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PRESENCE OF BLOOD CELLS IN RAINBOW TROUT MILT

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ABSTRACT. Blood cells were found in milt, collected at the end of spawning, of 2-years-old rainbow trout. Among blood cells, lymphoid cells dominated, followed by erythrocytes and phagocytic cells. Lymphoid cells were mainly present in clusters rather than as individual cells. Erythrocytes usually accompanied lymphoid cells. At present it is not clear if the presence of blood cells in milt is related to normal physiology of semen or rather to milt contamination.

Key words: RAINBOW TROUT, BLOOD CELLS, MILT

INTRODUCTION

Lumen of the sperm duct of rainbow trout contains large numbers of spermatozoa (average concentration $10 \cdot 10^9$/ml) suspended in seminal plasma. Sperm cells are produced up to two months before spawning, and sperm duct serves as a storage organ for male gametes. In order to protect spermatozoa during storage and prevent premature sperm activation, epithelial cells of the sperm duct secrete a number of mineral and organic substances important for optimal storage (Lahnsteiner et al. 1993). Data concerning the presence of somatic cells in the lumen of the sperm duct are incomplete and are mainly related to phagocytes involved in resorption of spermatozoa during the post-spawning period (Billard and Takashima 1983; Lahnsteiner et al. 1993). It is not clear, however, if blood cells are present in milt during spawning.

Recently we have identified many proteinase inhibitors in fish seminal plasma (Ciereszko et al. 1998). Proteinase inhibitors of rainbow trout seminal plasma are similar or the same as blood plasma inhibitors (Ciereszko et al. 1999). General role of proteinase inhibitors is the control of activities of various proteolytic enzymes. Target proteinases to fish seminal plasma proteinase inhibitors are not known as yet. The role of certain inhibitors in blood is related to the control of proteolytic enzymes of leukocytes, for example elastase (Hjelmeland 1983). If this function is also true for seminal plasma inhibitors, it implies the presence of these cells in the milt. To clarify this issue, we sought if blood cells were present in milt of rainbow trout.

MATERIAL AND METHODS

Rainbow trout semen was obtained from ten 2-year-old males raised in hatchery conditions (Czarci Jar, near Olsztyn). This population spawns in spring. According to the information provided by the hatchery personnel, the males were already sampled 2 - 3-times. Samples of milt were obtained by stripping at the end of spawning (April 2, 1999). The fish were not anesthetized during sperm collection. Special care was taken during milt collection to avoid any visible contamination with blood, slime, feces, urine or water. Milt was stored on ice for about three hours before analysis.

Smears of sperm (made like blood preparations) were fixed in methanol and stained in Giemsa (Klontz 1972). Differential cell counts were carried out by counting 200 - 400 cells from 7 slides for one fish. Cell identification was based on description of rainbow trout blood cell morphology by Lehmann and Sturenberg (1975). Cells with black granules, similar to those of kidney melano-macrophagic centers, were classified as phagocytic cells. Photos were taken with Nikon Optiphot 2 using a Multiscan programme.

RESULTS

Blood cells were found in all milt samples . Among blood cells, lymphoid cells dominated, followed by erythrocytes and phagocytic cells (tab. 1). Lymphoid cells were mainly present in clusters rather than as individual cells (photo 1). Erythrocytes usually accompanied lymphoid cells.

TABLE 1

Differential blood cell count in rainbow trout sperm, mean values/standard deviation (n=10)

Cells	Mean value (%)	SD	Range
Lymphoid cells	85.82	9.82	(74.2 - 95.1)
Erythocytes	13.53	9.59	(3.6 - 25.4)
Phagocytic cells	0.65	0.58	(0 - 1.5)

DISCUSSION

Our results indicate the presence of blood cells in rainbow trout milt. Lymphoid cells, which dominated in our material, were recognised by Lehmann and Sturenberg

