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Boar sperm with defective motility are discriminated in the backflow moments after insemination

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ABSTRACT

During insemination, a large number of spermatozoa are deposited in the female genital tract, but a very low percentage is able to colonize the site of fertilization. The influx of neutrophils into the uterine lumen and semen reflux (backflow) are known mechanisms that decrease the number of spermatozoa within the uterus. No report has attempted to ascertain whether the backflow is a random or selective process of the spermatozoa. In this work, sows were inseminated using two populations of spermatozoa in the same proportion: (1) unstained spermatozoa with high motility and (2) stained spermatozoa with low, medium, or high motility. Volume, number, and percentage of stained spermatozoa were evaluated in the backflow (collected at 0–15, 16–30, and 31–60 minutes after insemination). This article provides evidence that (1) the motility characteristics of the spermatozoa do not influence the percentage of sows with backflow, the volume and number of spermatozoa in the backflow; (2) the discarding of spermatozoa in the backflow is not specific during the first moments after insemination (0–15 minutes), whereas later (16–60 minutes), spermatozoa with defective motility (low and medium groups) are discarded in a higher proportion than high group in the backflow ([16–30 minutes: low, 85.13 ± 4.32%; medium, 72.99 ± 5.05%; and high, 54.91 ± 2.38%; $P < 0.0001$; 31–60 minutes: low, 87.16 ± 6.01%; medium, 87.02 ± 4.01%; and high, 59.35 ± 2.86%; $P = 0.001$]). Spermatozoa with poor motility are discarded in the backflow probably as a selective process, on the part of the female genital tract or as a result of the intrinsic low spermatozoa motility.

1. Introduction

When the spermatozoa are deposited in the female tract either as a result of natural or artificial insemination (AI), they have to travel a long way until they reach the ampullar-isthmic junction [1]. The process of sperm transport through the uterus and oviduct is complex and involves dynamic interactions between spermatozoa and the female genital tract. Sperm transport to the site of fertilization is thought to be a combination of both passive and active transport. The spermatozoa encounter different environments within the female reproductive tract. The initial steps of sperm transport mediated by the female tract (passive transport) seem to be most important in the transport from the site of deposition to the proximal uterus and the uterotubal junction (UTJ) [2]. The passive part of

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sperm transport is probably because of the flow of fluid (i.e., extender, seminal plasma, or uterine fluid) and contractile movement of the uterine horns during the estrous period [3]. Active sperm transport resulting from the intrinsic movement of sperm cells seems to be important for their migration from the proximal uterus to the UTJ and the oviduct [4,5], although uterine contractions may also be involved [3]. Several studies have indicated that to reach the oviductal environment, spermatozoa have to be intact and endowed with appropriate motility [6–9]. Whatever the case, sperm transport is rapid, and within minutes of insemination, spermatozoa are found in the oviducts [1,10].

During porcine insemination, a large number of sperm are deposited in the female tract but only a few 1000 reach the oviduct. For e.g., if a sow is cervical inseminated with 3000×10^6 spermatozoa (100% of deposited spermatozoa), about 142×10^3 will reach the UTJ and 1.5×10^3 the sperm reservoir (SR) in the caudal part of the isthmus [11], which represents 0.0047% and 0.00005%, respectively, of the total spermatozoa deposited.

The fact that most of the spermatozoa are lost during their passage through the female tract suggests that the uterus has certain mechanisms to discard and/or select spermatozoa. Two of the mechanisms known to be involved in spermatozoa losses are the influx of leukocytes from blood to the lumen of the uterus and backflow. Leukocytes are mainly polymorphonuclear neutrophils (PMNs) and the result of their influx into the uterine lumen is that the spermatozoa are phagocytized [12–14]. It has been hypothesized that PMNs take part in sperm cell selection, removing superfluous, nonmotile, or damaged spermatozoa [15]. Whether sperm cell phagocytosis is a selective or random process is still open to question [12]. Another known mechanism that provokes a reduction in sperm population in the female tract is backflow, which considerably reduces the volume and number of spermatozoa within 60 minutes of cervical AI (CAI; 54% and 25%, respectively) and post-cervical AI (post-CAI; 39% and 15%, respectively) [16]. Although there is some evidence that the spermatozoa in the backflow have been submitted to a selective process [16], further studies need to be performed to clarify how the spermatozoa are selected in the uterus on their way to the oviduct.

The presence of different subpopulations in mammalian ejaculate (reviewed by Holt and Van Look [17]) has a functional implication although the exact role is still unknown. There is a lack of evidence concerning the effect that nonfunctional sperm (in terms of motility) will have on their selection in the uterus immediately after insemination. The hypothesis is that nonmotile or low-motile sperm are selected after sperm deposition and then discarded through the backflow. This hypothesis is based on our previous finding that spermatozoa of lower quality were found in the backflow in comparison with those in the original dose [16].

The present study evaluates the influence of different levels of sperm motility in the insemination doses on the volume, number, and type of spermatozoa (based on their motility characteristics) collected in the backflow at different times. The porcine industry is developing new biotechnologies such as sex-sorting sperm and freezing thawing or sperm-mediated gene transfer that require significantly reduced sperm doses for AI. For these new procedures to be successful, new approaches, such as the use of post-CAI, are necessary [16,18–22] as this will decrease the number of spermatozoa used per dose. Besides, the quality of sperm obtained by sex sorting or freezing thawing is compromised [23], so it is important to know how the uterus selects sperm when the number of quality cells deposited is reduced.

2. Materials and methods

2.1. Ethics statement

The experimental procedures for the use of animals were approved by the Ethical Committee of the University of Murcia.

2.2. Experimental design

Nine ejaculates from seven boars (Duroc) of proven fertility and 45 multiparous sows (Landrace × Large White) were used in this study. The females were inseminated using a total of 1500×10^6 spermatozoa in 25 mL. Every insemination sperm dose was composed of two parts from the same ejaculate: (1) 750×10^6 unstained sperm in 12.5 mL with high motility (>70% motility) and (2) 750×10^6 spermatozoa in 12.5 mL previously stained with Hoechst and with different levels of motility (low, medium, or high). The average of the different sperm motility populations used for this study was as follows: low, 7.50 ± 4.33%; medium, 42.50 ± 12.50%; and high, 75.00 ± 2.88% of motility (%). The spermatozoa were stained to clearly and objectively identify the sperm population collected in the backflow because their motility characteristics can change between insemination and collection.

Two different populations were used: unstained sperm of high motility mixed just before post-CAI with stained sperm of low, medium, or high motility. An aliquot of 500 μL of the dose after mixing and before insemination was kept to count the percentage of sperm stained (approximately 50%) as a control. The backflow was collected in each individual sow at different times (0–15, 16–30, and 31–60 minutes) from the beginning of insemination. Once in the laboratory (within 1 hour of collecting the backflow), the volume (% of the dosage), concentration of spermatozoa (% of the dosage), and number of stained sperm (%) collected in the backflow were evaluated.

2.3. Sperm collection

Semen was obtained from each boar once a week using the gloved-hand technique and filtered to remove the gel. The number of spermatozoa in the ejaculates was counted in a hemocytometer (Neubauer counting chamber; VWR International, Haasrode, Belgium). Only ejaculates with a rich fraction volume, 75 mL or greater; concentration, 200×10^6 spermatozoa/mL or greater; motility, 70% or greater; and total abnormalities, 20% or lesser were used in this study. Immediately after evaluation, and before