

Expression of superoxide dismutases in the bovine oviduct during the estrous cycle

M. Roy^{a,b}, D. Gauvreau^{a,b}, J.-F. Bilodeau^{a,b,c,*}

^a *Unité de Recherche en Ontogénie et Reproduction, Centre de Recherche du Centre Hospitalier de l'Université Laval, Québec, Canada G1V 4G2*

^b *Centre de Recherche en Biologie de la Reproduction (CRBR), Université Laval, Québec, Canada G1V 4G2*

^c *Département d'Obstétrique et Gynécologie, Faculté de Médecine, Université Laval, Québec, Canada G1K 7P4*

Received 28 September 2007; received in revised form 30 January 2008; accepted 9 May 2008

Abstract

The superoxide dismutases (SODs) are first-line enzymatic antioxidants that dismutate superoxide anion ($O_2^{\bullet-}$) to produce hydrogen peroxide (H_2O_2). The primary objective was to characterize, by western blot analysis, the expression of two SODs, the cytosolic (Cu,ZnSOD or SOD1) and the mitochondrial (MnSOD or SOD2) forms in three sections of the oviduct, i.e. isthmus (I), isthmus–ampullary junction (IA), and ampulla (A), during the estrous cycle. The Cu,ZnSOD and MnSOD proteins were mostly expressed in the ampulla ($I < IA < A$; $P < 0.0093$). Expression of Cu,ZnSOD was lowest during metestrus ($P < 0.0041$) and it was mostly expressed in the oviduct contralateral to the CL ($P = 0.0019$). The expression of MnSOD was consistent throughout the cycle. Based on immunohistochemistry, the SODs were present in all cell types of the oviduct, with variations among oviductal sections. We inferred that the expression of Cu,ZnSOD was influenced by the hormonal milieu. Furthermore, variations among oviduct sections in protein expression profile of SODs suggested they have an important role in preserving and capacitating sperm. © 2008 Elsevier Inc. All rights reserved.

Keywords: Oviduct; Superoxide dismutase; Reactive oxygen species; Estrous cycle; Cow

1. Introduction

In cattle and other species, the oviducts play a critical role in early reproductive processes, including gamete maturation, fertilization, and early embryo development [1–3]. These processes require an appropriate balance between reactive oxygen species (ROS) and antioxidants, as shown by many *in vivo* and *in vitro* studies

[4–6]. Reactive oxygen species (ROS) exist in several forms ($O_2^{\bullet-}$, H_2O_2 , $OH^{\bullet}$, NO, $ONOO^-$) and can be either beneficial or detrimental to reproductive events [4,6,7]. Indeed, low levels of ROS can be beneficial in promoting the binding of sperm to the zona pellucida [8–10]. In cattle, the superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are essential for sperm capacitation and the acrosome reaction, respectively, *in vitro* [6,11]. However, high hydrogen peroxide (H_2O_2) concentrations reduced bull sperm motility *in vitro* [6] and may impair fertilization and embryo development [12,13]. Furthermore, interaction of $O_2^{\bullet-}$ and H_2O_2 can generate the hydroxyl radical ($OH^{\bullet}$), one of the most powerful ROS, which can damage lipids, proteins and nucleic acids [14]. The H_2O_2 can also form $OH^{\bullet}$ via the

* Corresponding author at: Département d'Obstétrique et Gynécologie, Faculté de Médecine, Université Laval, Québec, Canada. Tel.: +1 418 525 4444x46153; fax: +1 418 654 2765.

E-mail address: jean-francois.bilodeau@crchul.ulaval.ca (J.F. Bilodeau).

Fenton reaction in the presence of transition metals (e.g. iron) [14]. The $O_2^{\bullet-}$ can also react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), a powerful oxidant, which reduces the bioavailability of NO [15].

