respectively. There is no significant difference between them. **Conclusion:** This study confirms that IUI can be applied as a first-line treatment in infertile couples. Sperm parameters do not affect pregnancy rate when total rapid progressive sperms are > 500 million after gradient sperm washing.

E-mail: cailiuhong@hotmail.com

Pt7-10

Incubation of ejaculated and epididimal pig spermatozoa with oviductal fluid protein does not affect tyrosine phosphorylation pattern

Carmen Matás*, K Aviles-Lopez, FA Garcia-Vazquez

Departamento de Fisiología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Background: Capacitation, which represents the final maturational steps, renders mammalian sperms competent to interact correctly with the oocyte, and it occurs in the female genital tract *in vivo*. *In vitro*, this phenomenon can also be reproduced in defined media. During the capacitation, the membrane proteins and lipid organization change dramatically resulting in sperm binding to the zona pellucida and initiating acrosome reaction. Protein phosphorylation is known to regulate sperm function, such as motility and zona pellucida recognition. Mammalian capacitation is also probably mediated by protein phosphorylation. However, little is known about boar SP factors and porcine oviductal fluid (POF) that may influence the tyrosine phosphorylation (Tyr-P) of sperm proteins during capacitation. **Aim:** To determine if proteins from POF modify the Tyr-P pattern spermatozoa. **Methods:** POF was obtained by tubal aspiration with a micropipette from female just post-ovulation. After centrifugation, the epithelial cells was precipitated, the supernant was collected to determine the protein concentration. Ejaculated (EJ) and epididymal (EP) spermatozoa were divided into three groups: 1) spermatozoa without any treatment (control = C); 2) spermatozoa washed through Percoll® gradient (P); and 3) spermatozoa washed through Percoll® and incubated with porcine oviductal fluid protein (50 μg mL^{-1}) for 30 min (P-POF). All samples were incubated in sperm capacitation medium (TALP) after treatments. The plasma membrane was isolated according to the report (Bravo *et al.* 2005) and the soluble proteins were separated by 12% SDS-PAGE. Afterward, the proteins were transferred onto a nitrocellulose membrane and blocked with TBST-Albumin 5% overnight; after that the membranes were incubated with primary antibody (anti-phosphotyrosine antibody, 4G10 Upstate Biotechnology) (1:20 000), and then with secondary antibody (anti-mouse IgG antibody, Biorad®)(1:20 000)) and revealed with ECL+Plus (Amersham®). Seven replicates were conducted for this experience. **Results:** The results showed a band of tyrosine phosphoprotein of 19 kDa in all experimental groups except the EJ-C group. A band with a range of 40 43 kDa was present in all experimental groups although the intensity of phosphorilation was different. **Conclusion:** The incubation of ejaculated and epididimal pig spermatozoa with oviductal fluid protein did not affect protein tyrosine phosphorylation pattern in the incubation time studied.

Supported by Seneca 08752/PI/08 y AGL2006-03495.

E-mail: cmatas@um.es

Pt7-11

Increasing chromatin decondensation in boar's ejaculated and epididymal spermatozoa treated by Percoll® gradient and porcine oviductal fluid

