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Distribution of superoxide dismutase 1 and glutathione peroxidase 1 in the cyclic canine endometrium

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ABSTRACT

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are two important antioxidant enzymes involved in tissue homeostasis by protecting cells and tissues from an accumulation of reactive oxygen species. Information concerning antioxidant enzymes in the canine uterus is almost inexistent. This work intends to establish the pattern of distribution of SOD1 and GPx1 immunoreaction in canine endometrium throughout the estrous cycle, using 46 endometrium samples of healthy dogs representing different cycle stages (anestrus-10, proestrus-10, estrus-10, early diestrus-7, and diestrus-9). SOD1 distribution in canine endometrium showed cyclic variations ($P \leq 0.001$), with higher immunoscores in the progesterone-associated stages. Changing immunoreaction also concerned the different epithelial structures considered (surface epithelium, superficial glandular epithelium, and deep glandular epithelium) ($P \leq 0.001$), but it was always higher than in the stroma ($P \leq 0.001$). Deep glandular epithelial cells usually showed higher scores of immunoreaction compared with the other epithelial cells. Interestingly, in epithelial cells, distinct subcellular patterns for SOD1 were seen: the nuclear labeling was observed in estrus and early diestrus ($P \leq 0.001$), whereas an apical reinforcement was observed in estrus ($P = 0.011$) in the glandular epithelia but not in the surface epithelia. In general, GPx1 distribution in canine endometrium remained relatively unchanged throughout the estrous cycle ($P = 0.169$) despite the slight decrease observed from proestrus to early diestrus. The highest scores were found in anestrus and diestrus ($P < 0.05$), varying with of the structure considered. An apical reinforcement pattern was also found for this molecule, which peaked in proestrus and estrus ($P < 0.005$). In summary, the present study showed that SOD1 and GPx1 are consistently distributed in the canine endometrium. The cyclic changes registered for both molecules suggest that they may play important roles in endometrial physiology, probably in apoptosis and proliferation.

1. Introduction

The mammalian endometrium is a complex and highly dynamic tissue, with the ultimate purpose to guarantee embryo survival, implantation, and a successful pregnancy. In response to the changes in sex steroids, the endometrium undergoes cyclic remodeling, integrating morphologic, and functional changes. This process is ultimately controlled by several cytokines, interleukins, and growth factors, among other molecules [1–4].

As in other aerobic systems, endometrial cells continuously generate reactive oxygen species (ROS), as a consequence of their normal metabolism. The term ROS refers to radical and nonradical oxygen species formed by the partial reduction of oxygen, such as the superoxide anion ($O_2\bullet-$),

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the hydrogen peroxide (H_2O_2), or the hydroxyl radical (OH•) [5]. Physiological amounts of ROS are necessary for normal tissue function as they are involved in the regulation of different cellular signaling pathways [5,6] that affect different cellular processes, such as proliferation, differentiation, and programmed cell death (apoptosis).

ROS control is an important process sought to maintain tissue homeostasis [7]. The amount of ROS produced in tissues is maintained within physiologically balanced levels by a network of highly complex and integrated defense mechanisms, through specific scavenger reactions and detoxification pathways, that include endogenous enzymatic and nonenzymatic antioxidant systems [8]. The key endogenous enzymes directly involved in the control of ROS production include the superoxide dismutase (SOD), catalase, glutathione peroxidases (GPxs), glutathione reductase, and glutathione-S-transferase. In turn, the glutathione, the nicotinamide adenine dinucleotide phosphate ($NADP^+$) and the reduced form of $NADP^+$ (NADPH) integrate the nonenzymatic endogenous antioxidant systems [8,9]. A variety of other dietary antioxidant substances also exists, such as vitamin C, vitamin E, carotenoids, or the natural flavonoids [8].

