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Examination of Sperm Viability and Egg Fertilization Rates in Trout (*Oncorhynchus mykiss*) Breeding

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Abstract

The purpose of this review is to investigate various methods used to extend sperm viability and increase egg fertilization rates in trout. It aims to identify effective sperm protection and fertilization rate increasing strategies to increase variation in trout variation. Sperm samples taken from trout were examined with different preservative solutions and storage conditions. Sperm viability conditions and fertilization rates were compared using Cortland solution and other preservative solutions. Findings obtained through analysis show that some preservative solutions and storage methods significantly increase sperm survival time and egg fertilization rates. It has been observed that Cortland solution, in particular, is effective in prolonging sperm viability and increasing fertilization rates. The possibility of applying effective methods to increase the viability of trout sperm and the prolongation of fertilization and the positive effects on trout production are being investigated. Guidance for practical suggestions and changes is recorded.

Keywords: *Cortland, solution, trout, motility, sperm, egg*

1. Introduction

Trout (*Oncorhynchus mykiss*) is of great importance in aquaculture on a global scale. Its ability to be grown with high productivity in freshwater environments and its economic value have made this species a preferred subject of cultivation worldwide. Turkey is one of the leading countries in Europe in trout cultivation, producing approximately 150,000 tons of trout in portion sizes (280-320 gr) and above kg per year. This situation makes significant contributions to the local economy and also creates employment (Stankus, 2022). With the increasing importance of the concepts of sustainability and productivity in the global fisheries sector since 2000, modern techniques have been adopted in trout cultivation and biotechnological approaches have become rapidly widespread in the cultivation of this species. In this process, genetic improvement programs, the use of environmentally friendly feeds and optimized breeding protocols have been implemented in order to increase productivity in trout cultivation (Alavi and Cosson, 2006). In aquaculture conditions, ensuring high sperm viability and optimum fertilization rates increases the efficiency of the production process and enables the obtaining of healthy juvenile fish. Therefore, optimizing sperm quality and fertilization rates stands out as an important research area in aquaculture (Tzeng et al., 2002 ; Billard and Cosson, 2020).

Factors affecting sperm viability include storage conditions, preservative solutions used, and sperm collection methods. Sperm viability is generally defined as the ability of sperm cells to

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maintain their physiological functions, and this may affect the reproductive success of fish (Guerin et al., 2006). Preserving sperm viability is especially important for processes such as sperm freezing and long-term storage (Doğan, 2023).

Various methods have been developed to increase sperm viability and fertilization rates. Many studies have been conducted, especially on the effects of preservative solutions. Cortland solution is a common solution used to preserve sperm viability. This solution has been shown to be effective in increasing the vitality of sperm cells and improve fertilization rates (Kime et al., 2009).

Many studies have investigated many factors affecting sperm vitality and fertilization rates. These studies have revealed the effects of sperm storage techniques, temperature control and preservative solutions used on sperm quality. In particular, comparisons between different preservative solutions play an important role in determining the most effective methods (Wang et al., 2014; Lahnsteiner et al., 2016).

The methods used to increase sperm vitality and fertilization rates include factors such as cryopreservation (freezing) techniques, providing appropriate temperature conditions and solution formulations. Evaluating the effectiveness of these methods is a critical step in providing more successful and efficient production processes in fish farming (Pérez et al., 2018; Doğan, 2023).

The aim of this study was to evaluate the effectiveness of various methods used to extend sperm vitality and increase egg fertilization rates in trout. Different sperm storage techniques and preservative solutions will be compared and the most effective methods will be determined. This study aims to fill the gaps in current knowledge and to provide applicable recommendations in trout farming.

2. Material and Method

For the study, 20 healthy female and 5 functional male trout were provided from Ayhan Alp Alabalık Ürt. ve Tic. Trout farming facility in Seydikemer district of Muğla province. This study was carried out with an experimental research design in order to investigate sperm viability and egg fertilization rates in trout (*Oncorhynchus mykiss*) farming. In line with the purpose of the study, different solutions (Cortland liquid, Ringer solution, Hepes buffer (N-2-Hydroxyethyl]piperazine-N-2-ethanesulfonic acid), Sodium Hydroxide (NaOH) 0.038 g/l) will be used to investigate the effects of various environmental factors (water temperature, pH, water quality) on sperm viability and fertilization rates. Component ratios of Ringer solution: Sodium chloride (NaCl) 6.5 g/l, Potassium chloride (KCl) 0.42 g/l, Calcium chloride ($CaCl_2$) 24 g/l, Sodium bicarbonate ($NaHCO_3$) 0.2 g/l, Cortlant solution component ratios: Sodium chloride (NaCl) 8 g/l, Potassium chloride (KCl) 0.2 g/l, Sodium bicarbonate ($NaHCO_3$) 0.35 g/l, Glucose 1 g/l (Hatipoğlu and Akçay, 2010; Little and Rubinstein, 2012).

2.1. Egg Collection from Female Broodstock Fish

For experimental applications, sperm and eggs were collected from female and male trout during their reproductive periods. The process of collecting eggs from female fish was carried out by anesthetizing them with the method of immersion in water as 5 ml/50 l (phenoxyethanol/water) and calming the fish. After the anesthesia process, the female fish's abdomen was massaged in a way that would not damage the mucosa, as seen in Figure 1, and the eggs were collected in a sterile container, mixed and homogenized.

