Journal of Equine Veterinary Science 43 (2016) S49–S55

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Review Article

The Role of Oviductal Cells in Activating Stallion Spermatozoa

Bart Leemans [a,b,*], Bart M. Gadella [c,d], Tom A.E. Stout [c,e], Catharina De Schauwer [a], Hilde Nelis [a], Maarten Hoogewijs [a], Ann Van Soom [a]

[a] Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium
[b] Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX
[c] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[d] Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
[e] Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form 6 June 2016
Accepted 6 June 2016
Available online 14 June 2016

Keywords:
Sperm-oviduct interaction
Oviduct
Protein tyrosine phosphorylation
Hyperactivated motility
In vitro fertilization
Equid

ABSTRACT

Conventional in vitro fertilization is poorly successful with equine gametes. Although stallion spermatozoa bind to the zona pellucida in vitro, they fail to acrosome react and cannot therefore penetrate into the perivitelline space. Failed sperm penetration most likely relates to the absence in in vitro fertilization media of essential molecules required to fully support stallion sperm capacitation. In vivo, the oviductal lumen provides an environment that appropriately regulates interactions between the gametes and promotes fertilization. Identifying the oviductal "fertilization stimulating factors" would enormously benefit the development of equine in vitro fertilization media. This review focuses on the current understanding of equine sperm–oviduct interactions, which may hold essential clues to achieving successful in vitro fertilization with equine gametes.

1. Introduction

Horse breeding has evolved tremendously during the past century with the gradual removal of regulatory restrictions and the development of artificial reproductive technologies (ARTs). Based largely on successes achieved in human infertility clinics, a number of assisted reproductive technologies have also been introduced to aid the production of foals from subfertile horses. Applying ARTs can also accelerate genetic progress in horse breeding and support the conservation of endangered horse breeds and wild equids. Techniques like artificial insemination [1] and embryo transfer [2] are nowadays routine, while the in vitro production of equine embryos is gradually gaining popularity as a treatment for infertility and because it has a number of practical advantages. However, in vitro embryo production (IVEP) can currently only be applied commercially using intracytoplasmic sperm injection (ICSI) [3–6].

Conventional in vitro fertilization (IVF) could be an alternative to ICSI for producing IVEP equine embryos. Although two IVF foals were born in the early 90s [7,8], this initial success could not subsequently be repeated. While successful equine IVF experiments have occasionally been reported, none has proven to be repeatable between laboratories or even within a single laboratory [9–14]. The primary problem appears to be a failure of stallion spermatozoa to penetrate the zona pellucida in vitro. The zona pellucida is the glycoprotein layer surrounding the oocyte, which is normally penetrated by hyperactivated spermatozoa with the help of enzymes released during the acrosome reaction [15,16]. It is generally accepted that equine IVF fails because of inadequate sperm capacitation in vitro

* Corresponding author at: Bart Leemans, Salisburylaan 133, 9820 Merelbeke, Belgium.
E-mail address: bleemans@cvm.tamu.edu (B. Leemans).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2016.06.003

rather than because of oocyte maturation abnormalities. Indeed, in vivo matured oocytes cannot be fertilized in vitro [7] whereas transfer of in vitro matured oocytes to the oviduct of an inseminated mare yields similar pregnancy rates to normal artificial insemination [13]. In addition, if the zona pellucida is breached mechanically, for example, by ICSI, in vitro matured oocytes yield high rates of normal fertilization [17,18].

In vivo, fertilization takes place in one of the least accessible parts of the mammalian body, the oviduct [19]. Since it is difficult to study in situ, fertilization has been studied predominantly in vitro using oviduct models. Based on research in several mammalian species, Suarez [20] postulated the following hypothesis for in vivo capacitation. Millions of sperm cells are deposited in the uterine body to fertilize a single oocyte or a small number of oocytes. Of those millions of spermatozoa, only thousands reach the isthmus of the oviduct. As they arrive, the majority are trapped and held in a "sperm reservoir" near the caudal entrance to the oviduct until close to ovulation. In the period, just before ovulation, the fertilizing potential of spermatozoa is maintained and prolonged by binding to oviduct epithelial cells in the sperm reservoir. In the immediate preovulatory period, a number of physiological triggers induce the bound spermatozoa to undergo various capacitation-related events. As a result, the activated sperm cells escape from the reservoir by achieving hyperactivated motility and swim off toward the site of impending fertilization, that is, the isthmic-ampullary junction. Capacitated spermatozoa will penetrate the oocyte's extracellular vestments, that is, cumulus cells, zona pellucida, and oolemma, to eventually achieve fertilization. The sperm reservoir contributes to the prevention of polyspermic fertilization because, at a given time, only a limited number of sperm cells are released to reach the oocyte in the ampulla. This ensures that, once the oocyte is fertilized, it has time to secrete the cortical granules into its perivitelline space and thereby activate a functional block to fertilization by other spermatozoa. There are indications that proteins residing in the oviductal fluid, such as osteopontin [21], oviduct-specific glycoprotein and heparin [22] also help prevent the incidence of polyspermic fertilization [21,22].