K Aviles-Lopez, FA Garcia-Vazquez, L Vieira, JA Carvajal, J Gadea, Carmen Matás*

Departamento de Fisiología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Background: During spermiogenesis in mammals, the sperm chromatin structure undergoes replacement of nuclear histones, first by transition proteins and finally by protamines, leading to a highly packaged and condensed chromatin, which is resistant to oxidative insults. Further stabilization of the sperm chromatin structure is enhanced by the presence of seminal plasma components, such as free zinc ions and zinc-binding proteins. Moreover, the integrity of the sperm chromatin structure is an important factor in fertilization and embryo development, and can be affected by various factors. In previous studies we have showed that ejaculated spermatozoa washed by Percoll® gradient exhibited higher rates of head decondensation and pronuclei formation than epididymal spermatozoa or ejaculated spermatozoa without gradient treatment (by IVF and ICSI Matás *el al.*, 2003; Garcia-Vazquez *et al.*, 2009 respectively). **Aim:** To determine the sperm chromatin condensation after treated by Percoll® gradient and porcine oviductal fluid (POF). **Methods:** POF was obtained by tubal aspiration with a micropipette from female post-ovulation. After centrifugation, the epithelial cells was precipitated, and the supernatant was collected to determine the protein concentration. Ejaculated (EJ) and epididymal (EP) spermatozoa from different boars were washed through Percoll® gradient and incubated in sperm capacitation medium (TALP). EJ and EP spermatozoa were divided into three groups: 1) spermatozoa without any treatment (control = C); 2) spermatozoa washed through Percoll® gradient (P); and 3) spermatozoa washed through Percoll® and incubated with porcine oviductal fluid protein (50 μg/mL) in TALP medium for 30 min (POF). The samples were centrifuged and the pellet was resuspended in ethanol solution and phosphate-buffered saline to make permeabilization of membranes enhanced. After that, the samples were centrifugated and the pellet was resuspended in a propidium iodide (PI) solution in PBS. Measurements were expressed as the mean red intensity fluorescence units and it was used as an index of the state of the chromatin condensation, as this is directly related to the PI uptake by DNA. **Results:** The results were analysed by two-way ANOVA. Data showed that i) chromatin condensation of the control group were lower than experimental ones (EJ-C: 2.23 and EP-C: 16.69 *vs.* EJ-*P* = 22.68, EJ-POF = 22.48 and EP-*P* = 21.96, EP-POF = 23.37, $P < 0.001$). Although there were significantly differences between EJ-C and EP-C ($P < 0.001$), the chromatin condensation was similar after the spermatozoa were washed (EJ and EP). **Conclusion:** Sperm

source as well as sperm treatment appears to play an important role in the sperm chromatin condensation. The seminal plasma could be of significant benefit in improving the chromatin stabilization.

Supported by Séneca 08752/PI/08 y AGL2006-03495

Email: cmatas@um.es

Pt7-12

Enzymatic activity of glycosidases in boar sperm

A De Ondiz[1, 2], M Avilés[3], FA García-Vázquez[2], L Grullón[2], Salvador Ruiz[2]*

[1]Sciences Veterinary Faculty. Zulia University, Venezuela

[2]Department of Physiology, [3]Dept. Cell Biology and Histology, Murcia University, Spain

Background: The initial sperm-zona pellucida (ZP) binding is an important event that precedes sperm penetration into an oocyte. In several mammalian species, macromolecules such as lectin-like proteins and enzymes in spermatozoa are involved in the recognition of the oligosaccharides present in ZP glycoproteins. Spermatozoa glycosidases are thought to form stable enzyme-substrate complexes by binding to the oligosaccharide residues of the ZP glycoproteins with high specifity and affinity (Tulsiani *et al*., 1998). **Aim:** The objective of this study was to analyze and compare the presence of the glycosidases (α-D-Mannosidase, α-L-Fucosidase, β-D-Glucosaminidase, and β-D-Galactosaminidase) in fresh ejaculated and epididymal sperm from mature boars. **Methods:** Spermatozoa were washed three times in PBS by centrifugation at 800 × g for 10 min. The pelleted sperm were resuspended in the same buffer to obtain a final concentration of 250 × 10^6 spermatozoa /mL. The different enzymes were detected by incubating 2 × 10^6 spermatozoa (for α-D-Mannosidase, α-L-Fucosidase) and 20 × 10^6 spermatozoa (for the rest of enzymes) of sperm samples with the corresponding substrate conjugated to 4-methylumbelliferil for 2 h at 37ºC in PBS, pH 7.3. Fluorescence was read in units of fluorescence (UF/1 × 10^6 sperm) on a Fluostar Galaxy fluorimeter (BMG Lab Tecnologies) (using wavelengths of 340 and 450 nm for excitation and emission, respectively) and was corrected by subtracting tissue and substrate blanks. The results were analyzed using an one way ANOVA. **Results:** α-D-Mannosidase and α-L-Fucosidase are the enzymes of main activity present in the porcine ejaculated and epididymal sperm. A higher α-D-Mannosidase and β-D-Glucosaminidase enzymatic activity was observed in ejaculated sperm compared to epididymal sperm (α-D-Mannosidase: 8 810 ± 487.6^a, 5 363 ± 377.4^b and β-D-Glucosaminidase: 722.1 ± 37.76^a, 270.0 ± 42.36^b ($P < 0.05$), α-L-Fucosidase and β-D-Galactosaminidase activity was similar for both kinds of spermatozoa, 1 822 ± 89.2, 1 936 ± 223.2 and 176.0 ± 16.61, 129.4 ± 12.46, respectively. **Conclusion:** This study demonstrates that different glycosidases are present in the sperm plasma membrane. The higher enzymatic activities found in ejaculated sperm compared to the epididymal sperm suggests the binding of α-Mannosidase and β-D-Glucosaminidase coming from the seminal plasma to the epididymal spermatozoa. Supported by Seneca 08752/PI/08, AGL2006-03495 and 0452/GERM/06.

