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The time-dependent effects of prostate granules and seminal plasma on the capacitation, acrosome reaction, and motility of rabbit sperm

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ABSTRACT

The present study investigated the role of rabbit seminal plasma and prostate granules on capacitation, the acrosomal reaction (AR), and sperm motility. Semen ejaculates obtained from five mature New Zealand White rabbit bucks in three collection series were used in the study. Raw semen, Percoll-selected sperm alone and in presence of either seminal plasma or prostate granules were incubated for 120 min in capacitation medium. Chlortetracycline fluorescence (reflecting capacitation and the acrosome reaction) and sperm kinetic traits were analysed in each sample. All traits were observed from 0 to 120 min of incubation at 37 °C in 5% CO_2. Both seminal plasma and prostatic granules markedly improved the sperm motility but seminal plasma mainly inhibited the capacitation progress whereas prostatic granules inhibited the AR. In conclusion, prostatic granules and seminal plasma have specific roles in synchronising sperm capacitation and the AR with egg availability.

1. Introduction

Seminal plasma (SP) is a protective and nutritive medium for sperm cells. Some components of the SP are crucial for metabolism, function, survival and sperm transport in the female reproductive tract (Maxwell and Johnson, 1999). SP role is species dependent and it can stimulate sperm function in several animal species (ram: Ashworth et al., 1994; rabbit: Castellini et al., 2000; boar: Vadnais and Roberts, 2007), but it does not appear to have this effect on bull spermatozoa (Leahy and de Graaf, 2012). It also acts as a protective medium during *in vitro* processing (Maxwell et al., 1996; Maxwell and Johnson, 1999). In particular, the SP modulates some crucial steps such as sperm capacitation and the acrosomal reaction (AR). Indeed, in various animal species (e.g., ram, bull, boar, and rabbit) the SP contains molecules, termed decapacitation factors, that prevent or reverse spermatozoa capacitation (Chang, 1957; Okabe et al., 1993).

The seminal fluid also contains a particulate fraction that affects reproductive physiology in mammalian species (Stegmayr and Ronquist, 1982; Kravets et al., 2000). These particles are released by different organs in different species: the epididymis in humans, rats and sheep, the vesicular glands in cattle and the prostate in humans, horses and rabbits (Saez et al., 2003).

Rabbit semen contains a huge population of granules, which are mainly produced by the anterior part of the prostate (Castellini et al., 2006a,b, 2012). These granules have been proposed to synchronise sperm and oocyte availability, which is highly important for species in which ovulation is induced by mating. Rabbit prostate granules (PG) have large amounts of cholesterol and desmosterol (Mourvaki et al., 2010a), which protect spermatozoa

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against environmental shock and premature acrosome reaction (Zarintash and Cross, 1996; Serin et al., 2011). Further, these PG contain approximately 40% of the vitamin E in the semen (Mourvaki et al., 2008), which functions to protect sperm from free radicals (Kamal-Eldin and Appelqvist, 1996) and contributes to the modulation of spermatozoa capacitation and the subsequent AR (O'Flaherty et al., 2006).

Capacitation and the AR are highly complex mechanisms, and several hypotheses about the specific/synergic role of SP and PG components on responsiveness of sperm to capacitation factors and the AR (Lee et al., 1985; Cross and Mahasreshti, 1997; Pons-Rejraji et al., 2011; Breitbart et al., 1997) have been advanced, but no definite conclusions have been provided to date.

The aim of this study was to investigate *in vitro* the time-dependent effects of the SP and PG on rabbit sperm traits, with a special focus on capacitation and the AR.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

2.2. Animals and semen collection

Semen ejaculates ($n = 15$) were obtained from five mature New Zealand White rabbit bucks in three collection series using an artificial vagina. All animals used in this study werehoused at the experimental farm of the Department of Applied Biology of Perugia University. Specific guidelines for rabbit bucks (Boiti et al., 2005) and the International Guiding Principles for Biomedical Research Involving Animals have been applied.

