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Human sperm volume regulation. Response to physiological changes in osmolality, channel blockers and potential sperm osmolytes

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BACKGROUND: Volume regulation is an important sperm function because defective sperm cannot negotiate the female tract in an infertile mouse model and swollen human sperm cannot penetrate and migrate through mucus. METHODS AND RESULTS: The size of sperm from 52 donor ejaculates incubated in medium of female tract fluid osmolality (BWW290) was measured by flow cytometry to be identical to that in homologous semen osmolality (289–351 mosmol/kg), indicating effective volume regulation. Inhibition of anticipated regulatory volume decrease in BWW290 by the channel blocker quinine induced size increases and associated kinematic changes measured by computer-aided sperm analysis. Incubation in L-carnitine, *myo*-inositol and taurine did not change sperm volume or kinematics, but the presence of glutamate and K^+ decreased the efficiency of forward progression indicative of volume increase, suggesting them as potential osmolytes for human sperm. Linear regression suggested correlations of changes in cell volume and in kinematic parameters, and the association of faster forward progressive sperm with smaller cell size. CONCLUSIONS: Sperm volume and its regulation may be crucial to natural fertility. The identification of sperm osmolytes, ion channels and mechanisms involved would contribute to the understanding of male infertility and offer a lead for male contraception.

Key words: organic osmolytes/quinine/regulatory volume decrease/sperm function/sperm motility

Introduction

Mammalian sperm produced in the testis undergo maturation in the epididymis and are stored there until ejaculation (Cooper, 1998). This holds true for man despite claims to the contrary (see Cooper, 1990, 2002; Cooper and Yeung, 2000). In laboratory animals, the epididymis provides sperm with a unique milieu of high osmolality. Upon ejaculation into the female tract, sperm are subjected to a hypo-osmotic challenge that necessitates regulation of cell volume (see Cooper and Yeung, 2003). Defects in sperm volume regulation have recently been identified as the cause of infertility by natural mating in a transgenic mouse model, due to failure in sperm transport into the oviduct (Yeung *et al*., 2000), despite reproductive success with IVF (Sonnenberg-Riethmacher *et al*., 1996). Osmotic adjustment upon ejaculation would also be required of human sperm in the female tract, since there are changes in osmolality from ~340 mosmol/kg in the vas deferens (Hinton *et al*., 1981) to 280–290 mosmol/kg in the female tract environment. Inhibition of cell volume regulation, by in-vitro incubation of ejaculated sperm with the ion channel blocker quinine, leads to failure in the penetration and migration through surrogate mucus (Yeung and Cooper, 2001). Hence, human sperm volume regulation is an important factor in male fertility hitherto not well understood.

Epididymal fluid is characterized by progressive decreases in Na^+ and increases in K^+ concentrations along the duct. Osmolality is made up by high amounts of small organic molecules including amino acids such as glutamate and taurine, carnitine, glycerophosphocholine (GPC) and *myo*-inositol (see Cooper and Yeung, 2003). These organic molecules, as well as K^+, are commonly used by somatic cells as osmolytes for volume regulation (see Lang *et al*., 1998; Fürst *et al*., 2002). The increase in osmolality from testicular to epididymal fluid should induce uptake of osmolytes by epididymal sperm to counteract cell shrinkage. These compounds could then be utilized by sperm upon ejaculation into a relatively hypotonic environment in the female tract, by mechanisms of regulatory volume decrease (RVD) resulting in efflux of osmolytes and cellular water (Cooper and Yeung, 2003). In our preliminary study of murine sperm, it was revealed that the presence of such molecules in the incubation medium, with the exception of GPC, could prevent sperm RVD, thus supporting the hypothesis that these are sperm osmolytes. In the present study, these putative osmolytes were tested on human ejaculated sperm.

When challenged with physiological changes in osmolality, murine epididymal sperm swell considerably upon inhibition of RVD with induction of tail angulation (Yeung *et al*., 1999,

Table I. Semen parameters of the ejaculates studied (n = 52)

	Mean	Range	Lower–upper quartile
Ejaculate volume (ml)	3.5	1.5–8.3	2.9–4.3
Sperm concentration (10^6/ml)	61	9–238	33–80
Normal morphology (%)	22	12–37	18–26
Motility (%)	60	45–76	57–64
Grade a motility (%)	33	13–50	30–39
Grade b motility (%)	18	5–30	14–22
Semen osmolality (mosmol/kg)	318	289–351	311–329

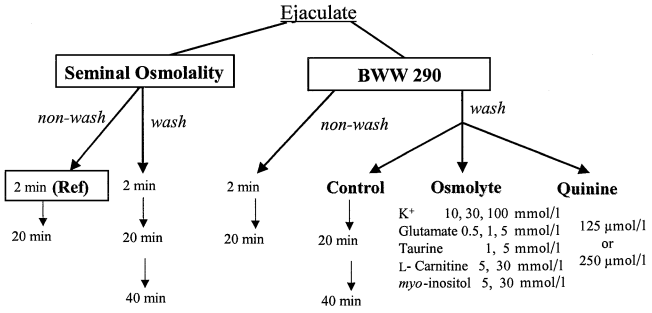

Figure 1. Flow chart of sperm preparation and incubation (see Materials and methods for details). Sperm were analysed with a flow cytometer for viability and laser forward scatter (FS) at all the time-points indicated. All washed and incubated samples were analysed for percentage motility and kinematic parameters at 20 min. (Ref) = FS reference value used in Figure 2.