E-mail: sruiz@um.es

Pt7-13

Expression of β-chemokine RANTES in human and mouse epididymis

Zhen Li*, Zhi-Jian Sun, Bin-Fang Ma, Yuan-Qiang Zhang

Department of Histology and Embryology, Fourth Military Medical University, Xi'an 710032, China

Aim: To investigate the expression and cellular distribution of RANTES (regulated upon activation normal T cell expressed and secreted; CCL5) in male reproductive system. **Methods:** RT-PCR, in situ hybridization, immunohistochemical staining and immunofluorescence staining were employed to examine the distribution of RANTES in human and mouse epididymis. Spermatozoa were collected from different segments of epididymis and immunofluorescence staining was used to detect RANTES association. Real-time RT-PCR and Western blot were used to quantitate the levels of RANTES expression in mouse epididymis on postnatal days 7, 14, 21, 28, 35, 42, 49, 56 and 140. **Results:** The mRNA for RANTES was not detected in human testis. In contrast, the epididymis produced high amount of RANTES transcripts. In situ hybridization and immunohistochemical studies revealed RANTES–positive staining in columnar ciliated cells in efferent ductules/initial segment and in narrow cells located in the caput epididymis, in both human and mouse. RANTES–positive basal cells, located underneath the columnar cells, were only identified in the corpus and cauda epididymis in human. Immunofluorescence studies also showed obvious segment-specific expression pattern of RANTES in epididymis. We observed that RANTES was bound on the post-acrosome region of epididymis spermatozoa. Qualitative analysis of tissues from postnatal days 7, 14, 21, 28, 35, 42, 49, 56 and 140 mice indicated that the signals of RANTES were first detected on day 7 and increased during sexual maturation. **Conclusion:** These data indicate that RANTES is mainly restricted to the columnar ciliated cells and narrow cells, and is secreted into the lumen of epididymis throughout sexual maturity. The exact physiologic, pathophysiologic and molecular mechanisms involved in this process require further studies.

E-mail: lizhenhe@fmmu.edu.cn

Pt7-14

Expression of recombinant hamster zona pellucida glycoprotein ZP4 in mammalian cells

MJ Izquierdo-Rico[1], AB Pérez-Oliva[2], Manuel Avilés[1]*, C Jiménez-Cervantes[2]

[1]Department of Cell Biology and Histology, School of Medicine, University of Murcia, 30100 Murcia. Spain

[2]Department of Biochemistry and Molecular Biology, School of Medicine, University of Murcia, Murcia, Spain.

Background: The initial interaction between gametes takes place at the sperm surface and the zona pellucida (ZP), an egg extracellular matrix in mammals. This extracellular matrix is formed by three or four glycoproteins depending on the specie (ZP1, ZP2, ZP3 and ZP4). ZP4 has been implicated in sperm