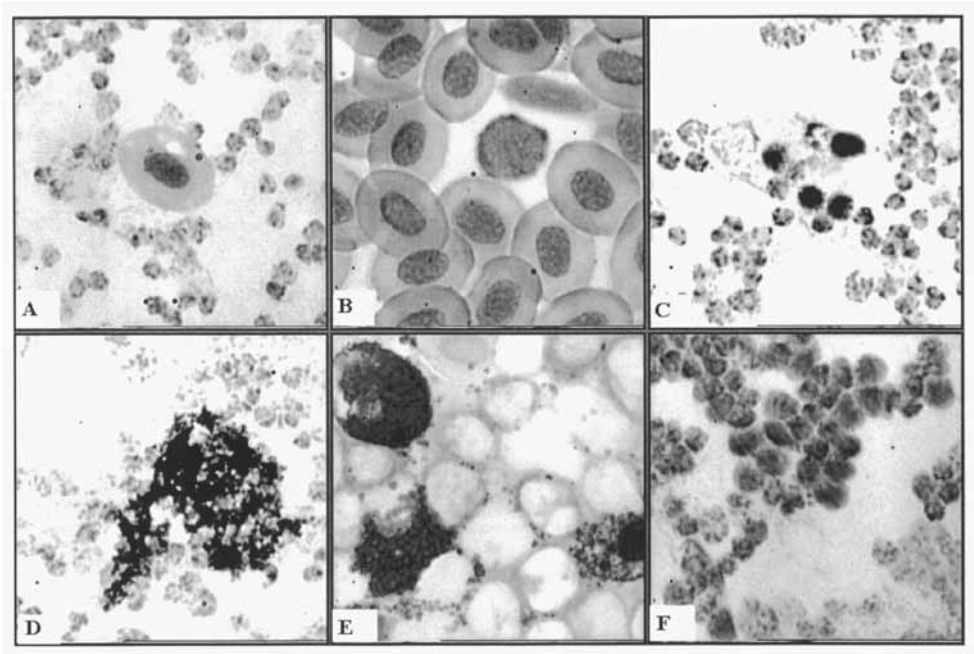

Photo 1. A, C, D, F - blood cells in rainbow trout milt. A - erythrocyte, C, H - lymphoid cells. D - destroyed phagocytic cell (for comparing see: E - macrophages from rainbow trout kidney). B - peripheral blood of rainbow trout: numerous erythrocytes, one lymphocyte and one thrombocyte (fusiform)

(1975) in peritoneal fluid of rainbow trout and in peripheral blood of fish acutely infected with VHS. Normally they are not frequently presented in blood. However, it is not clear at present, if these cells and erythrocytes were introduced to milt by handling, or if their presence in semen reflected normal sperm physiology, as in the case of mammals (Mortimer 1994). It has to be underlined, however, that presence of certain amounts of leukocytes in mammalian semen is considered normal, but presence of any erythrocytes is abnormal. Blood cells could be introduced to milt due to injures caused by handling of the fish, or lack of care during semen collection. It is common during the stripping procedure that rupture of blood vessels within male reproductive tract occurs. Although no visible damage to blood vessels happened during sample collection, we cannot exclude such a possibility during previous samplings. On the other hand, if blood cells in semen originated from ruptured vessels, one could expect erythrocytes to dominate. This was not the case in our study. Skin mucus is another possible source of erythrocytes. In the case of smaller fish (as in our study) it is not possible to avoid contact of milt with skin mucus. Wechsler (1984) indicated

that erythrocytes and lymphocytes may appear in skin mucus, especially during stress. Further studies concerning skin mucus as a potential source of milt contamination are necessary to clarify this issue.

In both male and female fish, spawning is accompanied by profound hematological changes. In mature male brown trout an increase in 11-ketotestosterone levels during spawning period resulted in erythropoesis stimulation (Pottinger and Pickering 1987). Spawning time, similarly to stress, may decrease activities of blood enzymes (Luskova 1997). In females, spawning may affect extreme involution of the thymus and suppress antibody production (Nakanishi 1986). Hematological as well as reproductive characteristics of males may be modified by diet quality during the prespawning period (Svobodova et al. 1998). The role of changes in hematological parameters during spawning period is not clear at present. If the presence of blood cells in milt is part of normal semen physiology, it needs to be established if there is a relationship between hematological indices of blood and milt.

Presence of blood cells in milt raises also a question of the relationship of these cells to semen quality. It has been shown for mammals that peroxidative damage to the sperm membrane may be an important pathological mechanism of male infertility, and the major source of the reactive oxygen species that damage the spermatozoa are white blood cells (Zalata et al. 1995). If blood cells gradually appear in the milt as a result of stress and injuries due to consequent milt collections, then blood cells may be an important factor responsible for a decrease of fish sperm quality (including its usefulness for short-term storage and cryopreservation, Billard 1986) at the end of spawning. To clarify this issue, further experiments on concentrations of blood cells in milt throughout the spawning period are necessary.

In conclusion, this study reports presence of blood cells in rainbow trout milt. This information may be important both for studies on sperm physiology and to improve handling of spawners. Presence of blood cells in milt suggests that they can be a potential source of many contaminating substances in the analyzed samples of seminal plasma and sperm . This has to be taken into account in biochemical studies on milt. Potential danger of introducing blood to milt during stripping calls for special care during semen collection.

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STRESZCZENIE

WYSTĘPOWANIE KOMÓREK KRWI W MLECZU PSTRĄGA TĘCZOWEGO

Znaleziono komórki krwi w mleczu dwuletnich pstrągów tęczowych w końcowym okresie tarła. Wśród komórek krwi najczęstsze były komórki limfoidalne; erytrocyty i fagocyty były rzadsze. Komórki limfoidalne występowały w skupiskach (rzadziej jako pojedyncze) w towarzystwie erytrocytów. Dyskutowano nad obecnością elementów morfotycznych krwi w mleczu, czy jest to zjawisko fizjologiczne czy zanieczyszczenie powstałe w trakcie wycierania pstrągów.

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