2002a). However, human ejaculated sperm increase in cell volume only to a minimal extent with little morphological changes in the tail (Yeung and Cooper, 2001). This contrasts with the conventional hypo-osmotic swelling (HOS) test for viability where sperm are subjected to a non-physiological osmolality of 150 mosmol/kg (Jeyendran *et al*., 1992). In the HOS test, sperm with intact plasma membranes form coils in the tails because of the rapid and drastic swelling, and lose their motility within 2 min (Hossain *et al*., 1998). On the other hand, human sperm swollen in physiological osmolalities display greater changes in kinematic parameters than in cell volume (Yeung and Cooper, 2001). Such kinematic changes can be detected with more sensitivity using computer-aided sperm analysis (CASA) than changes in cell volume measured by flow cytometry, which are readily detectable in the mouse (Yeung *et al*., 2002a). Therefore, both cell volume and kinematics were used as end-points in the present study in the search for putative human sperm osmolytes.

Materials and methods

Source of ejaculates and semen analysis

Fifty-two ejaculates were obtained at the Institute of Reproductive Medicine from healthy men volunteering for a study for hormonal male contraception, which was approved by the Ethics Committee of the University and the State Medical Board, Münster, Germany. The participants gave written informed consent. They were between 18 and 45 years old and did not suffer from chronic illnesses or infections of the urogenital tract and took no medications of any kind. Only prior-to-treatment samples were used for the present study. Semen samples were obtained by masturbation after ⩾2 days of abstinence. After liquefaction at 37°C for 30 min, routine semen analysis was carried out according to the protocol of the World Health Organization (1999). Osmolality of the semen sample was measured after liquefaction using a vapour pressure osmometer (Wescor Vapro 5520; Schlag GmbH, Germany) that does not require separation of cells and particles before measurement. Characteristics of the ejaculates are given in Table I.

Medium

Each ejaculate was treated with modified Biggers–Whitten–Whittingham (BWW) medium (Biggers *et al*., 1971) containing bovine serum albumin at 4 mg/ml, with osmolality adjusted to 290 mosmol/kg (that of cervical mucus and uterine fluid; BWW290) or that of homologous semen (BWWsemen). BWWsemen was prepared by mixing appropriate volumes of two BWW solutions with osmolality of 270 and 480 mosmol/kg to achieve the required seminal osmolality. Various BWW290 solutions, each containing a test osmolyte (see Figure 1 for substances and concentrations), were made freshly each day by adding a small volume of the stock solution and omitting the equivalent amount of NaCl, and the final osmolality was checked. Osmolyte stock solutions containing test substances that had an acidic pH (glutamate and taurine) were adjusted to pH 7.0 before use. All BWW solutions contained phenol red as a pH indicator, and were equilibrated in an incubator at 37°C with 5 % (v/v) CO_2 in air before use.

Sperm preparation and incubation

As indicated in the flow chart of the protocol (Figure 1), 10 µl aliquots of the freshly liquefied ejaculate were dispersed in 250 µl BWWsemen and BWW290 and analysed with the flow cytometer (2 min time-point) after addition of 3 µl propidium iodide (PI, 500 µg/ml phosphate-buffered saline) to stain non-viable cells, as described below. Another dispersed aliquot was incubated at 37°C with 5% v/v CO_2 in air and analysed after 20 min. Aliquots of the ejaculate were also washed (150 µl ejaculate in 1 ml medium, centrifuged at 450 *g* for 5 min at room temperature) with BWWsemen, BWW290 control medium or BWW290 containing quinine or a test osmolyte listed in Figure 1. The sperm pellet was resuspended in 100 µl fresh solution of the wash medium and incubated. For volume measurement with the flow cytometer at 20 and 40 min of incubation, 15 µl sperm suspension was diluted into 250 µl of the same medium containing 3 µl PI as above.

Evaluation of sperm volume by flow cytometry

The laser forward scatter signal of viable sperm as a reflection of sperm cell volume (Yeung *et al*., 2002a) was analysed by a flow cytometer (Coulter Epics XL, version 3.0; Germany) as described previously (Yeung and Cooper, 2001). The same flow cytometer