During a first step of the scavenger defense mechanisms that maintain the redox homeostasis, the dismutation of the superoxide anion (O_2•−) into H_2O_2 and O_2 by the SOD occurs. In mammals, SOD possess three different isoforms: a copper- and zinc-containing SOD (Cu,Zn-SOD or SOD1), a dimeric protein found in the cell cytoplasm; a manganese-containing SOD (Mn-SOD or SOD2), located in the mitochondrial matrix; and a Cu- and Zn-containing SOD (ecSOD or SOD3), a tetrameric glycoprotein that constitutes the major SOD isoform in extracellular fluids [10]. In a second step to avoid ROS propagation, the produced H_2O_2 is quickly converted to H_2O and O_2 by GPxs, typically through the oxidation of glutathione. There are several known GPxs isoforms (GPx1 to 8), some of them identified in specific tissues such as the gastrointestinal or the male reproductive tract or the lungs [11,12]. GPx1, located in the cell cytosol and mitochondria, is ubiquitously expressed in tissues and represents the major antioxidant enzyme of GPx family [13].

In the reproductive system, alike diverse other body systems, ROS are maintained under a delicate balance by the antioxidant mechanisms. It is now commonly accepted that ROS have a crucial role within the female reproductive system, actively contributing to fertility [14,15] and early pregnancy events [16,17]. In humans, in physiological concentrations, ROS mediates many significant incidences in the endometrium, including the hormone signaling, angiogenesis, apoptosis, cell proliferation, and prostaglandin secretion [18,19]. It has been reported that ROS are involved in progesterone-mediated physiological events, such as decidualization or menstruation [18,20].

Changes in the expression of ROS and antioxidants enzymes have been evidenced during the endometrial cycle in women [15,16,21]. References on ROS and antioxidants enzymes expression in the endometrium of domestic animals are, however, scarce. Nevertheless, the work of Al-Gubory et al. [17] in sheep and that of Ramos et al. [22], in cows, on the activity of oxidative stress enzymes in the endometrium, are suggestive of the existence of changes in antioxidant enzymes throughout the estrous cycle.

Dogs present a typical hormonal profiling during their estrous cycle that is considerably different from those of other domestic species. Dogs are monoestrous species, meaning that a stage of anestrus separates two consecutive reproductive cycles [23], which is fundamental to the female dog fertility [24]. Further unique features of the canine reproductive biology include a relatively prolonged follicular stage, the preovulatory luteinization of dominant ovarian follicles, the ovulation of an immature oocyte that takes close to 3 days to became fertilizable, and a relatively long diestrus, similarly lengthened in cyclic or pregnant diestrus [23]. The characteristic extended luteal stage favor the incidence of progesterone-associated diseases, such as deciduoma formation [25] or the cystic endometrial hyperplasia and pyometra complex [26].

The interest in studying the role of oxidative stress in female reproduction is increasing, particularly regarding its possible association with infertility and the development of uterine diseases. The balance of oxidative stress within the canine endometrium is poorly known, despite that recently Kobayashi et al. [27] described an increase in SOD activity in the uterine fluid of bitches in diestrus compared with anestrus or estrus. Therefore, this work intends to analyze the immunohistochemical pattern of distribution of two antioxidant enzymes, the copper- and zinc-containing SOD (SOD1) and the glutathione peroxidase 1 (GPx1) in the canine endometrium throughout the estrous cycle.

2. Material and methods

2.1. Tissue collection and preparation

This study included 46 postpubertal, healthy nonpregnant bitches, submitted to routine ovariohysterectomy for contraception purposes, under the owner request, in a private veterinary clinic in Famalicão (Portugal). Most animals were mongrels (n = 33) or crossbreds (n = 7; distributed as follows: Poodle [3], Portuguese Podenco [2], Pincher [1], Pequinois [1] crosses); purebreds were represented by Siberian husky (n = 3), Cocker Spaniel (n = 2), and Labrador retriever (n = 1). The ages ranged from 10 months to 8 year old, with an average of 2.5 years, distributed in the following age groups: 34 females from 10 months to less than 3 years old, eight females with ages between 3 and less than 6 years old, and three females being 6 to 8 years old.

Samples of canine endometrial tissue were collected immediately after the elective ovariohysterectomy and used with the owners' informed consent, following the International Ethical standards. Transversal fragments from each uterine horn, along with a transversal hemisections from each ovary, were collected immediately after the surgery. After fixation in 10% formalin and embedding in paraffin wax, sections of 3 μm were prepared and stained with hematoxylin and eosin. The uterine and ovarian sections were used for histologic staging of the estrous cycle and to exclude uterine disease.

Before surgery, a blood sample was collected from the jugular vein into a controlled vacuum tube (Serum-gel,