



Palmitoleate enhances quality of rooster semen during chilled storage



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ABSTRACT

The practice of artificial insemination is widely utilized in poultry; and this requires a broad use of semen storage techniques to prevent the reduction of fertilizing ability of stored semen. The antioxidant activity of palmitoleic acid with *in vitro* experiments has been shown. The present study was designed to evaluate the effect of palmitoleic acid on the quality of rooster semen stored at 4°C. Semen was collected from ten roosters twice a week. Ejaculates with greater than 80% forward spermatozoa motility were pooled and after dilution semen was enriched with 0 (control), 0.125 (P 0.125), 0.25 (P 0.25), 0.5 (P 0.5) and 1 (P 1) millimolar palmitoleate. Forward spermatozoa progressive motility and viability, as well as amounts of malondialdehyde (MDA) and total antioxidant activity (AOA) were evaluated in seminal plasma and spermatozoa at 0, 24 and 48 h of storage. Motility was 78.5 ± 2.21 , 77.5 ± 1.04 , and $69.5 \pm 2.32\%$ at 24 h and 58.66 ± 1.35 , 49.33 ± 1.36 and $43.00 \pm 2.08\%$ at 48 h in P 0.125, P 0.25 and control, respectively ($P < 0.02$). There were no significant differences in amount of MDA in the seminal plasma among groups, while the amounts of MDA in spermatozoa were less in the P 0.125, P 0.25 and P 0.5 groups compared to the control group at 24 and 48 h of storage ($P < 0.002$). Total amounts of AOA in seminal plasma were greater in palmitoleate treatment groups than the control at 24 and 48 h ($P < 0.01$). Moreover, palmitoleate treatment groups had greater values of total AOA in spermatozoa compared to the control group at 24 and 48 h of storage ($P < 0.05$). In conclusion, enrichment of rooster semen with small doses of palmitoleate has beneficial effects on the semen quality during cold storage.

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1. Introduction

The liquid storage of avian semen and artificial insemination are techniques utilized in poultry to optimize

the management of genetically superior males (Blesbois et al., 1999). Following *in vitro* storage of undiluted poultry semen fertilizing capacity of the semen decreases a few hours after collection (Blesbois et al., 1999). Therefore, it is important to develop an efficient system of semen storage for poultry. It is necessary to dilute the spermatozoa in a buffered and osmotically equilibrated saline diluent to maintain the quality of stored semen (Clarke et al., 1982).

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When the semen is stored at the refrigerator temperature (4 °C), there is a gradual decrease in the motility, functional integrity of spermatozoa membranes, and fertility (De Lamirande et al., 1997). One reason for this decrease may be the action of the reactive oxygen species generated by the cellular components of semen, namely a superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and lipid hydroperoxides, formed via lipid peroxidation of the membrane lipids of the spermatozoa (Alvarez and Storey, 1984, 2005; Del Maestro, 1980). The effects of the lipid peroxidation include irreversible loss in the motility, damage to the spermatozoa DNA and fertility (Aitken, 1994; De Lamirande and Gagnon, 1992; Maxwell and Watson, 1996). The antioxidant activity (AOA) system in cells, such as spermatozoa, is comprised of enzymatic and non-enzymatic antioxidants which have been described as a defense functioning mechanism against lipid peroxidation. The AOA is also an important system in maintaining spermatozoa motility and viability (Agarwal et al., 2007; Bilodeau et al., 2001; Gadea et al., 2004; Zini et al., 2009). However, this endogenous AOA may be insufficient to prevent the lipid peroxidation during prolonged storage (Aurich et al., 1997). Moreover, total AOA values were less in infertile than fertile men (Lewis et al., 1995).

Avian spermatozoa are susceptible to lipid peroxidation (Cerolini et al., 1997). Several attempts have been made to enrich semen by adding substances such as caproic acid (MacPherson et al., 1977), pentobarbital (Fiser et al., 1978, 1980), carnitine (Neuman et al., 2002), inosine and adenine (Esashi et al., 1969) in birds and ascorbic acid (Aurich et al., 1997), taurine (Alvarez and Storey, 1983), superoxide dismutase, catalase, trehalose, glutathione (Bucak and Tekin, 2007; Maxwell and Stojanov, 1996) glutamine and hyaluronan (Bucak et al., 2009) in other domestic animals to protect the spermatozoa against the harmful effects of the lipid peroxidation. Nevertheless, improvement in semen quality still appears to be a challenge in poultry production.

