Theriogenology 84 (2015) 566–574

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Morphometry of boar sperm head and flagellum in semen backflow after insemination

Francisco Alberto García–Vázquez [a,b,*], Iván Hernández-Caravaca [a,c], Wellington Yánez-Quintana [a], Carmen Matás [a,b], Cristina Soriano-Úbeda [a,b], María José Izquierdo-Rico [b,d]

[a] *Department of Physiology, Faculty of Veterinary Science, International Excellence Campus for Higher Education and Research "Campus Mare Nostrum", University of Murcia, Murcia, Spain*
[b] *Institute for Biomedical Research of Murcia (IMIB-Arrixaca), Murcia, Spain*
[c] *Boehringer-Ingelheim S.A., Barcelona, Spain*
[d] *Department of Cell Biology and Histology, Faculty of Medicine, International Excellence Campus for Higher Education and Research "Campus Mare Nostrum", University of Murcia, Murcia, Spain*

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 30 March 2015
Accepted 7 April 2015

Keywords:
Backflow
Flagellum length
Insemination
Morphometry
Sperm
Uterus

ABSTRACT

Once deposited in the female reproductive system, sperm begin their competition and undergo a selection to reach the site of fertilization. Little is known about the special characteristics of sperm that reach the oviduct and are able to fertilize, with even less information on the role of sperm dimension and shape in transport and fertilization. Here, we examine whether sperm morphometry could be involved in their journey within the uterus. For this purpose, sperm head dimension (length, width, area, and perimeter) and shape (shape factor, ellipticity, elongation, and regularity), and flagellum length were analyzed in the backflow at different times after insemination (0–15, 16–30, and 31–60 minutes). Sperm morphometry in the backflow was also analyzed taking into account the site of semen deposition (cervical vs. intrauterine). Finally, flagellum length was measured at the uterotubal junction. Sperm analyzed in the backflow were small (head and flagellum) with different head shapes compared with sperm observed in the dose before insemination. The site of deposition influenced head morphometry and tail size both being smaller in the backflow after cervical insemination compared with intrauterine insemination. Mean tail length of sperm collected in the backflow was smaller than that in the insemination dose and at the uterotubal junction. Overall, our results suggest that sperm size may be involved in sperm transport either because of environment or through sperm selection and competence on their way to encounter the female gamete.
© 2015 Elsevier Inc. All rights reserved.

1. Introduction

When millions of sperm are deposited in the female genital tract through natural or artificial insemination (AI), a journey begins to accomplish the objective for which this highly specialized cell has been created: oocyte fertilization. Of the number deposited (10^7–10^{10}), only few reach the oviduct [1,2] where the encounter between male and female gametes takes place. What is clear is the existence of a loss of genetic material (related to male gamete) because very few sperm are able to reach the oviduct compared with the number that was previously placed in the uterus. However, it is still not clear whether the sperm are lost through a discriminatory or a random process, or a combination of both. Either or both possibilities could be related in part to the uterine environment and/or properties intrinsic to sperm.

Sperm encounter different environments and obstacles on their way toward the oocyte. Although uterine fluid (UF)

* Corresponding author. Tel.: +34 868 888009; fax: +34 868 884147.
E-mail address: fagarcia@um.es (F.A. García–Vázquez).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.04.011

in general has been shown to have a deleterious effect on mouse sperm [3], the oviductal fluid has been seen to modulate sperm function [4]. Moreover, other factors such as temperature [5] or bicarbonate concentrations and pH [6,7], which modulate sperm capacitation, are constantly changing within the female genital tract. Besides the fluids and components mentioned previously, the sperm also encounter different cell types. Polymorphonuclear neutrophils are one such type, which invade the uterus lumen after insemination, decreasing the sperm population through phagocytosis [8–10]. Moreover, uterine and oviductal epithelial cells are also involved in the sperm journey to the oocyte. Such cell types act as sperm function modulators, especially if they attach themselves. These cells not only modify the sperm function but also modify oviduct proteome and transcriptome profiles in the presence of sperm [11,12]. Another important factor during the estrus cycle is uterine contractions, which vary greatly during the cycle [13] although they are primarily responsible for sperm transport to the oviduct. Nevertheless, contractions are also involved in sperm loss through the backflow (15%–25%) of deposited semen [14] that takes place for several hours after insemination.

