8. SUMMARY.

The fertilization process in mammals takes place in a complex microenvironment in the female genital tract, and the oviduct fluid (OF) represents the aqueous milieu in which oocytes, spermatozoon, and embryos are suspended during oviductal transit. Early events in fertilization require interactions between complementary molecules present on different cell surfaces. In this way, carbohydrates play a key role in different reproductive events such as sperm-oviductal cell interaction, a prerequisite for oocyte zona pellucida (ZP) penetration, and sperm-oocyte recognition, necessary for primary binding, among others.

It has been reported that sugar residues contained in the ZP have been identified as sperm receptors in bovine and porcine oocytes. The importance of carbohydrate-contained material such as glycoprotein and glycolipids on cell surfaces gives rise to several potential functions for the glycosidases in the reproductive process. Since these enzymes catalyze the hydrolysis of oligosaccharide chains thus modifying the interactions among cells. However, the enzymatic activity level of this enzymes or their fluctuation along the estrous cycle in both bovine and porcine oviductal fluids have not been studied in detail.

In the present research we study the variation along the oestrous cycle in the enzymatic activity of 7 exoglycosidases (α-L-fucosidase, β-N-acetyl-glucosaminidase, β-D-galactosidase, α-D-galactosidase, α-D-mannosidase, β-N-acetyl-galactosaminidase, and N-acetyl-neuraminidase), the protein levels and the volume of fluid collected from cows (bOF) and pigs (pOF) oviducts. This work was made with OF collected by the aspiration of oviducts from slaughtered females and classified according to ovary morphology taking into account the follicle size and the presence and aspect of the corpus luteum. All the BOF samples come from pubertal animals whereas the pOF came from gilts and sows.

The obtained OF samples were centrifuged (7.000g, 10 minutes, 4ºC), discarding the small cellular pellets and further calculating the mean volume collected per oviduct in each sample and phase of the estral cycle, both in bOF and pOF. Finally samples were kept (-80ºC) until assay for a maximum period of 3 weeks. In both species samples were collected throughout a year.

Enzymatic activity and protein level were studied in each sample. Enzymatic activity was determined by the fluorescence (340nm excitation, 450nm emission) emitted by substrates derived from 4'methylumbelliferyl used for each enzyme: α-L-fucoside, β-N-acetyl-glucosaminide, β-D-galactoside, α-D-galactoside, α-D-manoside, β-N-acetyl-galactosaminide and N-acetyl-neuraminic. The concentration of oviductal proteins was

studied by the bicinconinic acid assay (BCA) by absorbance (560nm) and employing BSA as standard. 1 Unit of specific enzymatic activity (U) was defined as the quotient among the amount of enzyme necessary to hydrolyze 1pmol of substrate (4'metylumbelliferyl-glicoside)/minute at 37ºC and pH=7, and the protein concentration of the sample.

The obtaining method by oviduct dissection and further content aspiration with an automatic pipette permits the obtaining of OF with enough volume and quality for further analysis in the studied species.

After analysis of a total of 237 bovine and 269 porcine oviducts, results showed that form the 7 studied exoglycosidases, neither bOF nor pOF displayed activity for α-D-galactosidase and N-acetyl-neuraminidase at any phase of the estral cycle, but it did for α-L-fucosidase, β-N-acetyl-glucosaminidase, β-D-galactosidase, α-D-mannosidase, β-N-acetyl-galactosaminidase.

The enzymatic activity in bOF did not show variations among follicular and luteal phases showing mean specific enzymatic activity of 39.7, 88.4 and 65.3U respectively for α-L-fucosidase, β-N-acetyl-glucosaminidase and β-D-galactosidase. However, the specific activity for α-D-manosidase and β-N-acetyl-galactosaminidase was higher in the follicular than in the luteal phase (92.8 *vs*. 83.1U and 66.5 *vs*. 59.4U, respectively for both enzymes). Regarding the protein concentration and bOF volume, no differences were observed during the estral cycle. Mean protein level in bOF was 55.0μg/μl and mean volume per oviduct 36.5μl. When total protein concentration (μg)/oviduct was calculated, values reached 1354.77 ± 172.78, 2118.57 ± 200.65, 1.680.49 ± 122.79 and 1563.20 ± 149.76 respectively for early follicular, late follicular, early luteal and late luteal phases. The highest concentration of protein/oviduct was in the moments close to ovulation, at the late follicular phase (P=0'02). The increase in the OF secretion and oviductal protein synthesis in the moments close to ovulation has been widely described in the literature and it suggests a steroid control on these functions.

Regarding porcine species, specific activity ranged along the estral cycle in the 5 active exoglycosidases. On one hand, α-L-fucosidase and β-N-acetil-glucosaminidase activity was maximum close to ovulation (late follicular phase) reaching values of 72.0 and 91.9U respectively for each enzyme and deeply decreasing after ovulation to be constant during late luteal and early follicular phases. On the other hand β-D-galactosidase, α-D-manosidase and β-N-acetyl-galactosaminidase specific activities were maximum at early follicular phase (70.3, 104.4 and 69.8U respectively for each

enzyme) and they strongly decreased after ovulation to keep constant during luteal phase.
The comparison among early follicular phase-pOF from gilts and sows showed a higher β-D-galactosidase specific activity in sows (70.3 *vs.* 57.6U respectively). Besides, pOF protein level varied along estral cycle having the higher values at late luteal phase (53.5μg/μl) and the smaller at late follicular phase (39.4μg/μl). The mean volume of pOF collected per oviduct also varied with the estral cycle collecting the higher volumes in the moments close to ovulation (late follicular phase, 50.62μl/oviduct) and decreasing after ovulation and with the progress of estral cycle.

From the results we may infer that both bovine and porcine oviductal fluids display α-L-fucosidase, β-N-acetyl-glucosaminidase, β-D-galactosidase, α-D-mannosidase, and β-N-acetyl-galactosaminidase activity with some variations in different stages of the oestrous cycle. So that the role of these enzymes in the reproductive process can be hypothesized, but further researches are necessary to demonstrate it. Thus, it is possible that α-L-fucosidase takes part in the releasing of the spermatozoa bound to oviductal epithelial cells, or in the remodeling of the oocyte's ZP during its passage through oviduct. β-N-acetyl-glucosaminidase could participate in the dispersion of the cumulus cells, in the penetration of the spermatozoon through the ZP and also in its remodeling. β-D-galactosidase could remove molecules of β-galactose from the surface of the oviductal cells, which could have a role both in the nutrition as in the releasing of some spermatic cells from the oviductal reservoir. α-D-manosidase could modify the oocyte's surface and also remove sperm receptors from the ZP, or it could act like a protein in the binding to the oviductal cells. Finally it is suggested a role of β-N-acetyl-galactosaminidase in the modification of receptors of the bovine ZP and in the interaction of spermatozoa with the pig ZP.