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Association of classical semen parameters, sperm DNA fragmentation index, lipid peroxidation and antioxidant enzymatic activity of semen in ram-lambs

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Abstract

The objective was to determine relationships among classical semen characteristics, sperm chromatin structure assay (SCSA), lipid peroxidation and antioxidant enzymatic activity in ram-lamb semen. Fifty-seven ram-lambs were electroejaculated, and routine semen evaluation was conducted (as part of a breeding soundness evaluation). The percentage of sperm DNA fragmentation index (%DFI) and the percentage of sperm with abnormally high DNA stainability (HDS; immature spermatozoa) were determined by SCSA using the metachromatic properties of acridine orange. Semen was centrifuged at 800 × *g* for 15 min to separate spermatozoa and seminal plasma and the aliquots were stored at −70 °C until analyzed. Lipid peroxidation, superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels in seminal plasma and spermatozoa were measured by spectophotometric assays. The classical semen parameters were negatively related to lipid peroxidation and GPx activity in spermatozoa; motility and morphology were negatively related to %DFI ($P < 0.05$). Based on Kruskal–Wallis pair-wise comparison of median values among breeding

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soundness outcome groups, %DFI was lower in the satisfactory group compared to other groups ($P < 0.05$) and the lipid peroxidation and GPx activity in seminal plasma and spermatozoa were lower in satisfactory and questionable groups ($P < 0.05$). However, the SOD was lower in the unsatisfactory group ($P < 0.05$). In summary, classical semen parameters were negatively related to % DFI, lipid peroxidation and GPx activity in ram-lamb spermatozoa and seminal plasma. There were indications that SOD and GPx have crucial protective roles against the toxic effect of reactive oxygen species (ROS) in ram-lamb semen.

Keywords: Spermatozoa; DFI; Lipid peroxidation; Antioxidant enzyme

1. Introduction

Breeding soundness evaluation in rams is performed to assess and categorize their potential breeding ability. Based on physical examination, scrotal circumference, and semen parameters, rams are classified into three categories: satisfactory, questionable and unsatisfactory for breeding potential [1–3]. Color, volume, gross and progressive motility, and morphology are the usual parameters used for semen evaluation. Among numerous variables involved in selecting a ram for natural service or cryopreservation of semen, certain crucial parameters such as motility and morphology are more indicative of semen quality. The ultimate goal of semen evaluation is to predict the fertilizing capacity of an ejaculate [4]. It is generally accepted that conventional sperm characteristics are poorly correlated with the fertilizing capacity of spermatozoa and that both inter- and intra-assay variability of these characteristics are high [5,6]. Hence, it is challenging for veterinarians to predict fertilizing capacity, as there is no single sperm parameter that accurately predicts fertility in vivo. Therefore, advanced evaluation techniques of semen are needed to increase the odds of achieving an accurate diagnosis [6,7].

The extent of sperm DNA fragmentation index (DFI) and the percentage of cells with abnormally high DNA stainability (HDS; immature spermatozoa) measured by the sperm chromatin structure assay (SCSA) are useful indices in evaluating semen [8–12]. These parameters are highly repeatable and provide important biological information about DNA defects for diagnostic and prognostic purposes for both human and animal subjects [8–10]. The SCSA variables were significantly related to male fertility in numerous species, including human [13,14], bull [15] and stallion [16]. A wide variation in these parameters was also observed among ejaculates of bulls with lower fertility potential [17].

Impaired sperm function is a general cause of male infertility. A balanced generation of reactive oxygen species (ROS) and antioxidant enzymes is associated with normal physiological functions [18–20]. An unbalanced, excessive production of ROS and decreased level of antioxidant enzymes cause decreased sperm motility and viability, and increased sperm defects by initiating an oxidation chain reaction damaging proteins, lipids and DNA [19,20]. Seminal plasma has an antioxidant system that seems to be very relevant to the protection of sperm. The sperm oxidative defence enzymes predominantly include superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase [19–24].

This study investigated the relationship of classical semen parameters with SCSA, lipid peroxidation and antioxidant enzymatic activity in ram-lambs undergoing breeding