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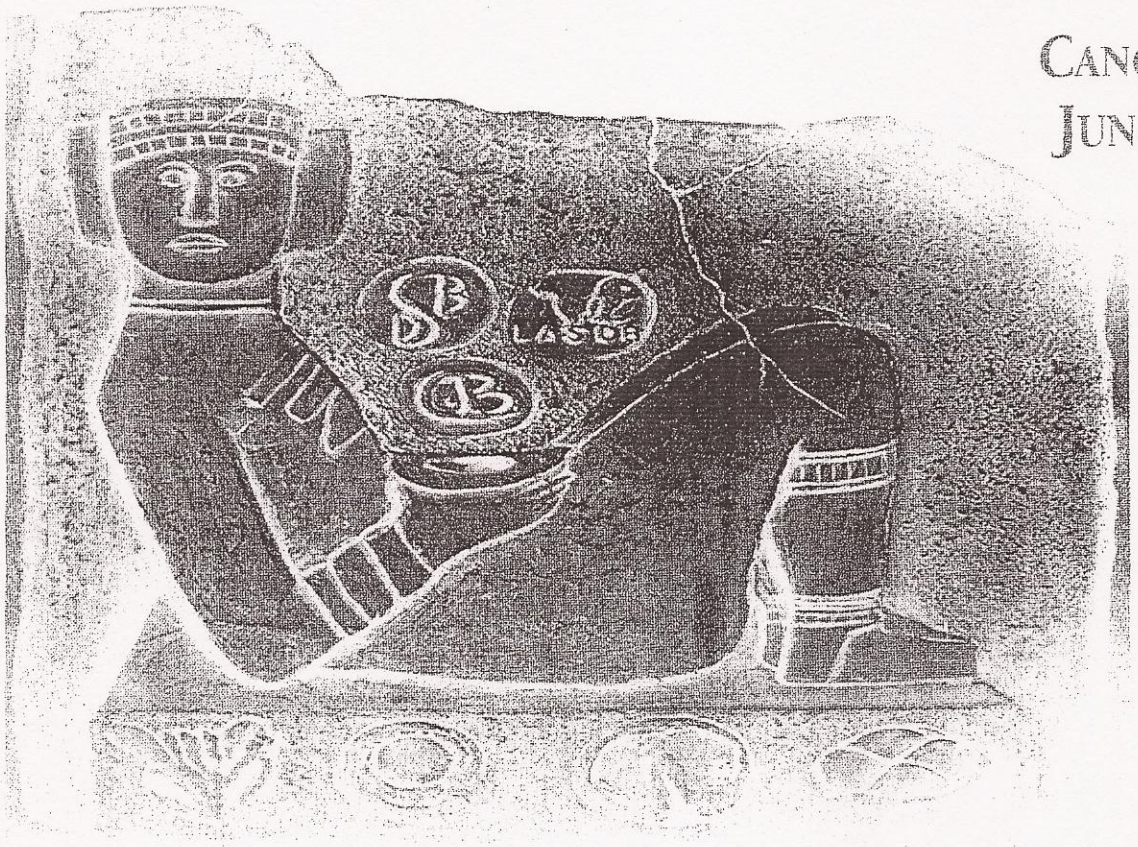

envelope modifications are caused by the action of a trypsin-like protease named oviductin. The aim of this work was to clone the oviductin complete cDNA and to analyze their functional domains. Total RNA was isolated from oviductal PR of hormonally stimulated animal. Sets of primers were designed based on homology sequences. We first amplified, cloned and sequenced an internal 530 pb partial cDNA. The 5′ cDNA end was amplified using a new group of specific designed primers. To complete the mRNA sequence, a 3′ rapid amplification of cDNA ends (3′ RACE) was performed. The overlapping sequence showed a 3203-bp-long oviductin cDNA with one open reading frame coding for a 980 aminoacids protein. The molecular structure comprise two protease domains (α and β) and three CUB domains. The α domain has three important aminoacids for catalytic activity (His, Asn, Ser), while in the β domain a His residue was replaced by Asn. Thus, this domain is not likely to be proteolytically active. These results would indicate that the Bufo arenarum oviductin α domain produce the partial hydrolysis of the envelope glycoproteins. At this moment, the exact function of the CUB domains and β domain is still unknown.