Between 1990 and 2000, American researchers studied sperm-oviduct interaction in horses using an oviduct monolayer model [23–37]. Since then, very little novel data have been reported on this topic. To develop in vitro oviduct models that more closely mimic the periovulatory situation in vivo, we recently [38–40] used alternative approaches to study equine sperm–oviduct interaction, namely oviduct explant and apical plasma membrane models. The aim was to generate new insights into (1) the molecular basis of equine sperm–oviduct binding, (2) the capacitation-related mechanisms that induce sperm tail-associated protein tyrosine phosphorylation, hyperactivated motility, and the acrosome reaction, (3) how hyperactivated, protein tyrosine phosphorylated spermatozoa are triggered to release from the oviduct epithelium, and (4) the fertilizing capacity of hyperactivated, protein tyrosine phosphorylated spermatozoa. In this review, the various aspects of equine sperm–oviduct interaction in the oviduct explant and apical plasma membrane models are discussed.

2. Equine Sperm–Oviduct Binding

In vivo, sperm binding to epithelial cells in the caudal part of the oviduct is probably essential for recruiting and storing viable and potentially fertile noncapacitated spermatozoa before fertilization, to establish the so-called "sperm reservoir" [20]. In several mammalian species including the cow, hamster, and pig, this molecular interaction between spermatozoa and oviduct epithelium is based on a Ca^{2+}-dependent carbohydrate-lectin recognition [41]. In cattle, glycosaminoglycans [42,43], S-S reductants [43,44], and capacitation triggers like albumin, Ca^{2+}, and HCO_3^- [42] alter the affinity of the sperm cells for the oviduct epithelium and thereby induce the release of bound spermatozoa. In the horse, D-galactose was previously reported to be a key-molecule in facilitating sperm-oviduct binding, on the basis of studies using an oviduct monolayer model [23]. Indeed, galactose-binding proteins have been isolated from equine sperm plasma membranes [45]. However, we recently reassessed the potential role of various carbohydrates, glycosaminoglycans, lectins, S-S reductants, and capacitation factors including albumin, Ca^{2+}, and HCO_3^- in equine sperm–oviduct interaction using both an oviduct explant and an oviduct apical plasma membrane model [40]. Although N-acetylgalactosamine, N-acetylneuraminicacid (sialic acid), and D-mannose or D-glucose were highly abundant in oviduct epithelium, D-galactose moieties were not detected. Using a competitive binding assay and pretreatment with N-glycosidase F, we were able to demonstrate that equine sperm–oviduct binding was not exclusively regulated by Ca^{2+}-dependent lectin or disulphide (S-S) binding. Moreover, the combination of the classical sperm capacitating factors albumin and HCO_3^- markedly reduced (>10-fold) the sperm binding density of oviduct epithelium. Instead, the between stallion spermatozoa binding affinity increased considerably, resulting in Ca^{2+}-independent head-to-head agglutination. In conclusion, combined albumin and HCO_3^- treatment induces head-to-head sperm agglutination which physically prevents equine sperm–oviduct binding.

3. Capacitation of Stallion Spermatozoa in an Oviduct Explant Model

In vivo, sperm-oviduct binding in the period before ovulation is also thought to be an essential step in the capacitation process that prepares spermatozoa for fertilization [20]. One important hallmark of sperm capacitation is tail-associated protein tyrosine phosphorylation. In many species, tail-associated protein tyrosine phosphorylation can be induced in vitro by exposing spermatozoa to a combination of HCO_3^-, Ca^{2+}, and albumin (mice [46], hamster [47], pig [48], and man [49]). These three triggers are, however, not sufficient in cattle and horses. In cattle, capacitation can be induced by adding heparin-like glycosaminoglycans to the other capacitation triggers [50]. Exposure to these four capacitation triggers in vitro does then induce a considerable increase in the percentage of