an incomplete mixing of the solutions initially.

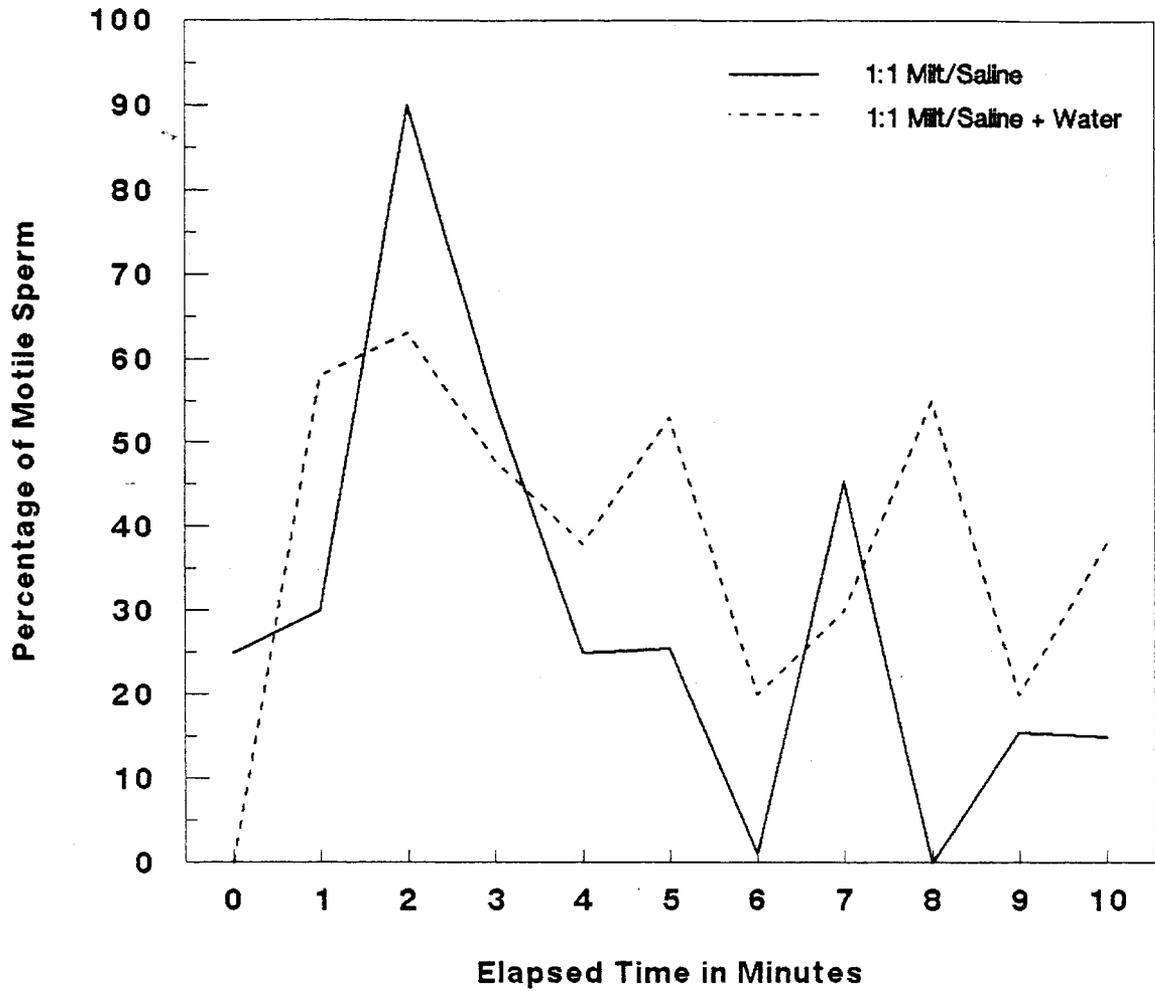


Figure 2. Percentage of progressively motile sperm using milt and saline in a 1:1 ratio and milt and saline (1:1) with water.

