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ScienceDirect
Theriogenology 69 (2008) 700–713
Theriogenology
www.theriojournal.com

Actin binding protein expression is altered in uterine luminal epithelium by clomiphene citrate, a synthetic estrogen receptor modulator

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Received 23 August 2006; received in revised form 30 November 2007; accepted 4 December 2007

Abstract

Clomiphene citrate (CC), a synthetic oestrogen, is often prescribed as a superovulator in treating infertility. Although CC works efficiently, pregnancy rates following CC treatment are ~10 times lower than ''natural'' rates. This study investigates how a dose of 1.25 mg CC given to ovariectomized rats before the implantation priming hormones (a single dose of progesterone for 3 days and a dose of estrodiol-17β on d3, P-P-PE), alters the expression and distribution of α-actinin, gelsolin and vinculin. Actin binding proteins show a specific distribution within the uterine epithelium during implantation, linking the actin cytoskeleton to integrin expression on the uterine surface and in this way aiding ''adhesiveness'' for blastocyst apposition to the uterine epithelium. In this study, immunocytochemistry on frozen uterine sections using mouse monoclonal antibodies against α-actinin, gelsolin and vinculin and peroxidase-conjugated secondary antibodies, show that CC, administered before the P-P-PE regimen, down-regulates the expression of vinculin, does not alter the expression of gelsolin and up-regulates α-actinin on the uterine apical surface, when compared to P-P-PE treated animals. All three proteins are down-regulated on the apical surface of the luminal epithelium and glands in all groups when compared to pregnant controls. Vinculin was only localized in the basolateral compartment of the uterine epithelial cells in the CC treated groups. By down-regulating these proteins on the uterine surface and up-regulating vinculin on the basolateral membrane of the epithelium, CC may impede adhesion and invasion of blastocysts at implantation. These results may aid the exogenous manipulation of uterine tissue to control fertility and improve assisted reproductive out-comes.

Keywords: Uterine epithelium; Clomiphene citrate; Actin binding proteins; Uterus; Implantation

1. Introduction

The period of uterine receptivity in mammals is limited to a few hours or days and is referred to as the window of receptivity, strictly under the control of the ovarian hormones, oestrogen (E_2) and progesterone (P_4) secreted in a particular sequence and precise ratio. The actual process of implantation is synchronised and depends on the exact expression and availability of matching receptors and signals, including gene expression, growth factors, cytokines, adhesion molecules and hormones from both embryonic and maternal tissue [1–3]. During the time of early implantation a series of defined and documented morphological changes are

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0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.12.002

seen and include alterations in epithelial cell shape and size, determined largely by the actin cytoskeleton and its associated proteins; as well as marked modifications to the apical, basal and lateral membrane compartments of the uterine luminal epithelial cells [4,5]. The changes seen during early implantation directly associated with the cytoskeleton, include the conversion of long regular microvilli on the apical plasma membrane of the uterine epithelial cells seen at oestrus or d1 of pregnancy, into flattened projections and pinopods. This uterine profile is present for a limited period at implantation in most mammals, including humans, and in rats specifically on d5.5 of pregnancy, at the time of implantation [6–9].

The cytoskeletal changes known to occur during the window of receptivity include reorganization of intermediate filaments associated with the disruption of the terminal web by d3 of pregnancy, leaving a relatively microfilament free cytoplasm [10,11]; changes in actin distribution from the apical surface of the uterine luminal epithelium on d1 of pregnancy to general dispersal at d6 of pregnancy, just after implantation [12]. Actin associated proteins, and linker proteins including α-actinin, vinculin, gelsolin, tropomysin, plectin, plakoglobulin and advillin have also been shown to be modified by the hormonal conditions governing the window of receptivity [12–15].

The cytoskeleton of a cell is central to many basic cellular functions including phagocytosis, cytokinesis, shape change, and polarity; all important in the implantation reaction in early pregnancy. Changes in cytoskeletal structure and cell shape associated with processes like implantation, can be initiated in a number of ways including the interaction of the actin binding proteins interacting with, (1) each other, (2) the Arp2/3 complex, (3) talin and (4) specific integrins triggering actin nucleation from inside the cell [16,17]. It is also known that the interaction of the actin cytoskeleton with integrins is differentially regulated in different parts of the cell [18].

Vinculin, a well characterized focal adhesion protein, is often localized in many different integrin mediated cell junctions as well as being co-localized with cadherins in cell–cell junctions. Importantly, vinculin is associated with cell protrusion and the coupling of β1-integrin receptors to actin polymerisation [18,19]. Vinculin binds actin through an association with α-actinin and therefore actin indirectly to the plasma membrane [20,21]. Vinculin also interacts with talin, an adhesion protein, providing an additional link between integrins and the actin cytoskeleton, integrins may therefore be activated by vinculin initiating integrin mediated cell adhesion from within the cell [17,18,22–25].

The second actin binding protein chosen for this study is α-actinin, a short rod shaped anti-parallel dimer that interacts with actin filaments cross-linking them to form bundles of filaments [20,24]. Additionally, α-actinin links actin fibrils to the cytoplasmic tails of transmembrane receptors, including cadherins, integrins and ICAMs situated in the plasma membrane; as well as interacting with vinculin [18,20,24]. Of the two non-muscle isoforms of α-actinin, α-actinin-1 is found in focal adhesions and cell–cell junctions like vinculin, while α-actinin-4 is found only in certain types of membrane ruffles [18] that are commonly associated with pinopods in rats [26]. Several proteins interact with α-actinin, suggesting that α-actinin plays an important role as a scaffolding protein [18].

Gelsolin is a Ca+-dependent protein and the best characterized member of the actin binding proteins. Gelsolin belongs to a super-family of proteins, that sever, fragment and cap actin filaments. Further, gelsolin is also associated both with actin filament reorganization by bundling (villin found in microvilli) or nucleating actin filaments during cell locomotion and vesicle trafficking [27–31]. Additionally, gelsolin is complicit in apoptosis, both through the death receptor and mitochondrial pathway, acting as a substrate for caspase-3. It is directly responsible for the characteristic cytoskeletal destruction and membrane blebbing seen during apoptosis.

Clomiphene citrate (CC) is the most frequently prescribed synthetic oestrogens or selective oestrogen receptor modulators (SERM); [32] used for treatment of infertility [33]. Clomiphene has been very successfully and extensively used for induction of ovulation in assisted reproductive procedures including *in vitro* fertilization (IVF) and gamete intra-fallopian tube transfer (GIFT); [34,35]. It however, appears to impede implantation when used in this manner [32]. Clomiphene has been shown to down-regulate pinopod formation [36], as well as dramatically change apical membrane architecture at the time of implantation [37,38]. Although CC, like estrogen acts via the oestrogen receptors (ER) it also appears to operate via anti-oestrogen binding sites (AEBS) and other pathways including growth factor cascades and signal transduction pathways [39–41]. Overall, CC has profound effects on the structure of the apical, lateral and basal plasma membrane domains of the luminal epithelial cells [37,38,42], all of which are cytoskeletally associated.

In the laboratory rat, luteal progesterone alone is insufficient to ‘prime’ the endometrium for successful