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Computer-assisted analysis of sperm motion in goats and its relationship with sperm migration in cervical mucus

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Abstract

In vitro sperm migration in cervical mucus relates to sperm concentration at the utero-tubal junction and to in vivo fertilization performance in goats. The present study aimed to characterize, using Computer-Assisted Sperm Analysis (CASA), motility patterns depicted by buck sperm and their relation to the migration efficiency in homologous (goat) and heterologous (heifer) cervical mucus in vitro. Semen was collected from 23 sexually mature bucks from three breeds by artificial vagina and sperm were assessed for motility parameters with a Hobson Sperm analyzer following extension in Sperm Analysis Medium (SAM). To study the relationship between kinematics parameters and the ability of sperm to migrate in cervical mucus, in a first experiment, motility performance of buck sperm suspended in SAM was compared against seminal plasma. In a second experiment, kinematics parameters of sperm were characterized. In a third experiment, bucks with sperm that differed in specific motion parameters were compared for the ability of their sperm to migrate through goat and bovine cervical mucus collected at estrus. In a fourth experiment, ejaculates that were compared in their migration ability and were assessed simultaneously for their motility parameters. Overall, sperm suspended in SAM medium had better velocity and similar linearity and lateral head displacement than those suspended in seminal plasma; furthermore, caprine sperm swam relatively fast (relative to bovine and ovine sperm), following a very linear trajectory. Under the conditions used, velocity parameters, linearity and lateral head displacement seemed to be related to sperm migration efficiency in homologous mucus but not in bovine cervical mucus.

Keywords: CASA; Sperm migration; Sperm motion; Cervical mucus; Caprine

1. Introduction

The prospective analysis of sperm-fertilizing ability has clinical and technological interest. In spite of initially focusing on the development of single tests to measure global prospective fertility, there was subsequently a shift to look for the minimal number of tests that measure reliably the most critical aspects of sperm function engaged in fertilization and development [1–3].

The ability of sperm to migrate through the female genital tract and penetrate the oocyte vestments depends upon the hydrodynamic potential conferred by the flagellar bending and the resistance exerted by the secretions present in the lumina of the genital tract [4]. The potential for sperm migration can be measured in vitro by testing sperm migration through estrous cervical mucus. The cervical mucus is a hydro gel, conformed by an insoluble fraction of aggregates of mucin surrounded by a soluble fraction more or less

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hydrated (silates), depending upon the stage of the ovarian cycle [5,6]. The large amount of mucus secreted by the cervical epithelium during the follicular phase combined with the abdominal pressure and the contractility of the genital tract organizes the mucus, in humans and cattle, as a three-dimensional fibrillar meshwork that allow the movement of sperm through inter-fibrillar spaces of low resistance [5–7]. This structure facilitates the orientation of sperm to the upper genital tract, as suggested earlier [8], and can be mimicked in vitro by human and bovine cervical mucus aspirated into capillary tubes [7]. Sperm migration in goat and bovine cervical mucus has been related to sperm concentration at the sperm reservoir (the utero-tubal junction) [9] and to in vivo fertilization performance in goat [9], cattle [10] and human [11,12].

It seems that the different migration efficiency seen in sperm of some males is based on kinematics properties that define their propulsive strength [13]. Computer-Assisted Sperm Analysis (CASA) has demonstrated to be a useful tool to assess kinematics properties of individual spermatozoa in an ejaculate (reviewed in ref. [14]). Provided semen manipulation procedures and analysis settings are standardized, image processors are able to identify, objectively and reproducibly, a number of algorithms associated to the sperm path, some of them well correlated to fertility [15–17].

There is very little information on motility analysis of goat sperm by CASA procedures. Furthermore, no attempts have yet been done trying to associate quality of sperm movement with sperm transport in the female genital tract. Since the fibrillar pattern of mucus aspirated into capillary tubes was similar to that seen in cervical mucus [7] and sperm migration through cervical mucus was related to the colonization of the sperm reservoir in the oviduct [9], the present study aimed to characterize the parameters of sperm motion in bucks by CASA analysis and their relation to the efficiency of sperm migration through homologous (caprine) or heterologous (bovine) cervical mucus in vitro.

2. Materials and methods

2.1. Experimental animals

The study was carried out during the breeding season. All goats used in the study ($n = 170$) were clinically and gynecologically sound. Sexually mature bucks ($n = 35$) were aged 1–3 years, of Saanen, British Alpine and Boer breeds and they were clinically and andrologically sound based on genital morphology and semen analysis. Teasers ($n = 2$) used for collection of semen were adult goats that received intravaginal devices of progesterone for 7 days (CIDR, InterAg, Hamilton, New Zealand) followed by weekly administration of 1 mg of estradiol cypionate i.m. (ECP, Intervet, Boxmeer, Holland) to maintain estrous behavior. During daytime, bucks and goats were maintained in different paddocks, separated visually and by night they were housed in pairs (bucks) and in groups (female goats) considering individual requirements for spacing, ventilation and free access to fresh water.

Crossbred beef heifers ($n = 5$), 2 years old, were maintained on pasture with free access to fresh water. They received supplements of vitamins and minerals and were clinically and gynecologically healthy during the experimental period.

2.2. Estrus synchronization and estrous detection

Estrus synchronization in female goats was performed by exogenous hormonal treatment. Following the hygienic introduction of a progesterone/release intravaginal device (CIDR) for 7 days, single doses of $PGF_{2\alpha}$ analogue (Tiaprost, 0.15 mg i.m.; Intervet, Wiesbaden, Germany) and eCG (300 IU i.m.; Folligon, Intervet, Boxmeer, Holland) were administered 24 h before the retrieval of the intravaginal device. Heifers were synchronized by a single administration of Tiaprost (0.75 mg i.m.) during the luteal phase, based on an ultrasound examination.

Estrus detection in goats was performed twice daily by direct observation of sexual behavior with the help of androgenized females. In heifers, estrous detection was solely done by the observation of mounting behavior between females. In either case, a female was considered to be in estrus when it accepted to be mounted.

2.3. Collection and handling of buck semen

Buck semen was collected with an artificial vagina using estrogenized teasers. The collected semen samples were extended 1:1 to 1:3 with Sperm Analysis Medium (SAM) using disposable 5-mL test tubes (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) and maintained at 30 °C until sperm concentration was determined by manual counting with a Neubauer haemocytometer. The composition of the SAM medium was: 2.65 mM calcium chloride; 0.49 mM magnesium chloride; 102.74 mM sodium chloride; 2.00 mM potassium chloride; 5.00 mM sodium bicarbonate; 0.28 mM sodium phosphate; 19.97 mM Hepes; 26.0 mM DL-lactic acid (60%) sodium salt; 5.55 mM glucose; 8.75 mM sucrose; 1.00 mg/mL polyvinyl alcohol; 1.00 mg/mL bovine serum albumin;