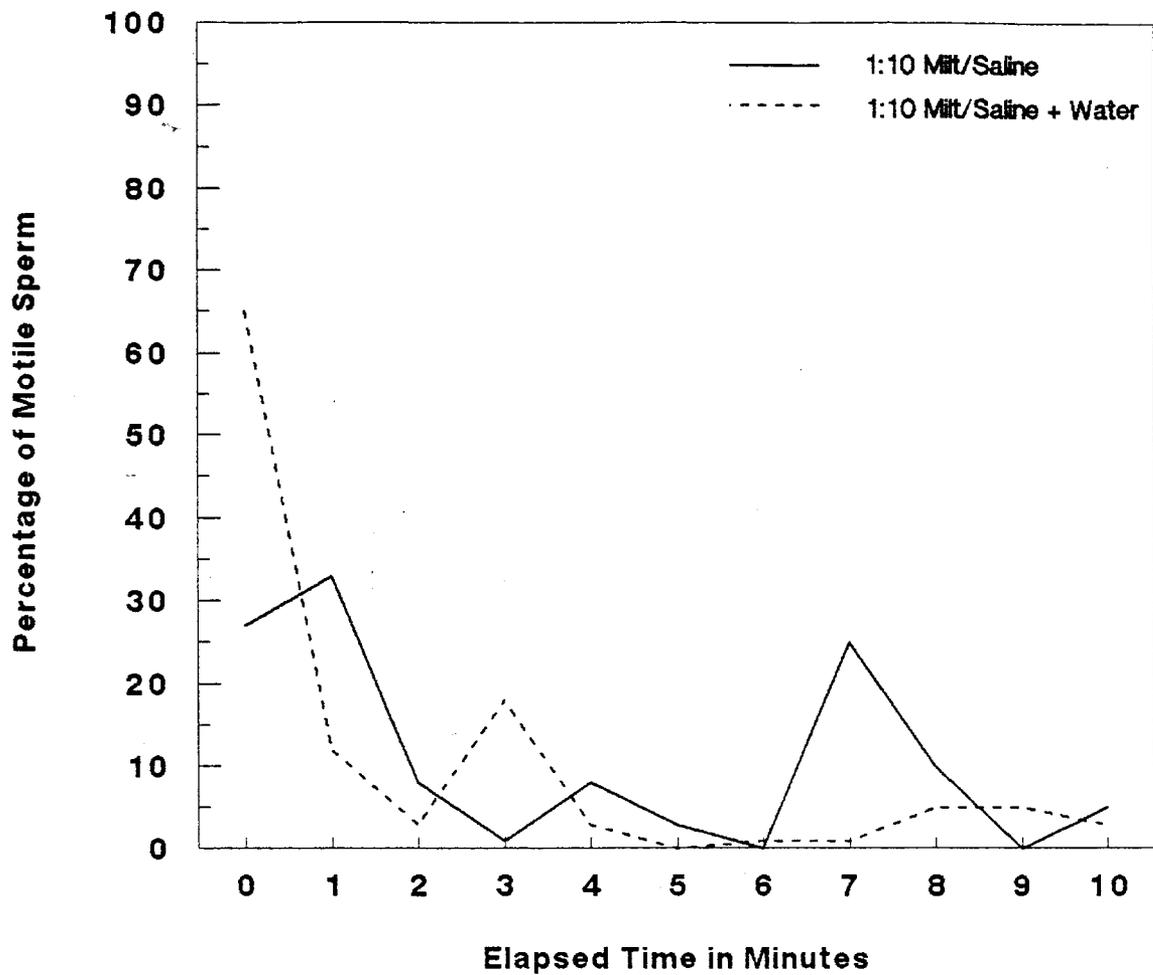


Figure 3. Percentage of progressively motile sperm using milt and saline in a 1:10 ratio and milt and saline (1:10) with water.

CONCLUSIONS

Although motility is not an absolute indicator of the sperm's ability to fertilize an egg, there is generally a strong correlation between sperm motility and fertility rate (Stoss 1983). Ovarian fluid alone can activate sperm motility, but we found that it usually only activated 10%-15% of the sperm. Consequently, we expected a high fertilization rate if we mixed sperm thoroughly with eggs before adding water. A few minutes delay between additions of milt from several males should not be detrimental to sperm motility as long as water is added to activate sperm within 8 min.

The spawning techniques at the BDC were modified before the 1987 egg take to reduce the time between addition of milt and subsequent addition of water to less than 5 min. Gametes were also thoroughly mixed before water was added. Subsequently, the rainbow trout egg fertilization rate was 93%.

If milt volume must be extended, we recommend using the lowest possible dilution ratio. In a 1:1 dilution with milt, up to 50% of the sperm can still be activated with water after 5 min. After a 1:10 dilution with saline, the sperm are activated immediately and motility virtually ceases after 3 min. In addition, using 0.8% saline as a diluent is only recommended when the milt is to be used immediately.

ACKNOWLEDGMENTS

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