Theriogenology 86 (2016) 1092–1101

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Immunolocalization of E-cadherin and β-catenin in the cyclic and early pregnant canine endometrium

R. Payan-Carreira [a,*], M.A. Pires [a], C. Santos [b], B. Ström Holst [c], J. Colaço [a], H. Rodriguez-Martinez [d]

[a] CECAV, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[b] DEBA, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[c] Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[d] Faculty of Medicine & Health Sciences, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 30 March 2016
Accepted 31 March 2016

Keywords:
E-Cadherin
β-Catenin
Canine endometrium
Estrous cycle
Early pregnancy
Dog

ABSTRACT

Putative changes in E-cadherin and β-catenin during implantation in dogs are of interest to study, as they are relevant proteins for epithelial integrity. E-cadherin and β-catenin were immunolocalized in the canine endometrium during the estrous cycle and early pregnancy, using monoclonal antibodies. Both proteins were detected in all types of endometrial epithelia (surface epithelium [SE], superficial glandular, and deep glandular epithelia) at all stages of the estrous cycle and in early placental structures. E-cadherin depicted a gradient of intensity apparently being lowest in the SE to progressively increase toward the deepness of the endometrial glands, regardless of the stage of estrous cycle. The overall immunostaining was, however, weaker at diestrus. In pregnant samples, the trophoblast was conspicuously immunolabeled compared with the endometrial surface lining epithelium. In the latter, the cytoplasmic pattern predominated over the membrane-bound, as was also seen in the decidual cells of the placental labyrinth. In the early placenta, only trophoblast cells and lacunae retained membrane signals. β-Catenin membrane labeling appeared relatively constant throughout the cycle, although a tendency toward a decrease in intensity was detected at the secretory stages. In addition, a dislocation of the immunoreaction from membrane to the cytoplasm was observed in both the SE and the glandular epithelia at particular stages of the cycle. In early pregnancy, a loss of the membranous pattern was observed in the SE and labyrinth, but neither on trophoblast nor in lacunae. The results show the existence of a softening of the adherens junctional complex in progestagen-dominated stages favoring embryo-maternal interactions and endometrial invasion during canine implantation.

1. Introduction

In the bitch, such as in other mammals, the endometrium consists of a mesenchymal stroma, composed of individual fusiform cells (fibroblasts) surrounded by extracellular matrix and 2 epithelial cell populations: the glandular epithelium and the surface epithelium (SE) or luminal epithelium [1]. Endometrial epithelium is of utmost relevance for the integrity of the organ, for the interactions with the spermatozoa and with the embryo, and for the establishment and maintenance of pregnancy. A complex sequence of signaling events, encompassed by the crosstalk between endometrial epithelium and stroma, is crucial to the establishment of pregnancy [2,3].

In response to cyclic variations in sex hormones, the mammalian endometrium evidences recurrent structural and morphologic modifications in what is frequently named the endometrial cycle [4]. Several molecules have

* Corresponding author. Tel.: +351 259350425; fax: +351 259350480.
E-mail address: rtpayan@gmail.com (R. Payan-Carreira).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.03.041

been shown to display cyclic patterns of expression, responding differently to the sex steroid dominance on target tissues, contributing to normal functioning of the endometrium and the endometrial receptivity to the embryo [4–7]. The endometrial remodeling processes entail complex changes in epithelial cell–cell interactions, which are required to maintain the continuity and integrity of the endometrium [8]. Adhesive interactions between neighboring epithelial cells are crucial during implantation and also for tissue morphogenesis and renewal [9]. Endometrial modifications in the expression of different adhesion molecules during the cycle or early pregnancy have been reported in humans [8,10–12], sheep [13], and pigs [12,14].

Adherent junctions play important functions in the maintenance of intercellular connections, which defines the characteristic architecture of the epithelial lining. Through cadherin-mediated epithelial cell-to-cell connections, the integrity and strength of the epithelial layer [15,16], as well as the polarity of the epithelia [17] are maintained. Consequently, it can be presumed that the plasticity of cell-to-cell adhesion of endometrial epithelial cells is crucial to female fertility.

