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Pig sperm preincubation and gamete coincubation with glutamate enhance sperm-oocyte binding and in vitro fertilization

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ABSTRACT

As the taste receptor for monosodium glutamate (umami) is expressed in both murine and human spermatozoa and the presence of α-gustducin and α-transducin, G proteins involved in the umami taste signaling, has been described in boar germ cells, the aim of this study was to evaluate if monosodium glutamate (MSG) would exert any effect on sperm-oocyte binding, in vitro fertilization (IVF) and sperm parameters during in vitro induced capacitation.

For sperm-zona pellucida binding assay, boar spermatozoa were preincubated for 1 h and then coincubated for 1 h with denuded in vitro matured oocytes in presence of different concentrations of MSG (0, 0.1, 1, 10 mM). MSG 1 and 10 mM significantly ($P < 0.05$) increased the mean number of sperm bound to ZP compared with control (12.3 ± 9.0, 17.8 ± 11.3, 17.6 ± 10.8, MSG 0, 1 and 10 mM respectively).

For in vitro fertilization trials, both sperm preicubation (1 h) and gamete coincubation (1 h) were performed in presence of different concentrations of MSG (0, 0.1, 1, 10 mM). After 19 h of culture in fresh IVF medium, oocytes were fixed. MSG 1 mM significantly ($P < 0.05$) increased the penetration rate compared with control (53.7 ± 20.4 vs. 36.8 ± 16.2).

The addition of MSG during in vitro induced capacitation of boar spermatozoa did not cause any significant difference, compared with control, on the percentage of viable cells, spermatozoa with intact acrosome and the percentage of spermatozoa displaying tyrosine-phosphorylation of sperm tail proteins.

In order to evaluate whether the effect elicited by MSG could be due to glutamate uptake in boar spermatozoa, fertilization trials were performed in presence of either 1 mM MSG or 1 mM MSG + 100 µM DL-*threo*-beta-hydroxyaspartic acid (THA), a non selective inhibitor of glutamate uptake. A significant increase ($P < 0.05$) in the penetration rate in both MSG and MSG + THA groups compared to control was recorded (39.8 ± 15.7, 53.7 ± 22.1, 52.2 ± 23.7, Control, MSG and MSG + THA respectively) while no difference in penetration rate between MSG and MSG + THA treatment was observed suggesting that sperm glutamate transporters are not involved in the pathway mediating this effect.

Our study demonstrates for the first time that glutamate exerts a positive effect on sperm-oocyte binding and fertilization. Further studies are needed to clarify the mechanism by which glutamate exert his effect.

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1. Introduction

During the transit along the female genital tract to reach the oocyte, spermatozoa encounter an environment that varies in composition. Glutamic acid was reported to be present at different concentrations in female reproductive tract fluids. In mouse glutamate concentrations decline from uterus to the ampullary region of the tube [1] and in pig the decrease is so prominent that glutamic acid is very low or undetectable in sow oviduct fluid [2,3]. However, Hong and Lee [4] reported that glutamate is one of the most abundant amino acids in pig follicular fluid (pFF) with concentrations at least two-fold higher than those of other amino acids. Follicular fluid entrapped in the cumulus oophorus after ovulation may therefore modify the microenvironment in the close vicinity of pig oocyte increasing glutamate concentration.

The two subunit of the umami receptor dimer (T1R1 and T1R3), which form the functional receptor for monosodium glutamate in

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tongue taste buds, are expressed in mature murine and human spermatozoa and in murine spermatids, while transcripts for sweet taste receptor (T1R2) were not detected [5,6]. Moreover we have recently demonstrated the expression α-gustducin and α-transducin, G proteins accepted as specific markers of chemosensitive cells, in boar spermatozoa [7]. The expression of α-gustducin was also demonstrated in mouse, rat, bull and human male gametes [6,8]. Both α-gustducin and α-transducin are involved in the umami taste signaling [9] in response to aminoacids and, among them, glutamate is the most extensively studied ligand for umami receptor in taste buds.

These studies suggest a specific function of umami signaling in spermatozoa that during spermatogenesis lose the majority of the cytoplasm thus eliminating what is non-useful for their final mission: the transport of male genetic material to the oocyte.

On these bases, the aim of our study was to evaluate whether monosodium glutamate (MSG) would exert any effect on in vitro sperm-oocyte binding and fertilization. The effect of MSG on sperm viability, acrosome integrity and immunolocalization of tyrosine-phosphorylated sperm proteins was also assessed.

2. Materials and methods

Unless otherwise specified, all the reagents were purchased from Sigma-Aldrich (Milan, Italy).

