

extracellular matrix proteins. Moreover, it was demonstrated that in the chicken embryo of 9 days of incubation, oogonia retain the capability to migrate. This work was supported by UNAM PAPIIT IN216807 CONACYT Q45030.

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Program/Abstract # 261

The process of oocyte death. New views

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The process of cell death of oocytes was studied in 1 to 28 days old rats, by means of light and electron microscope and cytochemical methods. We detected acid phosphatase, TUNEL, active caspase-3 and LAMP-1 in each oocyte. In all ages studied, small clear vesicles were the most constant structural trait. These vesicles were most frequently associated with acid phosphatase activity. Lamp-1, caspase-3, acid phosphatase and clear vesicles were present in the simultaneously in 64 out of 73 oocytes (88%) of follicles undergoing atresia in rat of different ages. Acid phosphatase and clear vesicles are the more constant traits of oocytes at initial stages of cell death, caspase-3 and lamp-1 appear progressively in more advanced stages of the process. TUNEL is more frequently found in atretic oocytes of rats older than 10 days. We propose that in the process of oocyte death co-exists features typical of apoptosis as active caspase-3 and TUNEL, with lamp-1, acid phosphatase and cytoplasmic clear vesicles characteristic of autophagy. Blebbing and apoptotic bodies are never found in the process of cell death of the oocyte.

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Program/Abstract # 262

The effect of superovulation on methylation and expression of imprinted genes in pre- and post-implantation mouse embryos

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The stimulation of follicular growth and ovulation via the administration of exogenous gonadotropins is commonly used in human assisted reproduction as well as animal research. The concern has been raised that this procedure may force oocytes to go through the final growth and maturation process too rapidly, possibly affecting the establishment of methylation imprints. The goal of this study was to examine the effects of superovulation on genomic imprinting in pre- and post-implantation mouse embryos. Naturally cycling or superovulated CD1 females were mated with CAST-7 males such that parental alleles were identifiable, and blastocysts and postimplantation (E9.5)

embryos were collected. Methylation of the imprinted genes *H19* and *Snrpn* was examined using bisulfite sequencing, and allele-specific expression was examined using fluorescent hybridization probes. Blastocysts isolated from superovulated female mice showed a loss of imprinting at *H19*, but not at *Snrpn*. Similarly, at E9.5, superovulation did not appear to disrupt the DNA methylation or allele-specific expression of *Snrpn*, however methylation and expression of *H19* were perturbed. Superovulation caused overcrowding of the uterus, and as such embryo transfer experiments were undertaken to determine whether uterine crowding was contributing to the changes observed in embryos from superovulated female mice. In conclusion, superovulation affects the maintenance of methylation and monoallelic expression of the paternally imprinted gene *H19* in the mouse (supported by CIHR).

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Program/Abstract # 263

Participation of the N-terminal and C-terminal region of the SRY in the DNA binding

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Introduction: Sex determination is a processes regulated by the *SRY*. This gene encodes a transcription factor containing a central HMG box domain, an N-terminal and C-terminal regions. The functions and structures of the regions outside of the HMG box domain are not clear. *Materials and methods:* We analyzed *in vitro* the DNA-binding activity of the full length *SRY* protein and three *SRY* mutants (N-terminal-HMG, HMG box and HMG-C-terminal), using crude extract proteins, GST-*SRY* fusion proteins and pure *SRY* proteins with three different methods (EMSA, optical density and *kd*). *Results:* All the assays have been undertaken with three different protocols: with crude extract, pure fusion and the pure non fusion *SRY* proteins. We demonstrated in all protocols that the C-terminal less *SRY* protein obtained from crude extract, pure fusion and pure non fusion proteins had been statistically diminishing the DNA-binding ($P < 0.05$). In contrast, this difference was statistically significant only when used the pure HMG box mutant ($P < 0.05$). Moreover, we found that N-terminal less *SRY* protein had a small impact in the *SRY*-DNA binding in all of the three protocols. *Conclusion:* To our knowledge, this constitutes the first report where demonstrate the importance of the full length *SRY*, as well as, carboxy-terminal region of the *SRY* in the DNA binding. Likewise, we suggested that the analysis of mutations in the *SRY* it would be done using *SRY* pure protein and in lesser degree with pure fusion *SRY* protein, in spite of underestimate the effect of these mutations.

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