E-Cadherin, a member of the cadherin family of calcium-dependent adhesion proteins, is a cell surface glycoprotein and an important determinant of tissue processes related to selective cell adhesion or detachment [15,16], which in the uterus involves changes designed to support the implantation and growth of an embryo [8,18,19]. The cyclic tissue remodeling that occurs in the endometrium during the cycle requires complex changes in cell–cell and cell-matrix interactions. Spatiotemporal changes in the E-cadherin adhesion system may be associated with the morphologic changes observed in the mammals' endometrial cycle [10,20]. Furthermore, down-regulation of E-cadherin has been associated to invasive processes, which in the uterus includes the disruption of the epithelial barrier and the progression through a permissive extracellular mesenchymal matrix, as occurs at implantation in species with decidual placenta [21]. Supporting this concept, Dawood et al. [22] reported that E-cadherin and its gene transcripts were expressed in peri-implantation phase endometrium in women, and Liu et al. [23] found a relationship between E-cadherin and metalloproteinase-2 and metalloproteinase-9 during the mouse embryo implantation process. It was found that E-cadherin expression is significantly reduced close to the maternal recognition period in sheep [13] and pigs [14], species in which it may also play a favorable role in embryo elongation. In dogs, Guo et al. [24], using *in situ* hybridization, described a reduction in E-cadherin mRNA expression until Day 20 of pregnancy compared with the expression levels recorded in estrus, thereafter showing a strong signal in the glandular epithelium.

The E-cadherin junctional complex also includes several other proteins, such as β-catenin, by which cadherin is anchored to the cytoskeleton. In the cytoplasm, β-catenin pairs bind strongly to the E-cadherin domain, binding the complex to the actin skeleton through the α-actin molecule [25–27]. In addition to its role in cell-to-cell adhesion, β-catenin also plays an important role in the canonical Wnt signaling pathway [28,29], which has been implicated in the regulation of the endometrial cycle [30] and in implantation [31]. β-Catenin participation in either cell adhesion or the Wnt pathway depends on the existence of a competitive binding of this molecule to E-cadherin and Wnt-signaling molecules, which is determined by the activity of different kinases [28,29,31].

Epithelial functional or physical integrity is a major issue regarding embryo invasion in early pregnancy, a process that in dogs involves the trophoblast invasion and the concurrent erosion and transformation of the upper half of the endometrium to establish an endotheliochorial placenta [2,32]. Thereby, changes in the E-cadherin and/or β-catenin adhesive complex are expected to occur at the initial moments of implantation in dogs. Disgregation of components of this complex will result in lateral cell–cell dissociation [2,33], which would facilitate embryo invasion, as it occurs during tumor progression [34,35]. A reduced endometrial epithelial barrier, as indicated by a reduction in E-cadherin expression or the compromised polarity of epithelia during the luteal or secretory stage might also favor the development of pyometra during progesterone dominance, facilitating the colonization of the endometrium by pathogen bacteria ascending from the vagina [36]. This risk is likely higher in species with a physiologically long diestrus, such as dogs.

Despite extensive research reported on cadherin and β-catenin activity in the uterus of different species [8,13,18,37], information about the immunolocalization of E-cadherin and β-catenin in the canine endometrium is sparse. Canine reproductive physiology depicts several characteristics distinguishing this species from other domestic animals, particularly noncarnivorous. Of particular interest is the relatively long estrous phase and a remarkable long luteal phase which is of similar length in pregnant and nonpregnant cycles [38]. The dog presents an endotheliochorial placenta type, in which the invasive trophoblast destroys the luminal epithelium and the underlying lamina propria in the process of implantation and placentation [39,40]. Consequently, the spatiotemporal pattern in E-cadherin and β-catenin molecules in the canine endometrium may differ from those established in other species with a shallower noninvasive placentation. Considering these concepts, the aim of this work was: one to analyze E-cadherin and β-catenin protein immunolocalization throughout the stages of the canine estrous cycle and to determine whether temporal changes exist during the uterine cycle and to; two ascertain possible modifications of cadherin and/or β-catenin adhesion pathways in the canine embryo apposition and adhesion to the endometrial lining epithelium.

2. Material and methods

2.1. Animals

The study included 50 mature, healthy cross-bred bitches, aged from 1 to 9-year-old, submitted to elective ovariohysterectomy for contraception purposes. In addition, 9 pregnancy samples were obtained from females with unwanted 3-week-long pregnancies, submitted to elective ovariohysterectomy. Before surgery, a vaginal