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Sperm treatment affects capacitation parameters and penetration ability of ejaculated and epididymal boar spermatozoa

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Abstract

This work was designed to study how this ability is affected by different sperm treatments routinely used for *in vitro* fertilization (IVF) assay. In this study, boar sperm samples from epididymal or ejaculated origin were processed by three different methods: left unwashed (NW group), washed in Dulbecco's phosphate-buffered saline supplemented with 0.1% BSA (BSA group), and washed on a Percoll® gradient (PERCOLL group). After preparation of semen samples, changes in motility patterns were studied by CASA, calcium uptake by spectrofluorimetry, and ROS generation, spontaneous acrosome reaction, and lipid disorder by means of flow cytometry. Finally IVF assays were also performed with the different semen samples and penetrability results evaluated at 2 and 4 h post insemination (hpi). Independently of the sperm treatment, epididymal spermatozoa showed higher values of progressive motility, percentage of live cells with low lipid disorder, and penetration ability at 4 hpi than the corresponding ejaculated spermatozoa. Ejaculated spermatozoa showed higher levels of calcium uptake, ROS generation and percentage of spontaneous acrosome reaction than epididymal sperm. Regarding sperm treatments, PERCOLL group showed the highest values for some motility parameters (linearity of the curvilinear trajectory, straightness, and average path velocity/curvilinear velocity), ROS generation and penetration ability at 2 and 4 hpi; however this same group showed the lowest values for sperm curvilinear velocity and lateral head displacement. From all experimental groups, ejaculated-PERCOLL-treated spermatozoa showed the highest fertilization ability after 2 hpi. Results suggest that capacitation pathways can be regulated by suitable treatments making the ejaculated sperm able to reach capacitation and fertilize oocytes in similar levels than epididymal spermatozoa, although most of the studied capacitation-associated changes do not correlate with this ability.

Keywords: Ejaculated spermatozoa; Epididymal spermatozoa; Capacitation; *In vitro* penetration

1. Introduction

Capacitation is a vital phenomenon that a spermatozoon must undergo before it can fertilize an oocyte. It is a lengthy process in which early changes take place as rapidly as 1 min whereas full capacitation is accomplished within hours (depending on the mammalian species of interest). Capacitation confers upon the spermatozoon an ability to gain hyperactive motility, interact with oocyte zona pellucida (ZP), undergo acrosome reaction (AR), and initiate oocyte plasma membrane fusion [1]. Whereas this concept can still be considered valid, many studies have been carried out trying to characterize the specific changes in the sperm cell through the capacitation process. Among them, motility patterns [2–6], alterations in the sperm plasma membrane architecture [7–9], ROS generation [10], calcium uptake [11–13], or spontaneous AR [14] have been

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proposed as indicators of capacitation. However, none of the designed assays discriminate non-responding from responding cells at different time intervals of the specific process [15] and their relation to full ability for further oocyte penetration is unknown.

In vitro, sperm capacitation can readily be accomplished when provided culture conditions facilitate and support membrane changes and signal transduction pathway activation similar to those occurring *in vivo*. We have previously demonstrated that the methods of processing pig spermatozoa further affect different *in vitro* fertilization (IVF) parameters [16]. However, there is no detailed characterization of changes that sperm suffer after these different sperm treatments. Additionally, a wide difference exists among boar ejaculates and use of epididymal spermatozoa has become a more consistent option for pig IVF [17]. It has been reported that, *in vitro*, epididymal spermatozoa can reach capacitation and fertilize oocytes much easier than ejaculated spermatozoa [1], but this ability could be affected by capacitation treatments. *In vitro*, boar spermatozoa pre-incubated with seminal plasma (SP) display a lower fertilizing ability and it has been suggested that SP has sperm-coating components that firmly stick to the sperm surface inhibiting their fertilizing ability [18]. During epididymal maturation and ejaculation many glycoproteins and peptides secreted by the accessory glands bind to the sperm surface with varying affinities [19,20]; therefore, it is anticipated that they will have a significant effect on membrane function (glycodelines, [21], AWN-1, [22]). Equally, their differential removal during washing procedure in artificial media as a prelude to IVF may reverse SP or epididymal secretion effects, influencing capacitation and shortening sperm survival. Thus, we hypothesize that ejaculated spermatozoa are able to reach a capacitation status and to fertilize oocytes in similar levels than the epididymal spermatozoa as far as the treatments to remove seminal plasma can induce the suitable membrane alterations and signal transduction pathways necessary to bind and penetrate the oocytes. Additionally, we propose that some of the so-called "capacitation-associated changes", such as calcium increase, lipid disorder, and ROS production, might not correlate with *in vitro* fertilization ability thus not being useful to predict the penetration ability of a sperm sample.

2. Materials and methods

All chemicals were obtained from Sigma-Aldrich Química, S.A. (Madrid, Spain) unless otherwise indicated.

2.1. Sperm collection and handling

2.1.1. Ejaculated spermatozoa

Semen was routinely collected from mature fertile boars using the manual method. The sperm-rich fraction was collected in a pre-warmed thermo flask and the gel-fraction was held on a gauze tissue covering the thermo opening. The semen was then extended 1:2 with isothermal Beltsville Thawing Solution (BTS, [23]) and sperm concentration, motility, acrosome integrity, and normal morphology were microscopically evaluated by standard laboratory techniques. BTS consisted of 37.0 g glucose, 1.25 g EDTA, 6.0 g sodium citrate, 1.25 g sodium bicarbonate, and 0.75 g potassium chloride in 1 L distilled water with a final pH of 7.2. After dilution in the extender, a pool of semen from 4 different boars was used for all the experiments to avoid individual boar effect on the results.

2.1.2. Epididymal spermatozoa

Within 30 min of slaughter, porcine cauda epididymal sperm were collected by perfusion of the *ductus epididymidis* by washing with 2 mL BTS. These spermatozoa were evaluated as the ejaculated ones and used under the same criteria. A pool of samples from epididymis from four different boars was used for all the experiments.

2.2. Preparation of spermatozoa

The spermatozoa (ejaculated and epididymal) samples were: i) left unwashed (NW group), ii) washed in Dulbecco's phosphate-buffered saline (DPBS) supplemented with 0.1% BSA (BSA group), or iii) washed on a Percoll® (Pharmacia, Uppsala) gradient (Percoll® group). After treatment, sperm samples were diluted in TALP medium [24] consisting of 114.06 mM NaCl, 3.2 mM KCl, 8 mM calcium lactate•$5H_2O$, 0.5 mM $MgCl_2$•$6H_2O$, 0.35 mM NaH_2PO_4, 25.07 mM $NaHCO_3$, 10 mL sodium lactate, 1.1 mM Na pyruvate, 5 mM glucose, 2 mM caffeine, 3 mg BSA mL (fraction V, A-9647), 1 mg/mL PVA and 0.17 mM kanamycin sulphate, previously pre-equilibrated overnight at 38.5 °C in 5% CO_2 in 100% humidified air and with a final pH of 7.4.

Preparation of sperm samples for the three sperm treatments was as follows: for the NW-group, the semen samples (both epididymal and ejaculated) were diluted in TALP medium and sperm concentration adjusted according to experiment. The sperm concentration was assessed by a photometer (Spermacue, Minitüb, Germany). For BSA-group, samples (epididymal and ejaculated) were washed with DPBS supplemented with 0.1% BSA (fraction V, A-9647) by centrifugation (900 × *g*, 10 min, three