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Short communication

Relationship of blood and seminal plasma ceruloplasmin, copper, iron and cadmium concentrations with sperm quality in Merino rams

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ABSTRACT

The aim of the current study was to investigate the concentrations of ceruloplasmin, copper, iron, zinc and cadmium concentrations in blood serum and seminal plasma obtained from Merino rams. In addition, their relationship with sperm parameters, fertility rate and litter size were also studied. Blood and ejaculate samples (6 replicates) were taken in October from 19 Merino rams, aged between 18 and 24 months. Ceruloplasmin, copper, iron, zinc and cadmium in blood serum and seminal plasma were determined. Sperm parameters including volume, mass motility, motility, concentration, Hos-test, viability, abnormal sperm and acrosome abnormality in semen, fertility rate and litter size were also evaluated. Highly positive correlation was found between blood ceruloplasmin and blood copper concentrations ($r = 0.812$, $p < 0.001$), whereas negative correlation were determined between these parameters in seminal plasma ($r = -0.195$, $p < 0.05$). Seminal plasma copper concentration was positively correlated with seminal plasma cadmium ($r = 0.206$, $p < 0.05$) and seminal plasma iron ($r = 0{,}305$, $p < 0.01$) concentrations. Negative correlation was determined between blood ceruloplasmin level and acrosomal defect ($r = -0.443$, $p < 0.05$). Seminal plasma ceruloplasmin level was positively correlated with volume ($r = 0.255$, $p < 0.01$) and negatively correlated with abnormal sperm ($r = -0.186$, $p = 0.058$) and acrosome abnormality ($r = -0.213$, $p < 0.05$). Seminal plasma iron concentration was positively correlated with other abnormality ($r = 0.257$, $p < 0.01$). Seminal plasma cadmium concentration was positively correlated with sperm abnormality ($r = 0.207$, $p = 0.052$) and other abnormality ($r = 0.262$, $p < 0.05$) and negatively correlated with fertility rate ($r = -0.449$, $p = 0.054$). Blood cadmium concentration was negatively correlated with litter size ($r = -0.579$, $p < 0.01$). In conclusion, blood and seminal plasma ceruloplasmin may be suggested to have positive influence regardless of copper with its antioxidant property whereas iron and cadmium have negative influence on sperm parameters and fertility in Merino rams.

1. Introduction

Seminal plasma is an important indicator with its content and quality on monitoring the male infertility. In this context, the important role of reactive oxygen species (ROS) is of interest because excess semen ROS have negative effects on sperm quality including sperm movement and its ability for fertilization (Aitken and Curry, 2011). As sperm membrane of rams has a higher polyunsaturated/saturated fatty acids ratio than other species such as rabbit, bull and human, it is more vulnerable to peroxidative damage (Evans and Maxwell, 1987; Saleh and Agarwal, 2002).

Ceruloplasmin, mainly synthesized in hepatocytes, has multilateral function in the organism—is important in transporting copper (Cu), involved in iron (Fe) metabolism, antioxidation and acute phase response during inflammation (Halliwell, 1991). About 70 to 90% of the Cu is associated with Cp in blood plasma (Blakley and Hamilton, 1985). Copper is involved in biochemical reactions especially oxidation–reduction processes. The Cu^{2+} in diet is absorbed from duodenum with aminoacids or small proteins, competitively with Fe^{2+}, zinc (Zn) and cadmium (Cd) (Craig et al., 2009) and Cu

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antagonists include sulphide, molybdenum (Mo), Zn and Fe (Suttle, 2012). Copper homeostasis is maintained carefully by regulation of Cu^{+} specific membrane transporter and several metallochaperones (Prohaska, 2011). Among the farm animals, sheep are the most susceptible species to Cu toxicity because they do not have ability to increase biliary Cu excretion (Saylor and Leach, 1980). Ceruloplasmin can be inactivated during oxidative stress and Cu can be released leading to excess hydroxyl radical generation. Therefore ionic Cu is extremely low in the organism (Choi et al., 2000; Prohaska and Gybina, 2004). Many of the toxic effects of Cu, including increased LPO levels in cell membranes and DNA damage, are related to its role in generation of ROS (Bremner, 1998). Negative influence of high semen Cu concentrations were reported on spermatozoa motility and viability in water buffaloes (Tabassomi and Alavi-Shoushtari, 2013).

Zinc deficiency results in disorders of testes development and course of spermatogenesis (Cigankova et al., 1998). Excess of cadmium was reported to have degenerative alterations in testes (Toman and Massanyi, 1996).

To our knowledge, the relationship of blood serum Cp and Cu with seminal plasma Cp and Cu in ram and their relationship with sperm parameters and fertility rate was not reported, so far. Additionally, the relationship of Cp and Cu with Fe, Zn and Cd remain absent. The aim of the study was to determine the concentrations of Cp, Cu, Fe, Zn and Cd in blood serum and seminal plasma and their correlation with sperm parameters, fertility rate and litter size in Merino ram.