More closely related with sperm themselves, the ejaculate is composed of millions of sperm with different characteristics related to motility, acrosome integrity, morphology, vitality, or morphometry (shape, size). In previous reports, our group reported some degree of selection in the uterus related to sperm morphology and motility [14,15]. Moreover, several researchers have observed interindividual variations in sperm dimensions [16–18], suggesting their basis for competition. However, the significance of such variations is difficult to understand, and it is still unknown what "special" characteristics successful sperm need to fertilize compared with others within the same ejaculate.

Intramale ejaculates have different sperm subpopulations related to dimension and shape [19], although the functional implications of these remain unknown. In this study, we report that sperm of certain dimensions (head and tail) and shape are found in the backflow or within the uterotubal junction (UTJ; for sperm tail length) after insemination. Specifically, the aims of the present study were to examine (1) whether the sperm collected in the backflow after insemination have different morphometry (head and tail) from the insemination dose; (2) whether potential morphometry changes are related to osmolality, acrosome status (morphology and acrosome reaction), and UF; (3) whether the place in which are deposited within the uterus (cervical vs. intrauterine AI) influence sperm morphometry (head and tail) collected in the backflow; (4) whether tail length is different for sperm reaching the UTJ than in those collected in the backflow.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guiding Principles for the Care and Use of Animals (DHEW Publication, National Institutes of Health, 80-23). The protocol was approved by the Ethical Committee for Experimentation with Animals of the University of Murcia, Spain (Project number: AGL2012-40180-C03-01). All surgeries were performed under analgesic and anesthetic protocol [20], and all efforts were made to minimize suffering.

2.2. Sperm collection

A total of seven mature boars (Duroc) with proven fertility were used in this study. Ejaculates were obtained after at least 3 days of abstinence. The method used was the gloved-hand technique, and semen was filtered to remove the gel. Only ejaculates of good quality were used with respect to the criteria followed in this study (sperm-rich fraction volume, $\geq$75 mL; concentration, $\geq$200 $\times$ 10^6 sperm/mL; motility, $\geq$70%; and total abnormalities, $\leq$20%). The samples were stored at 16 °C and used within 24 hours.

2.3. Estrus detection and artificial insemination assays

Multiparous sows (Large White $\times$ Landrace; 3–6 parities) used for breeding were weaned 28 days after farrowing. Estrus detection was performed twice daily by experienced workers by allowing sows nose-to-nose contact with vasectomized mature boars and applying back pressure. The occurrence of estrus was defined by the standing reflex in front of a teaser boar and reddening and swelling of the vulva. Only sows with clear signs of estrus were used for the experiment. Sows were inseminated with a single dose of the diluted semen 24 hours after estrus detection.

AI was carried out by two methods, cervical artificial insemination (CAI) and intrauterine deposition (post-CAI) [14]. Briefly, CAI was performed with disposable multi-ring tip catheters (Import-Vet SA, Barcelona, Spain) and 3 $\times$ 10^9 sperm/dose, in 80 mL of a commercial extender (Zoosperm ND4; Import-Vet SA). Post-CAI was achieved with a combined Catheter Cannula Kit (Soft & Quick, Import-Vet SA), with doses of 1.5 $\times$ 10^9 sperm in 40 mL.

Before insemination, the sow perineal area was carefully cleaned with 0.5% chlorhexidine gluconate wipes (Despro; Import-Vet SA, Barcelona, Spain). In the case of CAI, the insemination dose was introduced gently and slowly in the sow's uterus to minimize the backflow during the procedure. For post-CAI, the insemination dose was introduced quickly (only in a few seconds).

2.4. Backflow collection and analysis

After insemination, the perineal area was thoroughly cleaned and dried. The semen backflow was collected in human colostomy bags (Hollister Ibérica SA, Madrid, Spain; fixed around the vulva and secured with tape) during the 60 minutes after insemination. If a sow urinated into the colostomy bag or if the colostomy bag was damaged, the corresponding value was deleted from the data.

2.4.1. Backflow volume and sperm concentration assay

Volume was measured in a graduated tube, whereas the number of sperm was assessed in triplicate per sample

Download English Version:

https://daneshyari.com/en/article/10891843

Download Persian Version:

https://daneshyari.com/article/10891843

Daneshyari.com