Production of ROS is controlled by various antioxidants. Nonenzymatic antioxidants include glutathione (GSH), α -tocopherol, β -carotene, ascorbate, and vitamin C [15,16]. The main antioxidant enzymes include superoxide dismutases (SODs) which neutralize superoxide anion ($O_2^{\bullet-}$), catalases which scavenge hydrogen peroxide (H_2O_2), and glutathione peroxidases (GPx) which detoxify H_2O_2 and lipid hydroperoxides (ROOH) [15]. The SODs exist in three forms: the cytosolic (Cu,ZnSOD or SOD1), the mitochondrial (MnSOD or SOD2) and the extracellular form (EC-SOD or SOD3) [17]. The SODs are the first line of defense against ROS in dismuting two superoxide anions ($O_2^{\bullet-}$) into one hydrogen peroxide (H_2O_2) [18], which can be metabolized by either catalases or GPx [15]. Thus, SODs are important defense enzymes against $O_2^{\bullet-}$, preventing the formation of $OH^\bullet$ and $ONOO^-$. That Cu,ZnSOD female knock-out mice had reduced fertility implicates SODs in reproduction in the female [19]. Although the balance between antioxidants and ROS is critically important for reproductive success, few *in vivo* studies have characterized antioxidants in the oviduct, especially SODs in cattle. In mammalian oviducts, Cu,ZnSOD and MnSOD were best characterized in the mouse, rat, rabbit, and human [20–22].

We previously reported that antioxidant enzymes such as glutathione peroxidases (GPx) and prooxidant enzymes such as nitric oxide synthases (NOS) that produce NO were spatially and temporally regulated in the bovine oviduct during the estrous cycle [23,24]. However, the specific protein regulation of superoxide dismutases (SODs) remains to be determined in the cow. The primary objective of the present study was to determine the expression of Cu,ZnSOD and MnSOD proteins during the estrous cycle in three sections of each oviduct (both ipsilateral and contralateral to the CL). The secondary objective was to determine cellular localization of these SODs.

2. Materials and methods

2.1. Oviducts

Genital tracts were recovered from Holstein cows/heifers at an abattoir and transported on ice to the laboratory within 4 h after death. Tracts with visible anomalies were eliminated. Based on visual examina-

tion of the ovaries, the tracts were divided according to stage of the cycle (3): metestrus (Days 0–3), diestrus (Days 10–15), and proestrus (Days 18–21). The locations of the current and previous CL were determined; only tracts with these CL on opposite ovaries were used. Furthermore, oviducts ipsilateral and contralateral to the current CL were studied separately. Oviducts were dissected (to remove blood vessels, ligaments, and other tissues) and cut in three sections of 2 cm: the isthmus was adjacent to the uterotubal junction; the isthmio-ampullary junction was the midpoint of the oviduct; and the ampulla was adjacent to the fimbria. Each section were frozen in liquid nitrogen and subsequently kept at -86°C until analyzed. For each stage of the estrous cycle, five pairs of oviducts (ipsilateral and contralateral to the CL) were used (three sections per oviduct).

2.2. Western blot analysis

A sample (~ 0.5 cm) from each section of the oviduct was mixed in ice-cold PBS (Invitrogen, Carlsbad, CA, USA) containing a protease inhibitor cocktail (1 mM EDTA, 0.5 mg/mL Leupeptin, 1.4 mg/mL Pepstatin A, 70 mg/mL PMSF; Boehringer Mannheim, Laval, QC, Canada) and homogenized with an Ultra Turrax T25 (IKA Works, Inc., Wilmington, NC, USA). Homogenates were mixed in Laemmli sample buffer (5% (v:v) 2-mercaptoethanol) and boiled for 10 min [23,24]. The quantity of proteins was determined using a BCA protein assay, according to the manufacturer's instructions (Pierce, Rockford, IL, USA).

An aliquot (30 μg) of proteins from each oviductal section were loaded on a 15% polyacrylamide-SDS gel. After electrophoresis, the proteins were transferred to a nitrocellulose membrane (0.2 μm ; Bio-Rad Laboratories, Montréal, QC, Canada). The membranes were incubated for 1 h at room temperature with 5% dry milk in TBS-T, then incubated for 2 h at room temperature with primary rabbit polyclonal antibodies against bovine SOD1 (dilution 1:10,000) [23] and SOD2 (dilution 1:5000) (GeneTex, San Antonio, TX, USA). The signal was revealed using ECL Trade Mark (GE Healthcare Bio-Sciences, Baie d'Urfé QC, Canada) and a densitometry was done with an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA, USA).

2.3. Immunohistochemistry

Immunohistochemistry was performed as previously described [24]. Briefly, immediately after dissection, a portion of each section of the oviduct (isthmus, isthmio-

Download English Version:

<https://daneshyari.com/en/article/2098224>

Download Persian Version:

<https://daneshyari.com/article/2098224>

[Daneshyari.com](https://daneshyari.com)