2. Materials and methods

Semen and blood samples from 19 Merino rams (18–24 months of age) were used in the current study. The rams, belonging to the Bahri Dağdaş International Agricultural Research Institute, Konya–Turkey (located at 37.857063 north latitude and 32.567036 east longitude) were maintained under uniform feeding, housing and lighting conditions. Rams were fed with a same ration composed of alfalfa hay, concentrated feed and dried grape, had ad libitum access to fresh water. Group feeding was accessed. Starting 15 days prior to blood and sperm sampling ($n = 4$ for each type of feed; alfalfa hay, concentrated feed, dry grape), feed samples were taken with an interval of 1 week. By determining the total amount of consumed feed by the group, the average amount of feed consumed per animal was determined. Refusal of the feed was not collected. The study was approved by Bahri Dağdaş International Agricultural Research Institute Local Animal Research Ethics Committee (No: 22.07.2013/2).

2.1. Evaluation of sperm parameters

Ejaculates were collected from the rams using an artificial vagina, in October as 6 replicates (with an interval of 1 day) according to AI standard procedures (Paulenz et al., 2002). The volume of ejaculates was evaluated in a conical tube graduated at 0.1 ml intervals.

Immediately after collection, semen was assessed for semen wave motion (mass motility) at 40× magnification using a phase-contrast microscope, graded on a subjective scale ranging from 1 to 5, where 1 was scored when there was no mass movement and 5 represented vigorous waves of sperm motion (Evans and Maxwell, 1987).

Spermatozoa motility was estimated subjectively using a phase-contrast microscope (100×), with a warm stage maintained at 37 °C. Semen was diluted with PBS (1/10 w/w) and, a wet mount was made using a 5 μl drop of this dilution, placed directly on a microscope slide and covered by a cover slip. Sperm motility estimations were performed in three different microscopic fields for each semen sample by the same researcher. The mean of the three successive estimations was recorded as the final motility score.

Sperm concentration was determined via Hemositometric method, briefly sperm was diluted at ratio of 1:200 with Hayem solution (5 g Na_2SO_4, 1 g NaCl, 0.5 g $HgCl_2$, 200 ml bicine) and density was determined using a 100 μm deep Thoma haemocytometer (TH-100, Hecht-Assistent, Sondheim, Germany) at 400× magnification with using a phase-contrast microscope and expressed as spermatozoa $\times 10^9$ ml^{-1} (Bearden et al., 2004).

The hypo-osmotic swelling test (Hos-Test) was used to evaluate the functional integrity of the sperm membrane. This was performed by incubating 30 μL of semen with 300 μl of a 100 mOsm hypoosmotic solution (9 g fructose + 4.9 g sodium citrate per liter of distilled water) at 37 °C for 60 min. After incubation, 0.2 ml of the mixture was spread with a coverslip on a warm slide. Four hundred sperms were evaluated for each sample and the percentage of spermatozoa with swollen and twisted tails were recorded underphase-contrast microscope (400×) (Revel and Mrode, 1994).

For the assessment of sperm abnormalities, a minimum of three drops of each sample were added to Eppendorf tubes containing 1 ml Hancock solution (62.5 ml formalin (37%), 150 ml saline solution, 150 ml buffer solution and 500 ml double-distilled water) (Schafer and Holzmann, 2000). One drop of this mixture was put on a slide and covered with a cover slip. The percentage of total sperm abnormalities (acrosomal and other abnormalities) was recorded by counting a total of 400 sperm under phase-contrast microscopy (1000× magnification, oil immersion).

Sperm viability rate was determined using eosin–nigrosin staining method (Evans and Maxwell, 1987). The sperm suspension smears were prepared by mixing a drop of the semen sample with 2 drops of the stain on a warm slide and spreading the stain with a second slide immediately. The viability was assessed by counting 200 cells under the phase-contrast microscope. Sperm showing partial or complete purple colourisation were considered nonviable and only sperm showing strict exclusion of the stain were considered to be alive.

Then, the ejaculates were centrifuged at 800 × g 20 min at 4 °C and seminal plasma was separated from spermatozoa for the analysis of trace element concentrations within 2 h. Blood samples were collected from the jugular vein prior to sperm collection.

2.2. Evaluation of trace elements

Serum and seminal plasma samples were stored at −86 °C until the analysis of Cp, Cu, Fe and Cd. Serum, seminal plasma and feed Cu, Fe, Zn and Cd concentrations were determined with ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer—Varian-Vista AX, Australia) using reference material European Reference Materials-LGC (ERM DA120a, Teddington, UK).

The samples of serum and seminal plasma were diluted with deionized water (total volume 1 ml) and 5 ml 65% HNO_3 + 2 ml 30% H_2O_2 (Merck) was added before digestion in the microwave oven (CEM MarsXpress, Matthews, NC, USA) at 210 °C at 200 PSI. Food samples were digested with 7 ml 65% HNO_3 + 3 ml 30% H_2O_2. The flame conditions were those recommended by the instrument manufacturer for Cu, Fe, Zn and Cd (wavelength 327.396 and 238.204, 213.856 and 228.802 nm and detection limit 0.6, 0.35, 0.3 and 0.3 ppb, respectively). All data were obtained using 10 s integration time based on 3 standard deviations and in general compromise conditions were used. Analyzing reference material ERM-DA120a tested the reproducibility of the method. Reference values for Cu and Zn are presented to be 1130 and 658 ppb,