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Expression of the GM-CSF receptor in ovine spermatozoa: GM-CSF effect on sperm viability and motility of sperm subpopulations after the freezing–thawing process

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Abstract

The granulocyte–macrophage colony stimulating factor (GM-CSF) is a pleiotropic cytokine capable of stimulating proliferation, maturation and function of haematopoietic cells. Receptors for this cytokine are composed of two subunits, alpha and beta, and are expressed in myeloid progenitors and mature mononuclear phagocytes, monocytes, eosinophils and neutrophils, as well as in other non-haematopoietic cells. We have previously demonstrated that bull spermatozoa express functional GM-CSF receptors that signal for increased glucose and vitamin-C uptake and enhance several parameters of sperm motility in the presence of glucose or fructose substrates. In this study, we have analyzed the expression of GM-CSF receptors in ovine spermatozoa and studied the effect of GM-CSF on sperm viability and motility after the freezing–thawing process. Immunolocalization and immunoblotting analyses demonstrated that ovine spermatozoa (Xisqueta race) expressed GM-CSF receptors. In addition, GM-CSF partially counteracted the impairing action of freezing/thawing on the percentage of total motility, as well as on the specific motility patterns of each of the separate, motile sperm subpopulations of ram ejaculates subjected to this protocol. These results suggest that GM-CSF can play a role in the resistance of ram spermatozoa to environmental thermal stress.

Keywords: GM-CSF; Cryopreservation; Ovine spermatozoa; Motility; Sperm subpopulations

1. Introduction

We have recently demonstrated that bovine spermatozoa express functional low- and high-affinity GM-CSF receptors that signal for increased glucose and vitamin-C uptake [1]. GM-CSF is a glycoprotein with several molecular-weight species ranging from 18 kDa to over 30 kDa with hormone-like properties [2], capable of stimulating the proliferation of multipotential cells as well as inducing the proliferation, differentiation, maturation and functional activation of granulocytes and macrophages [3]. GM-CSF is expressed by haematopoietic, and also in several non-haematopoietic cell types such as osteoblast, smooth muscle, endothelial and epithelial cells [4], and murine foeto-placental tissue [5,6].

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The biological activity of GM-CSF is mediated through specific cell-surface receptors which consist of two interacting subunits, alpha and beta [7,8]. Receptors for GM-CSF are also present in non-haematopoietic cells such as placental trophoblasts, endothelial cells, oligodendrocytes of the central nervous system [9–12], follicular fluid and ovarian cells [13] and fallopian tubes [14]. Although there is evidence indicating that GM-CSF is expressed in some non-haematopoietic cells, the physiological role of GM-CSF in male reproductive tissues is unknown. To further analyze the role of GM-CSF in male germ cells, we have provided evidence that seminiferous tubule cells of human and bovine testes express GM-CSF, suggesting that this cytokine might have a potential role in the proliferation and differentiation process of spermatogenesis. We have also demonstrated that this growth factor plays a role in sperm motility and suggest that it may probably control sperm fertilizing ability [15,16].

It is herein reported that GM-CSF receptors are expressed in ovine spermatozoa. Furthermore, the presence of GM-CSF partially counteracted the impairing action of freezing/thawing on the percentage of total motility, as well as on the specific motility patterns of each of the separate, motile sperm subpopulations of ram ejaculates subjected to this protocol. These results seem to indicate that GM-CSF can play a physiological role in sperm as an agent which collaborates in sperm resistance to environmental stress, like that induced by extreme changes of temperature.

2. Materials and methods

2.1. Samples collection

Ovine ejaculated semen was collected with an artificial vagina from two 3-year-old sheep from the Centro de Inseminación Artificial of the Universidad Austral de Chile and from two adult (2–3 years old) Xisqueta rams housed at the experimental farm of the Universitat Autònoma of Barcelona. Xisqueta is a rustic, native sheep breed of Catalonia (Spain) in danger of extinction. Ejaculates were obtained twice a week from November 2004 to December 2004. After collection in the farm, the tubes were placed in a bath at 37 °C and taken to the laboratory. Concentrations of the samples were determined at 200× with either a Neubauer or Thoma haemocytometer cell chamber. Mass motility was examined at 40× and graded on a scale of 0–5 (0 = non-motile, 5 = dense semen with highly vigorous motility). Any sample with a mass motility below 3 was discarded. The ejaculate of each male was analyzed separately and an aliquot of each one was pooled and analyzed also. Motility, viability and membrane integrity were analyzed on each sample before further processing, applying the techniques described in Section 2.5.

2.2. Immunoprecipitation and immunoblotting

Spermatozoa were washed three times with a solution (pH 7.4) containing 150 mM NaCl, 10 mM sodium phosphate (PBS) and 1 mM phenylmethylsulfonyl fluoride (PMSF) at 4 °C. Cells were homogenized in lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.4, 2 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulphate; SDS) containing several protease inhibitors (100 μg/mL PMSF, 2 μg/mL pepstatin A, 2 μg/mL leupeptin and 2 μg/mL aprotinin). The cell lysate was pre-cleared with Protein A–Sepharose CL-4B beads (Pharmacia; Uppsala, Sweden) for 60 min at 4 °C. For immunoprecipitation, equal amounts of protein (400 μg of total cell lysate) were incubated overnight at 4 °C with 2 μg of rabbit polyclonal antibodies of either the anti-alpha or anti-beta GM-CSF receptor (Santa Cruz Biotechnology; Sta. Cruz, CA, USA), followed by the addition of Protein A–Sepharose beads and incubated for a further 2 h at 4 °C. Bound immune complexes were washed three times with lysis buffer containing protease inhibitors and detergents. The pellet was eluted by boiling for 5 min with 2× Laemmli sample buffer [17]. Supernatant proteins were separated by SDS-PAGE as explained below, transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore Corporation; Billerica, MA, USA), and immunoblotted with anti-alpha or anti-beta GM-CSF receptor subunits (1:500) (Santa Cruz Biotechnology; Sta. Cruz, CA, USA). Specific bands were visualized by ECL® (enhanced chemiluminescence; Amersham Biosciences; Arlington Heights, IL, USA) following [18].

2.3. Immunostaining procedures

Spermatozoa were washed three times with 1× PBS pH 7.4, 1 mM PMSF at 4 °C and incubated overnight at 4 °C with anti-alpha (1:100 dilution) or anti-beta (1:50 dilution) GM-CSF receptor subunits in 1% BSA–PBS pH 7.4 and 0.3% Triton X-100, followed by donkey anti-rabbit IgG-Alexa 488 (Invitrogen; Los Angeles, CA, USA). As controls, spermatozoa were incubated with antibodies pre-absorbed with the respective peptide used to generate the antibodies. Specifically,