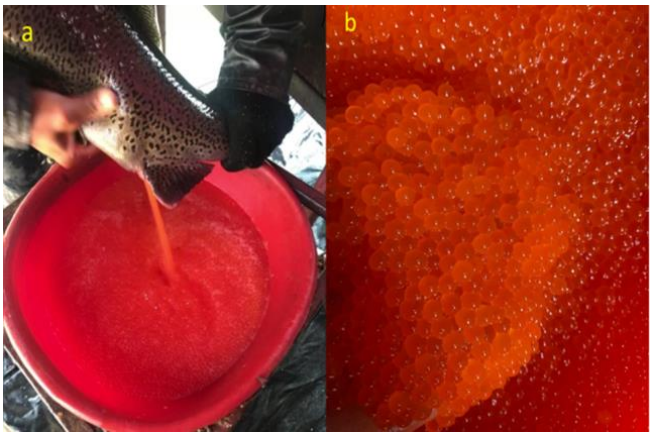

Figure 1. Egg collection from broodstock a,b, (Original)

2.2. Sperm Collection from Male Fish

The sperm collection process from male fish was carried out with the same method as in females, but since male fish were functional, they were dissected instead of milked and the gonads were removed as in Figure 2 and sperm was collected. 8-10 ml of sperm taken from each male was put into the same container and mixed as in the eggs. The collected sperm and eggs were kept in the hatchery without contact with water until the fertilization process took place.

Figure 2. Gonad removal from male fish, a b (Original)

2.3. Fertilization Procedure

After the egg and sperm collection procedures were completed, the fertilization procedure was performed. It was planned to have 1000 eggs in each group, including the control group, with 3 replications. 0.9% NaCl (physiological salt solution), salt water was added to the eggs taken into the containers, then 5 ml of sperm was added to the egg and salt water mixture and left to fertilize by mixing gently. After approximately 5 minutes, the operating water was added to harden the eggs. Then, they were placed in the hatchery cabinets, observed and opened.

3. Findings and Discussion

The findings of this study show the critical importance of sperm viability and egg fertilization rates in trout farming and how they are affected by various factors.

In our study, it was determined that sperm viability varies in different conditions. Sperm motility and viability vary depending on the storage methods used, temperature changes, and sperm

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collection techniques (Carleton and Drouillard, 2014; Miller and Armstrong, 2021). In particular, the viability rates of sperm cells stored at low temperatures show a significant decrease compared to fresh sperm (Álvarez and García, 2019; García et al., 2020). This finding emphasizes the importance of using and developing appropriate methods to preserve sperm quality during long-term storage processes (Huang et al., 2018; Doğan, 2023). The results of this study are shown in Table 1.

Table 1. Effects of different solutions on sperm and egg.

Solution Groups	Control %	Motilite Oranı			Motilite Süresi			Egg Eyed Rate			Egg Hatch. Rate		
Cortlant	86	76	72	80	62	59	65	85	88	86	92	94	95
Ringer	82	71	70	73	55	58	61	69	74	71	86	81	84
Cortlant +Hepes	93	84	87	90	94	93	101	87	90	93	93	95	90
Cortlant +NaOH	91	86	90	92	97	110	103	86	91	90	95	97	94
Ringer+Hepes	84	80	83	79	74	80	76	79	80	78	86	88	90
Ringer+NaOH	80	81	85	82	77	82	90	82	83	83	91	95	93

It has been observed that as the sperm vitality rate and duration increases, it also positively affects the rate of mole-shaped eggs being observed and opening. Motility is considered an important indicator in determining sperm vitality, and high fertilization rates have been observed in cases where a large portion of sperm cells are motile (Saito and Nakagawa, 2015; Jiang et al., 2019). When the solutions used in the study were compared, higher results were obtained for Cortland fluid compared to its own groups and other groups. Sodium hydroxide used for pH balancing was also found to be more effective than Hepes buffer. It has been observed that in addition to sperm vitality, egg quality is also an important factor in increasing egg fertilization rates (Jones et al., 2017). Egg size, shape and general quality directly affect fertilization rates (Morris et al., 2021; Morales et al., 2002). Large and regularly shaped eggs have higher fertilization rates than small and irregular eggs. This reveals the necessity of implementing appropriate nutrition and care strategies to improve egg quality (Smith et al., 2017).

Therefore, the genetic characteristics and health status of female fish also have an important effect on fertilization rates. Breeding studies should be given importance to obtaining superior individuals in enterprises. Eggs obtained from healthy and genetically suitable females have higher fertilization rates (Nguyen et al., 2022). Environmental factors, especially water temperature and pH levels, can also affect fertilization rates; it is important to keep these factors under control (Lee et al., 2021).

As a result, optimizing sperm viability and egg fertilization rates is critical for a successful production process in trout farming. Studies on methods to maintain sperm viability for a long time, viability tests and egg quality support the implementation of various strategies to improve these processes.

4. Suggestions

Trout (*Oncorhynchus mykiss*) has an important place in the aquaculture industry, and a good understanding of its genetic and breeding copies is required for successful production. This is reasonable, the viability of the sperm and the fertilization rates of the egg are two critical factors towards the sexual intercourse of trout. While sperm viability determines the sex of the male fish, fertilization rates also play a role in how the baby trout are kept. The aim of this study is to determine the most suitable conditions for maintaining sperm viability and egg fertilization rates in trout. High sperm quality and efficient fertilization parts are very important for sustaining production and economically sustainable trout performance.

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