The baseline characteristic (mean ± standard deviation) of raw semen was the following: volume 0.55 ± 0.08 mL and sperm concentration $415 \pm 32 \times 10^6$/mL; other sperm characteristics were reported in Table 1.

Immediately after collection, semen samples were pooled and diluted 1:10 in 50 mM Tris–HCl buffer (pH 7.0) and transported to the laboratory.

Table 1
Comparison of main kinetic parameters of rabbit sperm after different treatments at the beginning of incubation.

Treatment	Motile cells (%)	VCL (μm/s)	LIN (%)	ALH (μm)
Raw semen	83.5	209.7	35.5	2.95
Percoll-selected sperm	73.1	185.6	33.7	2.00
Percoll-selected sperm + PG	78.5	198.0	33.7	3.00
Percoll-selected sperm + SP	78.2	195.9	34.8	2.83
Percoll-selected sperm + SP + PG	82.0	210.8	33.4	2.87
Pooled SE	8.5	31.5	10.1	0.25

$n = 15$; VCL, curvilinear velocity; LIN, linearity (defined as the percentage of VSL/VCL ratio; VSL being the straight linear velocity); ALH, amplitude of lateral head displacement.

2.3. Experimental design

Four different experimental groups were compared: Percoll-selected sperm (without PG and SP); Percoll-selected sperm plus PG; Percoll-selected sperm plus SP; and Percoll-selected sperm plus PG and SP.

Sperm from raw semen were also used as a positive control.

The seminal plasma was separated by centrifugation at 700 *g* for 15 min the pellet was re-suspended in 1 mL Tyrode's albumin lactate pyruvate (TALP) and sperm were obtained by centrifugation at 500 *g* for 10 min in two-layer Percoll® gradients (Mourvaki et al., 2010a). PG were obtained from semen of vasectomised bucks of the same genotype and age to ensure the non-testicular origin of the PG with the centrifugation procedure reported by Castellini et al. (2006a,b).

Percoll-selected sperm were re-suspended in a suitable volume of a capacitation medium (TALP) with bovine serum albumin (BSA) alone, or containing respectively, SP, PG, or SP plus PG. Prostate granules were added back to the sperm at 800 μg protein/mL (Mourvaki et al., 2010b). Seminal plasma was re-added to Percoll-selected sperm at a final concentration of 20% (v/v) (Vadnais and Roberts, 2007).

All the samples were incubated at 37 °C in 5% CO_2 for 120 min, and data were recorded every 15 min. The kinetic traits and chlortetracycline (CTC) patterns were analysed in each semen sample.

2.4. Evaluation of sperm kinetic traits

Sperm kinetic characteristics were analysed using a computer-assisted sperm analyser (CASA, model ISAS, Valencia, Spain) in pooled semen samples that had been diluted 1:30 in TALP buffer. This system consisted of a negative phase contrast optics system (Olympus CH-2) equipped with a CCD Sony camera. The set-up parameters of the CASA were established previously (Castellini et al., 2011), and the acquisition rate was set at 100 Hz. For each sample, two drops and three microscopic fields were analysed (approximately 500 spermatozoa). The main sperm motion parameters acquired were motility rate (%), curvilinear velocity (VCL, μm/s), linearity (LIN, %) and amplitude of lateral head displacement (ALH, μm).

2.5. CTC fluorescence assay

The CTC fluorescence assay was performed as described by Kaul et al. (2001). The CTC solution was made by dissolving 750 μMCTC-HCl in a buffer containing 20 mM Tris–HCl, 130 mM NaCl, and 5 mM cysteine-HCl pH 7.0. A sperm suspension (100 μL) was put into a 1.5 mL foil-wrapped Eppendorf tube to which 100 μL CTC stock was added. The cells were immediately fixed by adding 8 μL of 12.5% paraformaldehyde to 0.5 mM Tris–HCl buffer (pH 7.5). Slides were prepared by combining 10 μL of the fixed solution with one drop of 0.22 1, 4-diazabicyclo[2.2.2]octane dissolved in glycerol/PBS to retard the fading of fluorescence. A coverslip was placed on top of the slide, and the sperm cells were gently compressed to allow excess fluid, if any, to be removed.