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Multiple collections of common carp *Cyprinus carpio* L. semen during the reproductive period and its effects on sperm quality

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ABSTRACT

Multiple collections of semen during the reproductive period of the common carp *Cyprinus carpio* L. were used to analyse changes in semen quality. Semen collection was performed on June 1 (first collection), 12 (second collection), and 19 (third collection) from individual males (n = 11) by gentle abdominal massage. Semen quantity (semen volume and sperm count), quality (sperm motility and sperm viability), as well as seminal plasma parameters (pH of seminal plasma and seminal plasma osmotic pressure) and its enzymatic activity, e.g., lactate dehydrogenase (LDH) and ß-*N*-acetylglucosaminidase (ß-NAG) were determined. Moreover, for the first time, the percentage of live, dead, and apoptotic sperm, as well as the proteolytic activity of seminal plasma, were determined using flow cytometry and zymography, respectively, at specific times during the common carp reproductive period. The lowest volumes of semen and sperm concentration were noted during the first semen collection (June 1). Analysis of computer-assisted sperm analysis parameters revealed the greatest sperm motility, sperm velocity, as well as amplitude of lateral head displacement, were evident in the third collection (June 19). There were no differences in progressively motile sperm, movement linearity, wobbling index, and beat cross frequency between the different collection times. The lowest percentage of live sperm was found in the first collection, although with the passage of time values of this parameter increased. Seminal plasma pH and seminal plasma osmotic pressure were at the lowest values in the second collection (June 12), which corresponded with the lowest concentration of sperm. In the first collection, seminal plasma contained the highest values of LDH and ß-NAG activity, whereas there were no differences in the proteolytic activity of seminal plasma determined between the different collections of semen. The results presented here indicate that during the reproductive period, males of common carp produce a large amount of semen of moderate quality. Low sperm motility noted in the second collection might be explained by a significant increase in sperm production during this period, followed by a low seminal plasma pH and high hydration rate. The high LDH and ß-NAG activity noted in the first collection of semen may reflect a reduced stability of the sperm cell membrane and its viability. The significant difference in the percentage of live sperm at June 1 compared to that at June 19 supports this hypothesis.

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1. Introduction

The common carp *Cyprinus carpio* L. is the main fish species produced in global aquaculture. It has gained popularity among fish producers due to its wide trophic spectrum and its rapid growth rate. It is also an important target of anglers. The culturing of fish in ponds faces difficulties during the last stage of oogenesis and sperm maturation. For instance, under captive conditions some cyprinids, such as the common carp, do not spawn spontaneously and reproductive dysfunctions, including a lack of ovulation and/or spermiation, can be observed in both genders (Mylonas et al., 2016). On the other hand, the percentage of mature gametes is determined by the appropriate timing of collections. In common carp, spermatogenesis is very short and the morphological changes of sperm are limited (Billard et al., 1995). It is known that practical laboratory methods used to evaluate semen quality do not always correlate highly with fertilisation capacity. Therefore, detailed analyses taking into account not only basic semen parameters but also morphology and viability may be helpful in the proper evaluation of gamete quality and its fertilisation ability.

Semen quality varies during the reproductive period, with a gradual increase in sperm motility and concentration at the beginning, and a decrease by the end of the spawning period (Lahnsteiner et al., 1998; Liley et al., 2002). Kruger et al. (1984) reported significantly lower values of common carp sperm concentrations in the late spring (1.6×10^6 mm^{-3}) than during the early phase of spring (8.2×10^6 mm^{-3}). Such differences may result from sperm hydration, which occurs during the last phase of sperm maturation. Christ et al. (1996) reported that common carp semen volumes were significantly highest (5.5–6.5 ml kg^{-1}) during the breeding season (May–June) in comparison with the early phase (2.8–3.0 ml kg^{-1}) of male maturation (March). Similarly, the percentage of motile sperm was significantly higher in samples collected during the summer months (May–June) than in samples collected during early spring (March) (Christ et al., 1996).

There are many parameters of semen quality that directly indicate sperm maturation and fertilisation ability. The most important include sperm motility, sperm concentration, osmolality of seminal plasma, and seminal plasma pH (Rurangwa et al., 2004). On the other hand, the composition of seminal plasma (ions, lipids, proteins, and sugar), as well as its enzymatic and proteolytic activity, might also influence sperm quality (Butts et al., 2010a,b). Flow cytometry has become an established method to obtain sperm diagnostic information as it allows the characterisation of each sperm in terms of cell function and the integrity of its components (Ogier de Baulny et al., 1997). Nowadays, flow cytometry is applied to the evaluation of sperm viability, acrosomal integrity, mitochondrial function, capacitation status, membrane fluidity, and DNA status (Gillan et al., 2005).

In Poland, the reproductive period of common carp takes place from mid-May to the end of June (Kucharczyk et al., 2008). During this time males release semen, although the quantity and quality of obtained gametes varies significantly within this period. It is possible to collect semen multiple times during the reproductive period from the same males, although due to variation in its quality, it must be collected at an appropriate time. The aim of the present study was to analyse the changes in sperm quantity and quality common carp semen collected June 1, 12, and 19 and to evaluate the relationship between semen parameters determined at different times during the reproductive period.

2. Materials and methods

2.1. Origin of fish and semen collection

Common carp males (n = 11) with an average body weight of 1.33 ± 0.28 kg were obtained from the Knieja Fishery Farm located near Częstochowa, Poland (50°49′31.6″N 19°29′34.6″E). Fish were harvested from the earth pond in mid-May when the water temperature achieved 14 ± 1 °C. Next, fish were moved to the hatchery and placed in an artificial tank and the water temperature was gradually raised to 18 ± 1 °C. Prior to relocation to the tank, the males were weighed and marked individually. Semen was collected multiple times beginning June 1 (first collection) by the gentle abdominal massage method. Semen collection from the same males was performed again on June 12 (second collection) and June 19 (third collection). Before semen collection, fish were anaesthetised with 2-phenoxyethanol (Merc, Darmstadt, Germany) administered at a dose of 0.5 ml l^{-1}. Contamination of the semen with water, urine, faeces, blood, or mucus was carefully prevented with thorough drying of the abdominal coating.

2.2. Analysis of semen volume and quantification of sperm

Total semen volume was measured directly after semen collection using syringes calibrated every 0.01 ml. Fish were weighed prior to semen collection. Sperm concentration ($\times 10^9$ ml^{-1}) was determined in accordance with the procedure described by Ciereszko and Dabrowski (1993). The values of semen volume and sperm concentration were used to determine total sperm production ($\times 10^9$).

2.3. Analysis of sperm motility

To analyse sperm motility parameters, the computer-assisted sperm analysis (CASA) system was applied. A 10 mM Tris buffer containing 100 mM NaCl with 0.5% albumin at pH 9.0 and osmolality of 200 m Osm kg^{-1} was used as an activation solution. Sperm motility was activated by mixing 1 µl of sperm with 25 µl of the activating solution buffer, and 1 µl of the activated sperm was placed on a microscope slide (a Teflon-coated glass slide with 12 wells, 30 µl in depth, and 5 mm in diameter) (Tekdon, Inc., Myakka City, FA, USA). Recordings were taken approximately 6 s after sperm activation. Sperm motility was registered using a Basler 202 K camera (Basler, Ahrensburg, Germany) integrated with an Olympus BX51 microscope (Plan FL N 20×/0.5 NH ph1 lens) (Olympus,