

HS/DS and sperm chromatin decondensation: new role for old friends?

Juan Carlos Calvo

University of Buenos Aires, Argentina

Dr. Juan Carlos Calvo was born in Buenos Aires, Argentina, on August 11, 1954. At age 25 he received his PhD degree in Chemistry from the University of Buenos Aires for his work on "Hormonal Regulation of Testicular Function". He is Full Professor of Biological Chemistry in the Department of Biological Chemistry, School of Exact and Natural Sciences, University of Buenos Aires and Senior Researcher at the Institute of Biology and Experimental Medicine, CONICET (National Research Council from Argentina). He has coauthored 79 research papers in peerreviewed journals, 17 papers and 1 book about scientific topics addressed to general audience. He has supervised 8 PhD Thesis and is currently supervising 2 more. In 1987 he moved to the National Institutes of Health in Bethesda, Maryland, USA and stayed 7 years working on molecular endocrinology, adipocyte differentiation, epithelial cell-adipocyte interaction and, finally on synthesis of proteoglycans and glycosaminoglycans during adipocyte differentiation, with Dr. Masaki Yanagishita as his direct supervisor. Upon his return to Argentina, Dr. Calvo continued working on extracellular matrix involvement in mammary and prostate cancer progression, as well as on the role of heparan sulfate and dermatan sulfate in chromatin decondensation in human and murine sperm nucleus.



Immunocytochemistry of mouse oocyte using an anti-Heparan Sulfate monoclonal antibody. Denuded and permeabilized oocytes were incubated with anti-HS antibody followed by fluorescein isothiocyanate-labeled anti-mouse immunoglobulin M. Image is representative of 270 oocytes observed in four different experiments.



lucida: A: Oocytes in GV stage with zona pellucida were incubated in the presence of a primary monoclonal antibody against HS and a second antibody anti-mouse IgM coupled to FITC. Intense and homogeneous staining is observed in the ooplasm. B: Control oocytes: incubated without primary antibody. Photograph is representative of 26 oocytes analyzed in 6 experiments.

Chromatin in mammalian sperm nucleus is unusually condensed, due to replacement of the majority of histones by protamines. Soon after fertilization, decondensation of this densely packed chromatin must occur to allow formation of the male pronucleus and syngamy. Decondensation involves protamine disulfide bond reduction by oocyte glutathione and replacement of protamines by oocyte histones with the aid of an acceptor molecule. In our laboratory we demonstrated that heparan sulfate present in the ooplasm would be functioning as

protamine acceptor during human sperm decondensation in vivo. We have also analyzed the role of heparin, structural analogue of heparan sulfate, and dermatan sulfate in murine sperm chromatin decondensation in vitro, including the possibility of a synergistic effect between both glycosaminoglycans. We assessed decondensation under phase-contrast microscopy following incubation of murine spermatozoa with glutathione and either heparin, dermatan sulfate or a combination of both. Both were able to promote decondensation of mu-

rine spermatozoa *in vitro* but the decondensing ability of heparin was significantly higher, thus revealing the existence of a synergistic effect observed in both capacitated and non capacitated spermatozoa and supported by transmission electron microscopy. These results indicate a new potential role for dermatan sulfate in murine sperm decondensation and provide evidence of differences in the degree of chromatin condensation throughout the murine sperm nucleus.

References

- Heparan sulphate: a putative decondensing agent for human spermatozoa in vivo. Romanato M, Cameo Mónica S, Bertolesi GE, Baldini C, Calvo JC and Calvo L. Human Reproduction 18: 1868-1873, 2003.
- 2) Further evidence on the role of heparan sulfate as protamine acceptor during the decondensation of human spermatozoa in vivo. Romanato M, Regueira E, Cameo MS, Baldini C, Calvo L and Calvo JC. Human Reproduction 20 (10): 2784-2789, 2005.
- 3) The presence of heparan sulfate in the mammalian oocyte provides a clue to human sperm nuclear decondensation in vivo. Romanato M, Julianelli V, Zappi M, Calvo L and Calvo JC. Human Reproduction

23(5):1145-1150, 2008

- 4) Heparin enhances protamine disulfide bond reduction during in vitro decondensation of human spermatozoa. Julianelli V; Farrando B; Alvarez Sedo C; Calvo L; Romanato M; Calvo JC. Human Reproduction 2012, 27 (7): 1930-1938.
- 5) Dermatan sulfate synergizes with heparin in murine sperm chromatin decondensation. Sanchez MC, Alvarez Sedo C, Julianelli V, Romanato M, Calvo L, Calvo JC and Fontana VA. Systems Biology in Reproductive Medicine (2012, in press).