Reproductive BioMedicine Online (2013) 27, 690–701

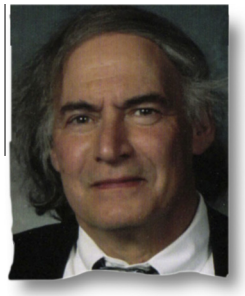
www.sciencedirect.com
www.rbmonline.com

SYMPOSIUM: FUTURES IN REPRODUCTION
ARTICLE

Sperm attachment and penetration competence in the human oocyte: a possible aetiology of fertilization failure involving the organization of oolemmal lipid raft microdomains influenced by the $\Delta\Psi$m of subplasmalemmal mitochondria

Jonathan Van Blerkom [a,b,*], Kyle Caltrider [a]

[a] *Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, United States;*
[b] *Colorado Reproductive Endocrinology, Rose Medical Center, Denver, CO 80220, United States*
[*] Corresponding author. *E-mail address:* jonathan.vanblerkom@colorado.edu (J Van Blerkom).



Jonathan Van Blerkom is a professor in the department of molecular, cellular and developmental biology at the University of Colorado and Laboratory Directorat Colorado Reproductive Endocrinology in Denver. His studies have focused on molecular and cellular aspects of early mammalian development including human follicles, oocytes and embryos and has published numerous research articles, reviews, and coauthored or edited books dealing with early mammalian development, including the human. His current research centres on the role(s) of mitochondria in early development, and the molecular organization of the oocyte and embryo plasma membrane as related to developmental competence.

Abstract The roles of oolemmal lipid raft microdomains enriched in the ganglioside GM1 and the tetraspanin protein CD9 were investigated as causative agents in fertilization failure in human IVF where spermatozoa progress to the oolemma but fail to attach or, if attached, to penetrate. The findings show that specific configurations of GM1 lipid raft microdomains are consistent with attachment and penetration, while microdomains composed of CD9 lipid rafts, a protein known to be critical for penetration, do not appear to have a central role in the initial stages of attachment. The relative magnitude of the potential difference across the inner membrane ($\Delta\Psi$m) in mitochondria localized to a stable subplasmalemmal domain appears to influence the organization of GM1 but not CD9 lipid raft microdomains in the corresponding oolemma. The findings present a novel view of how fertilization competence may be established in the human oocyte and a means by which certain fertilization failures that occur after conventional clinical IVF can be identified and explained in the unfortunate instance of fertilization arrest at the oolemma.

KEYWORDS: CD9, fertilization, GM1, lipid rafts, mitochondria
VIDEO LINK: http://sms.cam.ac.uk/media/1400893

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.09.011

Introduction

Fertilization failures that occur with normal human spermatozoa and meiotically mature (metaphase II, MII) oocytes after conventional insemination *in vitro* are an unexpected outcome in clinical IVF and can significantly reduce the likelihood of pregnancy if a high proportion of oocytes are affected. Failures of this type have persisted despite significant improvements in fertilization protocols, including stringent attention to culture conditions. One common form of fertilization failure in clinical IVF is characterized by sperm penetration but then the absence of second polar body and failure of the formation of a paternal or maternal pronucleus, or both. Failure of this type has been referred to as 'occult' fertilization and has been reported to affect a surprisingly high proportion of normal-appearing MII oocytes after conventional IVF (Van Blerkom et al., 1994) and may perhaps result from defects in preovulatory nuclear or cytoplasmic maturation (Miyara et al., 2003).

Here, this study investigated a different type of fertilization failure, namely, the presence on the surface of the oolemma of a spermatozoon that failed to penetrate a normal-appearing, meiotically mature human oocyte. How frequently this phenomenon occurs in clinical IVF is difficult to assess because most programmes expend little or no effort to identify possible causes of fertilization failure and simply discard unfertilized oocytes. When detected, however, its occurrence can be particularly difficult for patients to cope with because no reason can be given as to why an apparently functional spermatozoon, as indicated by its ability to undergo the acrosome reaction and pass though the cellular (cumulus and coronal cells) and acellular (zona pellucida) compartments that surround the oocyte, cannot penetrate the last barrier, the oolemma. While an unrecognized sperm defect cannot be precluded, in these instances it is not unreasonable to suspect that fertilization failure resides at the level of the oolemma, especially when other MII oocytes within a cohort and with similar morphologies fertilize normally.

