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Changes in activities of superoxide dismutase, nitric oxide synthase, glutathione-dependent enzymes and the incidence of apoptosis in sheep corpus luteum during the estrous cycle

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Abstract

Anti-oxidative enzymes play a role in protecting cells from oxidative stress-induced cell death. The present study was conducted to evaluate whether the anti-oxidant and pro-oxidant enzymatic capacities of the sheep corpus luteum (CL) are correlated with steroidogenic and structural status of the gland during the estrous cycle. Steroidogenic activity, apoptosis and superoxide dismutase (SOD1 and SOD2), nitric oxide synthase (NOS), glutathione peroxidase (GPX), glutathione reductase (GSR) and glutathione S-transferase (GST) activities were determined in the CL at specific developmental stages of the luteal phase. The intensity of apoptotic DNA fragmentation, characteristic of physiological cell death, was much greater in CL at late luteal phase than at early and mid-luteal phase, concomitantly with the diminution in the plasma progesterone concentrations from mid-to late luteal phase. SOD1 and GPX activities increased from early to mid-luteal phase, and increased further at late luteal phase. SOD2 and GST activities were not different between early and mid-luteal phase, but increased at late luteal phase. GSR activity was not different between any luteal phase examined. NOS activity decreased from early to mid- and late luteal phase. These results show that the activities of SOD1, SOD2, NOS, GPX, GSR and GST in the sheep CL are subject to major changes during the estrous cycle, and that the anti-oxidant and pro-oxidant enzymatic capacities of luteal cells are not correlated with cell steroidogenic status and integrity during the late luteal phase.

Keywords: Anti- and pro-oxidant enzyme; Superoxide radical; Hydrogen peroxide; DNA fragmentation; Apoptosis; Corpus luteum

1. Introduction

Superoxide dismutase (SOD) and glutathione (GSH)-dependent enzymes are the key anti-oxidants that protect cells against toxic and damaging effects of reactive oxygen species (ROS), mainly superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2), and their product lipid peroxide (LPO). Copper-zinc SOD (SOD1), which is located in the cytosol [1], and manganese SOD (SOD2) which is located in the mitochondria [2], both belong to the first enzymatic step that plays a vital protective role by catalyzing the conversion of O_2^- into H_2O_2. Glutathione peroxidase (GPX) is the principal peroxidase in mammals [3] which is present in significant amounts in the cytoplasm and catalyzes the conversion of H_2O_2 to H_2O. GPX detoxifies H_2O_2 to H_2O through the oxidation of reduced GSH [4]. In addition, GPX can metabolize lipid hydroperoxides to less reactive hydroxy fatty acids [4]. Glutathione reductase (GSR) is an important component of the cellular anti-oxidant defense mechanism. This enzyme catalyzes the reduction of the oxidized form of glutathione (GSSG) to GSH with NADPH as the reducing agent [4]. Therefore, GSR is essential for the glutathione redox cycle that maintains adequate levels of

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reduced GSH. The ability of GPX to reduce H_2O_2 or other hydroperoxides is therefore dependent on the activity of GSR. Glutathione S-transferases (GST) are a polyfunctional family of enzymes that play an important role in detoxifying reactive metabolites by catalyzing their conjugation with reduced GSH. They are involved in the intracellular transport of compounds and their delivery to sites for subsequent transformation and/or excretion [5].

These is substantial evidence that ROS play a number of significant and diverse roles in mammalian reproductive biology [6] and serve important physiologic roles within the ovary [7]. During recent years, evidence has accumulated to suggest that locally produced anti-oxidant enzymes within the corpus luteum (CL) are involved in the maintenance of luteal steroidogenic activity and structure. The activities of total SOD (SOD1 and SOD2) and catalase in the bovine CL during the estrous cycle [8] and the activity of SOD1 in the human CL during the menstrual cycle [9] show patterns similar to peripheral concentrations of progesterone, therefore a role of these anti-oxidant enzymes in the maintenance of progesterone production by luteal cells during the active luteal phase was suggested. Although glutathione-dependent enzymes have been suggested to play a role in anti-oxidative processes associated with reproduction [10], little attention has been given so far to the study of this important ROS scavenging system in ovarian cell integrity and steroidogenic activity. The changes in the activity of glutathione-related enzymes, namely GST and GPX, in different size of ovarian follicles during different reproductive phases in goat and sheep suggest that these enzymes may have functional role in the steroid hormone synthesis and metabolism in ruminant ovary [11]. The induction of apoptotic DNA fragmentation in bovine luteal cells by down-regulation of GPX [12] and by simultaneous treatment with H_2O_2 and with a specific inhibitor of GPX support a role for this anti-oxidant enzyme in the protection of luteal cell from H_2O_2-induced apoptosis [13].