2.1. In vitro maturation of porcine oocytes (IVM)

Porcine cumulus–oocyte complexes (COCs) were aspirated using a 18 gauge needle attached to a 10 mL disposable syringe from 4 to 6 mm follicles of ovaries collected at a local abattoir and transported to the laboratory within 1 h. Under a stereomicroscope, intact COCs were selected and transferred into a petri dish (35 mm, Nunclon, Denmark) prefilled with 2 mL of modified PBS supplemented with 0.4% BSA. After three washes in NCSU 37 [10] supplemented with 5.0 mg/mL insulin, 0.57 mM cysteine, 10 ng/mL epidermal growth factor (EGF), 50 μM β-mercaptoethanol and 10% porcine follicular fluid (IVM medium), groups of 50 COCs were transferred to a Nunc 4-well multidish containing 500 μL of the same medium per well and cultured at 39 °C in a humidified atmosphere of 5% CO_2/7% O_2 in air. For the first 22 h of in vitro maturation the medium was supplemented with 1.0 mM db-cAMP, 10 IU/mL, eCG (Folligon, Intervet, Boxmeer, The Netherlands) and 10 IU/mL hCG (Corulon, Intervet). For the last 22 h COCs were transferred to fresh maturation medium [11]. At the end of the maturation period the oocytes were denuded by gentle repeated pipetting.

2.2. Semen collection and preparation

Sperm-rich fraction of ejaculates were collected by gloved-hand technique from three mature boars of proven fertility and extended in equal volume of Androhep TM (Minitub, Tiefenbach, Germany). Only ejaculates with sperm viability higher than 85% were used in the experiments. In order to minimize the boar effect, ejaculates were pooled.

Semen was washed twice with PBS supplemented with 0.4% BSA and finally resuspended with Brackett & Oliphant's medium [12] supplemented with 12% fetal calf serum (Gibco, Invitrogen, Italy) and 0.7 mg/mL caffeine (IVF medium) [13].

2.3. Effect of MSG sperm-zona pellucida binding and fertilization parameters

2.3.1. Effect of MSG sperm-zona pellucida binding

For the sperm-zona pellucida binding assay, spermatozoa were diluted to a concentration of 500×10^3 spermatozoa/mL and 500 μL of the sperm suspensions were preincubated for 1 h in presence or absence of different concentrations of MSG dissolved in water (0, 0.1, 1, 10 mM).

At the end of the maturation period 30–35 denuded oocytes were added into each well and after 1 h of gamete co-incubation at 38 °C in 95% humidity and 5% CO2 in air the oocytes were washed four times in PBS 0.4% BSA with a wide bore glass pipette in order to remove the spermatozoa loosely attacked to zona pellucida. The oocytes were then fixed in 4% paraformaldehyde for 15 min at room temperature and then incubated with 8.9 μM Hoechst 33342 for 10 min in PBS 0.4% BSA in the dark, washed twice in the same medium, and individually placed in droplets of Vectashield (Vector Laboratories, Burlingame, CA, USA) on a slide, and covered with a coverslip. The number of spermatozoa attached to the zona pellucida of each oocyte was assessed by using the above described microscope and was expressed as mean number of spermatozoa per oocyte.

2.3.2. Effect of MSG fertilization parameters

For in vitro fertilization trials, spermatozoa were diluted to obtain 750×10^3 spermatozoa/mL and 500 μL of the sperm suspensions were preincubated for 1 h in presence or absence of different concentrations of MSG (0, 0.1, 1, 10 mM).

At the end of the preincubation period, 45 to 50 in vitro matured oocytes were transferred to each well. After 1 h of coculture, oocytes were transferred to fresh IVF medium and cultured for 19 h until fixation in acetic acid/ethanol (1: 3) for 24 h and stained with Lacmoid.

The oocytes were observed under a phase-contrast microscope and the following parameters were assessed:

(1) penetration rate (number of oocytes fertilized/number of inseminated oocytes);
(2) monospermy rate (number of oocytes containing only one sperm head–male pronucleus/number of penetrated oocytes);
(3) total efficiency of fertilization (number of monospermic oocytes/number of inseminated oocytes);

Degenerated and immature oocytes were not counted.

2.4. Effect of MSG addition during in vitro induced sperm capacitation

Sperm cells were incubated for 2 h in a Nunc 4-well multidish at a final concentration of 50×10^6 sperm/mL in presence or absence of different concentrations of MSG (0, 0.1, 1, 10 mM). At the end of incubation period, spermatozoa were subjected to the evaluation of the parameters below.

2.4.1. Evaluation of plasma membrane integrity and acrosome status

Twenty 5 μL of semen were incubated with 2 μL of a 300 μM propidium iodide (PI) stock solution and 2 μL of a 10 μM SYBR green-14 stock solution, both obtained from the live/dead sperm viability kit (Molecular Probes, Inc., Eugene, OR, USA) for 5 min at 37 °C in the darkness. At least 200 spermatozoa per sample were scored with a Nikon Eclipse E 600 epifluorescence microscope (Nikon Europe BV, Badhoeverdop, The Netherlands). Spermatozoa

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