Beneficial effects of palmitoleic acid, the n-7 monounsaturated fatty acid with a 16-carbon chain, on AOA and signal transduction alteration have been well documented in different cell types (De vries et al., 1997; Dimopoulos et al., 2006; Erbay et al., 2009; Maedler et al., 2003; Sauma et al., 2006). The purpose of the present research was to investigate the effect of palmitoleic acid on spermatozoa survival and motility during cold storage of rooster semen. To accomplish this, values for malondialdehyde (MDA), an indicator of lipid peroxidation, and total AOA were measured. To the best of the authors' knowledge, this is the first report of the semen enrichment with palmitoleic acid in roosters.

2. Materials and methods

2.1. Birds, diets and semen collection

Ten White Leghorn roosters (38 weeks of age) were housed in controlled environmental conditions (14L: 10D, light density: 20 lux, 22 °C) and maintained as required by the guidelines of the Animal Care of the Urmia University. A standard commercial breeder male diet restricted to 110 g feed/day was fed and water was supplied *ad libi-*

tum. The diet contained 11.5 MJ metabolizable energy/kg of 13.0% protein and 3.3% fat. Roosters were adapted over a 3-wk period to the diet and to dorso-abdominal massage for semen collection. Semen was collected twice a week into a graduated collection tube by the same person using the dorso-abdominal massage method for semen collection (Lake, 1957). Care was taken to avoid any contamination of the semen with the cloaca1 contents and blood. Seminal volume was measured in graduated collecting tubes and recorded on individual case report forms for each rooster.

2.2. Treatment and semen evaluation

Ejaculates (with greater than 80% forward progressive motility) obtained from the roosters were pooled and evaluated as a single sample. Each pooled ejaculate was partitioned into five equal aliquots to treat with different concentrations of palmitoleate. Semen was diluted with phosphate buffer diluent (Wilcox et al., 1961) either without palmitoleic acid (control) or supplemented with 0.125 (P 0.125), 0.25 (P 0.25), 0.5 (P 0.5) and 1 (P 1) millimolar palmitoleic acid, which was conjugated with bovine serum albumin (BSA) at a final concentration of 2×10^9 spermatozoa per ml. Both diluent and semen were at room temperature (18–22 °C) when mixed. Semen of all treatment groups was stored for 48 h at 4 °C. The determination of percentage of spermatozoa with forward progressive motility and viable spermatozoa was conducted at 0, 24 and 48 h of storage. Moreover, total amounts of AOA, MDA and total protein were measured in the seminal plasma and spermatozoa separately at the 0, 24 and 48 h storage time points. Palmitoleic acid was purchased from Sigma Company (P9417, Sigma–Aldrich) and the other materials used in this project were purchased from Merck Company.

Forward progressive motility of spermatozoa was assessed by placing a portion of ejaculate diluted with 2.9% sodium citrate solution (1:200) on a slide with a coverslip being placed over the sample, with using an Olympus (BX41, Japan) compound light microscope (400× magnification), equipped with a warm stage being used for spermatozoa assessments. The percentage forward progressive motility was assessed on 200 spermatozoa. Motility was expressed as a percentage of spermatozoa progressively exhibiting moderate to rapid forward movement (Ommati et al., 2013). Each experiment was replicated at least three times.

The viability of the spermatozoa was evaluated using a portion of the ejaculate stained with eosin-nigrosin solution (Bakst and Cecil, 1997). The stained seminal preparation was prepared in duplicate, and 200 spermatozoa per slide were evaluated. The slides were evaluated for cell viability, where unstained spermatozoa were considered as live cells. Each experiment was replicated at least three times. Concentration of spermatozoa was determined in duplicate, using a Neubauer hemocytometer.

The spermatozoa and the seminal plasma were separated by centrifugation. Semen was centrifuged for 10 min at 550 × g. The resulting pellet was used as the concentrated spermatozoa aliquot for purposes of the present study. The supernatant was centrifuged two more times, first for 10 min at 550 × g, and then for 30 min at 3000 × g.

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