There is also evidence that the free radical nitric oxide (NO), generated from L-arginine by the action of NO synthase (NOS), acts as an important regulator of many physiological events [14], including the regulation of functional and structural luteolysis. NO–NOS system is found to be a potential mediator of lipid peroxidation induced in rat CL by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during luteolysis [15] concomitantly with a diminution in the progesterone concentrations [16] and GSH content [17]. NO has an anti-steroidogenic action in human [18], rabbit [19], bovine [20] and rat [21] luteal cells in vitro. Treatment of human CL in vitro with a NOS inhibitor decreased the number of apoptotic cells [22]. Infusion of a NOS blocker prolonged the duration of the estrous cycle [23] through the prevention of $PGF_{2\alpha}$-induced luteolysis in cattle [24].

Collectively, these studies suggest that anti-oxidant and pro-oxidant enzyme systems are critical for the functional activity, development, maturation and regression of mammalian CL. Thus, activity of anti-oxidant and pro-oxidant enzymes would be expected to change dramatically in the CL of the estrous cycle during its lifespan. In the present study, we tested therefore the hypothesis that anti-oxidant and pro-oxidant enzymatic capacities would be correlated with steroidogenic and structural status of the sheep CL during the estrous cycle. Experiment was designed to determine the activity of key intracellular anti-oxidant enzymes involved in the defense mechanism (i.e. SOD1, SOD2, GPX, GSR and GST) and pro-oxidant enzymes (mainly NOS) in the CL collected from ewes at early, mid- and late luteal phase of the estrous cycle. Plasma progesterone concentrations, CL masses and the degree of in situ DNA fragmentation within the nuclei of luteal cells also were determined at these specific developmental stages of the luteal phase.

2. Materials and methods

2.1. Animals and tissue collection

All procedures relating to care and use of animals were approved by the French Ministry of Agriculture according to the French regulation for animal experimentation (authorization no. 78–34). The study involved 16 cyclic ewes of the Préalpes-du-Sud breed. Only ewes with a single CL were used. All the ewes were treated for 14 days with intravaginal sponges containing 40 mg fluorogestone acetate (Intervet, Angers, France) to synchronize estrus as described [25]. Each ewe received immediately after removal of the sponges an intramuscular injection of 400 IU of equine chorionic gonadotropin (eCG, Intervet). The ewes were killed at a local abbatoir in accordance with protocols approved by the local institutional animal use committee, then the reproductive tracts were collected and immediately transported to the laboratory. The ovaries were obtained 7 days, 12 days and 17 days following injection of eCG. Taking in account the time of estrus (day 0) to appear (45.3 h) after eCG injection [25], these stages correspond to early ($n=5$ ewes), mid ($n=6$ ewes) and late ($n=5$ ewes) luteal phase of the estrous cycle. The CL were dissected from the surrounding ovarian tissue and weighed. For the determination of apoptosis, a piece of each CL was fixed overnight in freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS), pH 7.4, washed in PBS, dehydrated through a series of increasing concentrations of ethanol (70%–100%), cleared in xylene and embedded in paraffin wax. The rest of CL was snap-frozen in liquid nitrogen and then stored at −80 °C until processed for enzyme activities.

2.2. In situ detection of apoptosis by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) method

Luteal apoptotic cells were identified with the In Situ Cell Death Detection Fluorescein Kit (Roche Diagnostics, Mannheim, Germany). This assay detects nuclear DNA fragmentation in apoptotic cells by TUNEL method, and