Preliminary studies of the molecular organization of the mouse and the human oocyte have provided evidence for a plasma membrane composed of lipid raft microdomains in general, and those containing two known lipid raft components in particular, the ganglioside monosialotetrahexosylganglioside (GM1) and the cell membrane tetraspanin CD9 (Van Blerkom, 2013). GM1 is a sphingolipid constituent of lipid rafts that is directly involved in a variety of the cell surface activities including facilitation of intercellular interactions, virus docking, signal transduction and protein binding (Babiychuk and Draeger, 2000; Brown and London, 2000; Godoy and Riquelme, 2008; Harder et al., 1998; Janes et al., 1999; Kawano et al., 2011; Landry and Xavier, 2006; Lopez and Schnaar, 2009; Luna et al., 2005; Neu et al., 2008; Olofsson and Bergstrom, 2005). CD9 is a member of the tetraspanin family (Ishii et al., 2006) that has been suggested to be the single most important oolemmal component involved in sperm penetration in mammals (Jejou et al., 2011; Miyado et al., 2008).

This study investigated whether fertilization failure at the level of the oolemma could be related to defects in lipid raft microdomains enriched in GM1 or CD9, or both, and the extent to which certain cytoplasmic factors could influence the organization of these lipid raft microdomains and, therefore, oolemma function.

Materials and methods

Human oolemma and fertilization failure

Ovarian stimulation, ovulation induction, follicular aspiration and conventional IVF followed protocols previously described (Van Blerkom et al., 2002, 2008). According to protocol, the first inspection for fertilization after conventional IVF in non-severe male factor cases (i.e. those not normally requiring intracytoplasmic sperm injection, ICSI) is made at 10–12 h. Normal fertilization is confirmed by an extruded second polar body and early evidence of pronuclear formation (Van Blerkom et al., 1995). If pronuclear formation is not detected, a second inspection occurs between 18 and 21 h. All MII oocytes classified as unfertilized at the second inspection were thoroughly examined by light microscopy to determine whether a sperm head was present on the oolemma and by DAPI staining to determine whether penetration without activation had occurred (Van Blerkom et al., 1994).

Fluorophore-conjugated cholera toxin B subunit (CTB), because of its high specificity and binding affinity for the pentasaccharide chain of GM1 (located in the exoplasmic leaflet), is commonly used to report lipid rafts enriched in this ganglioside in living cells and was used here for the same purpose with MII human oocytes (Brown, 2006; Harder et al., 1998; Janes et al., 1999; Kawano et al., 2011; Lagerholm et al., 2005). Preliminary studies established a standard protocol of labelling in which intact oocytes (zona enclosed) were stained for 35 min with Alexa Fluor 488- or 565-conjugated CTB at 5 μg/ml (Molecular Probes, Eugene, OR, USA) at 37°C in Quinns' Advantage Medium supplemented with 10% human serum albumin (HSA; Sage, Trumbull, CA, USA) in an atmosphere of 5% CO_2 and 95% air. In a second protocol tested for analytic use, unfertilized human MII oocytes were denuded of the zona pellucida by brief exposure to acid Tyrode's solution (Van Blerkom and Davis, 2007), washed four times for 3 min with HEPES-buffered Quinns' Advantage Medium containing 10% HSA and stained with Alexa 488- or 565-CTB for 15 min at a concentration of 1 μg/ml.

After staining, intact and zona-free oocytes were washed in HEPES-buffered medium and then either (i) examined by scanning laser confocal microscopy (SLCM; Zeiss LSM 510) in 30 μl of the same medium under oil in ΔT dishes designed for fluorescence microscopy (glass thickness 0.17 mm) with temperature maintained at a constant 37°C by a ΔT controller (Bioptics, Butler, PA, USA) as previously described (Van Blerkom and Davis, 2007; Van Blerkom et al., 1995) or (ii) fixed in freshly prepared 3.7% formaldehyde for 15 min, washed in HSA-supplemented HEPES-buffered medium and stored in fresh medium at 4°C for up to 4 days until examination.

The relative intensity of oolemmal CTB fluorescence was quantified as relative fluorescence units (RFU) from fully compiled projections of 2 μm SLCM sections taken at 5 μm intervals through representative oocytes with values and