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Biochemical characterization and sperm motility parameters of ostrich (*Struthio camelus*) semen

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ABSTRACT

The aim of the study was to obtain baseline values for biochemical parameters of ostrich seminal plasma and sperm motility parameters measured by CASA. Biochemical characteristics of ostrich semen included a high protein concentration (29.3 ± 9.1 g/l) and high amidase (280.6 ± 130.8 U/l) and LDH activity (1880.0 ± 983.6 U/l). On the other hand antioxidant, superoxide dismutase, anti-proteinase and acid phosphatase activity were low. Biochemical parameters of semen were variable. Motility of ostrich sperm was characterized by low linearity (23.0 ± 6.2%). The quality of undiluted semen stored at room temperature deteriorated within an hour due to agglutination and gelation. On the other hand, ostrich semen could be stored up to 4 h at 5 °C without loss of motility after which loss of motility occurred but could be partially mitigated using semen extenders (EK and Ovodyl).

1. Introduction

Ostrich farming has been recognized as a promising venture for the production of leather, meat, oil and feathers. However, success of ostrich farming has been variable due to several obstacles, including specific biological constraints with this ratite. These constraints include egg production, fertility and hatchability, high embryo mortality, and low chick survival (Malecki et al., 2008). Contrary to major livestock industries such as poultry, swine and dairy cattle, the ratite industry lacks structured breeding program that would facilitate rapid and significant increase in productivity (Malecki et al., 2006; Kawka et al., 2007). One approach for overcoming these constraints could be to develop artificial insemination (AI) technology (for recent review see Malecki et al., 2008). As the first step toward implementing this technology, semen collection methods with use of the artificial cloaca (AC) have been developed either using a female teaser or a dummy (a "non-teaser" approach for males that direct their courtship toward humans, Rybnik et al., 2007). Both methods appear useful for regular collection of ejaculates (Malecki et al., 2008).

Technologies of short-term and long-term storage of ostrich semen are in their infancy and are the next important step in the development of effective AI programs. Preliminary studies suggest that although the quality of fresh semen is good, a considerable loss of sperm quality occurs during storage in vitro (Malecki et al., 2008). Usefulness of different poultry diluents for short-term storage has been tested and in general successful storage appeared to be restricted to 24 h, however these data were not published. Therefore further studies are necessary to understand sperm quality changes during the storage.

Knowledge concerning physiological and biochemical characteristics of ratite semen is limited and informa-

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tion concerning motility characteristics and biochemical parameters of seminal plasma is lacking. Computer-aided sperm analysis (CASA) has been applied to studies of avian sperm motility mostly turkey (King et al., 2000; Kotłowska et al., 2007). CASA allows an objective quantification of sperm motility parameters, such as sperm velocities and trajectory changes that cannot be measured subjectively. CASA parameters are useful indices of sperm quality because they correlate well with fertility (King et al., 2000; Blesbois et al., 2008). Biochemical parameters of avian semen that are being identified and characterized include components of seminal plasma, such as proteins, acid phosphatase (AcP), anti-proteinase activity (APA) and components of antioxidative protection (Surai et al., 1998). Some of these characteristics appeared useful for characterization of the quality and seasonal changes in poultry semen (Hess and Thurston, 1984; Kotłowska et al., 2005a). Protein concentration has been linked to low quality of semen (Hess and Thurston, 1984), and LDH and AcP can be indicators of damage to spermatozoa (Buxton and Orcutt, 1975; Kotłowska et al., 2005a). Amidase activity and APA are related to serine proteinases of semen and their control through specific inhibition (Thurston et al., 1993). Proteases are represented in semen in several forms (Kotłowska et al., 2005b). Components of antioxidative protection include antioxidant activity and superoxide dismutase activity and are an important defense system against damage caused by oxygen radicals (Surai et al., 1998). Establishing the knowledge concerning motility and biochemistry of ostrich semen is important to better understand mechanisms responsible for decreasing semen quality during storage and to develop strategies to minimize the loss of sperm viability. In this study we determined several biochemical parameters of the ostrich seminal plasma and sperm motility parameters with CASA as well as changes in motility parameters during short-term liquid storage.