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Program/Abstract # 267

Capacitation-like changes in external fertilization: correlation of physiological modifications with fertilizing capacity acquisition in *Bufo arenarum* spermatozoa

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During its short life in the female tract, mammalian sperm must accomplish a series of cellular processes named capacitation to acquire fertilizing capacity. In animals with external fertilization as amphibians, gamete interactions are first established between sperm and molecules of the egg jelly coat released into the medium. Since dejellied oocytes are not normally fertilized, the aim of this study was to determine if the jelly coat of the toad *B. arenarum* promotes a "capacitating" activity on homologous sperm. We found that incubation of sperm in diffusible substances of the jelly coat (Egg Water) for 90–180 s is sufficient to render sperm transiently capable of fertilizing dejellied oocytes. Similar to mammalian sperm, the fertilizing state was correlated with an increase of protein tyrosine phosphorylation and a decrease of the sperm cholesterol content. Inhibition of either the increase in tyrosine phosphorylation or cholesterol efflux affected the acquisition of fertilizing capacity. Phosphorylation and fertilization could be promoted with $NaHCO_3$, and also by addition of the cholesterol binding compound beta cyclodextrin. Moreover, sperm could gain the ability to fertilize dejellied oocytes in the presence of these compounds. These data indicate that *B. arenarum* sperm could undergo a series of molecular changes to gain fertilizing capacity; these changes are reminiscent of mammalian sperm capacitation. Supported by NIH HD38082 and HD44044 (to PEV); ANPCyT (PICT0108545) and CONICET (PIP6428) (to MOC and SEA).

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Program/Abstract # 268

Involvement of calmodulin on guinea pig sperm nuclei decondensation

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Actin was implicated in diverse nuclear activities including transcription, chromatin remodeling and nucleocytoplasmic trafficking. Our group, detected actin myosin, spectrin and cytokeratin as guinea pig sperm nuclear matrix components. A retarding effect of nuclear decondensation, caused by heparin, is produced by phalloidin and/or diacetyl-monoxime treatment, suggesting a role for F-actin and myosin in the maintenance of nuclear stability in sperm. We detected calmodulin (CaM), myosin light chain and tubulin in the pure nuclei proteins of guinea pig sperm. We found actin–myosin interaction in the nuclear matrix. To define if CaM has a function in nuclear stability, we performed experiments of nuclear decondensation by heparin in absence or in CaM antagonists (W5, W7 or calmidazolium) presence. All three CaM antagonists assayed retarded heparin nuclear decondensation; the effect began to be clear and significant ($p<0.05$) after 2 min heparin treatment. The highest decondensation stable values were observed at 4 min of treatment; nuclear decondensation values were: 9.4 μm of diameter and 51.9 μm^2 of area, after this time the sperm number decline, which might mean nuclei destruction. Furthermore, an interesting result is, at 10 min of heparin/CaM antagonists treatment, approximately 80% (W5: 5.3; W7: 5.7 and calmidazolium: 5.7×10^6) of de original nuclei number (6.3×10^6) remained. While in the sperm nuclei sample treated with heparin or heparin/DMSO, original nuclei number was reduced in 80% (1.3×10^6 and 1.8×10^6, respectively).

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Program/Abstract # 269

Chromatin remodeling in mouse metaphase II oocytes independently of meiotic exit

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In mammalian fertilization, paternal chromatin becomes exhaustively remodeled, however, the maternal contribution to this process is still unclear. To address this, we prevented the induction of meiotic exit by microinjecting inactivated sperm heads into metaphase II (mII) oocytes. This permitted us to examine oocyte- (as opposed to embryo-) mediated sperm