2. Materials and methods

2.1. Animal housing and collection of semen

The study took place in June 2008 at the Stypułów Ostrich farm near Zielona Góra in Poland. Four male ostriches (3–4 years of age) were used. Birds were maintained individually in specially constructed enclosures and fed with ostrich breeder ration consisting of a whole grain mix, vitamin mineral supplement at morning and fresh lucerne chaff at afternoon. Water was provided ad libitum. Semen was collected using a dummy method (Rybnik et al., 2007). Average sperm concentration was $4.33 \pm 1.07 \times 10^9$ spermatozoa ml^{-1}. Seminal plasma was prepared by centrifugation twice for 10 min at $7950 \times g$ and was stored at −76 °C.

2.2. Short-term storage of semen

Immediately after collection semen samples were diluted (1:2, total volume 150 μl) either in avian semen extender (Ovodyl, IMV, l'Aigle, France) or EK extender (Siudzinska and Lukaszewicz, 2008). Samples of extended semen were stored for 20 h under refrigeration (5 °C). Undiluted semen was incubated at the same conditions and served as control. From each samples aliquots were removed at 0, 4, 8 and 20 h after for measurements of sperm motility.

2.3. Computer-assisted sperm analysis

Video recordings of motile sperm were made using a microscope with a 10× negative phase objective and a Sony CCD black and white video camera (SPTM108CE). MicroCell 20 μm, two-chamber slides (Conception Technologies, San Diego, CA, USA) mounted on a heated stage (39 °C) were used. Conditions of sperm dilutions, recording and Hobson Tracker settings followed those described by King et al. (2000) and Kotłowska et al. (2007) for turkey spermatozoa. However, the motility buffer (50 mM Tris buffer pH 7.4, 120 mM NaCl, 10 mM glucose, 2 mM $CaCl_2$) was supplemented with 0.5% bovine serum albumin (BSA) in order to prevent adherence of spermatozoa to the glass slides.

Video recordings were analyzed using Hobson Sperm Tracker (Hobson Vision Ltd., Baslow, UK). Motility parameters measured in this study were: curvilinear velocity (VCL) – velocity over the actual sperm track; average path velocity (VAP) – velocity over a calculated smoothed path; straight-line velocity (VSL) – velocity over the straight-line distance between beginning and end of the sperm track; linearity (LIN) – the straight-line distance divided by the incremental deviations of actual path; beat-cross frequency (BCF) – the frequency with which the sperm crosses the smoothed path; and the amplitude of lateral head displacement (ALH) – the time average of absolute values of the instantaneous turning angle of sperm head along its curvilinear trajectory.

2.4. Biochemical analysis

Osmolality of seminal plasma was measured with a Vapor Pressure Osmometer 5520 (WESCOR). The protein concentration of seminal plasma was measured by the Lowry method (Lowry et al., 1951). Anti-proteinase activity was determined by inhibition of bovine trypsin (Sigma Chemical Co., St. Louis, MO) and amidase activity according to Kotłowska et al. (2005b). One inhibitory unit (U) corresponds to the apparent amount of inhibitor able to block 1 unit of trypsin activity (defined as hydrolysis of 1 μM of N-α-benzoyl-DL-arginine-*p*-nitroanilide/min). Before measuring anti-proteinase activity seminal plasma was boiled for 4 min to denature amidase activity. No amidase activity was detected after this treatment and all electrophoretic forms of inhibitors were still visible. Acid phosphatase activity in seminal plasma was measured by the method of Bessey et al. (1946). Activity of LDH was measured according to Vassault (1983).

Electrophoretical detection of anti-proteinase activity was performed according to the method of Kotłowska et al. (2005b). Native-PAGE electrophoresis was conducted using 10% gel under non-reducing conditions. The gels (1 mm thick) were electrophoresed for 90 min at 200 V in an SE 250 vertical Mighty Small II electrophoresis system (Amersham Pharmacia Biotech., Sweden). Aliquots