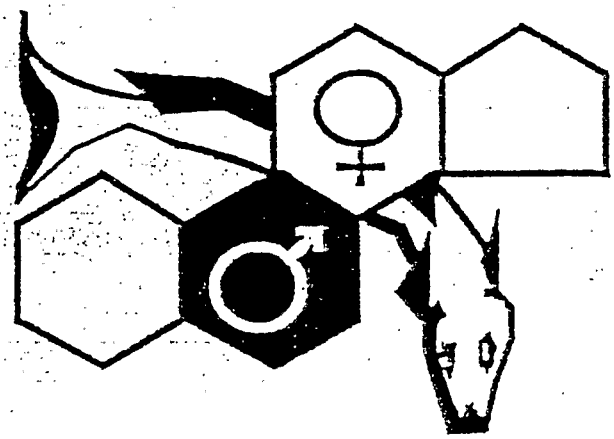
4th International Symposium

on

Reproductive Physiology of Fish



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RECEPTORS MEDIATED ENDOCYTOSIS OF VTG IN FISH FOLLICLE.

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Introduction

Oocyte vitellogenesis is one of the best examples of cell specialization for specific endocytosis of proteins. Indeed, vitellogenin, a hepatically synthesized lipophosphoglycoprotein which constitutes the main plasma yolk precursor reaches ovaries by the blood stream to be selectively taken up by the oocytes.

Vitellogenin enters the oocyte follicle by capillary vessels located in the theca, the outer layer surrounding the oocyte. It reaches the germinal cell passing through meso-epithelial cells of the theca and basement lamina, through intercellular spaces of granulosa cells, then into the extra-cellular matrix located between granulosa and the oocyte plasmic membrane, and finally along the oocyte microvillosities in the channels of the zona radiata

auratus, rainbow trout Oncorhynchus mykiss, sole Solea vulgaris and Siberian sturgeon Acipenser baeri.

Our experiments have been performed with homogenized follicles devoid of yolk, frozen in liquid nitrogen and stored for up to several months at - 20°C.

Purification of vitellogenin from plasma of estradiol pre-treated fish was obtained with one-step DEAE cellulose chromatography using Tris buffer at pH 7.8 in presence of calcium chloride and PMSF. Elution was performed with a linear gradient of sodium chloride (0-150 mM). Fractions of the main peak were identified as vitellogenin on polyacrylamide electrophoresis, pooled and concentrated on Amicon cell until the desired protein concentration.

apparent molecular weight of 100 kDa. Fifty fold excess of cold trout VTG completely extinguished the signal, demonstrating the specificity of the binding.

b - Characterization of vitellogenin receptor.

Characterization of the VTG receptor was performed by binding of ^{125}I-trout vitellogenin to crude salmon oocyte membranes using a solid-phase filtration assay.
Adjunction of suramine which blocks receptors gives rise to a linear non specific binding. Specific binding is determined by substraction of non specific binding from total binding and is saturable (Fig.1a). Transformation of data to Scatchard plot indicates the existence of a single class of binding site for vitellogenin (Fig.1b).

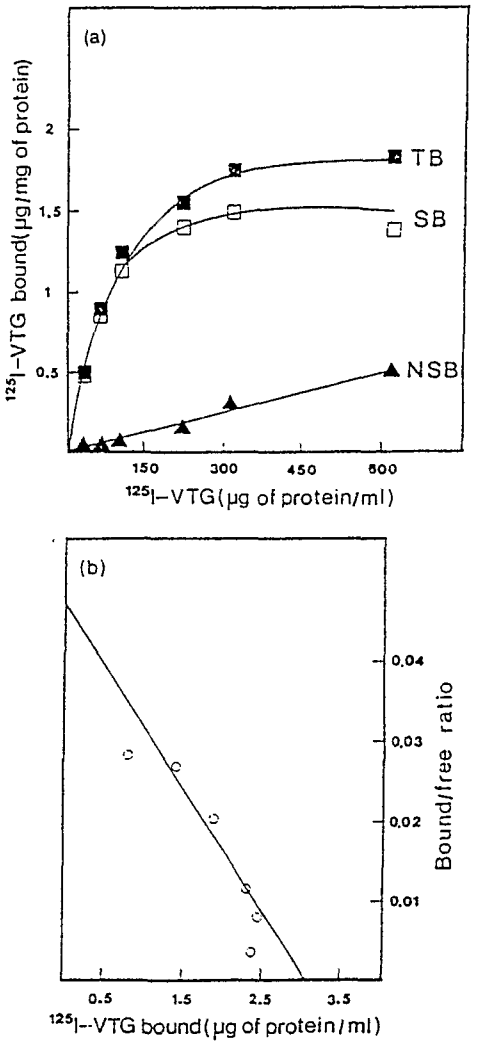

Fig. 1. a- Saturation curves. b - Scatchard transformation.

Competition binding studies were performed with increasing concentration of cold ligand. The binding is significantly reduced by adjunction of increasing amounts of unlabelled trout VTG or unlabelled chicken VTG demonstrating the specificity of the binding. In other hand competition with high concentration of HDL is ineffective (Fig.2).

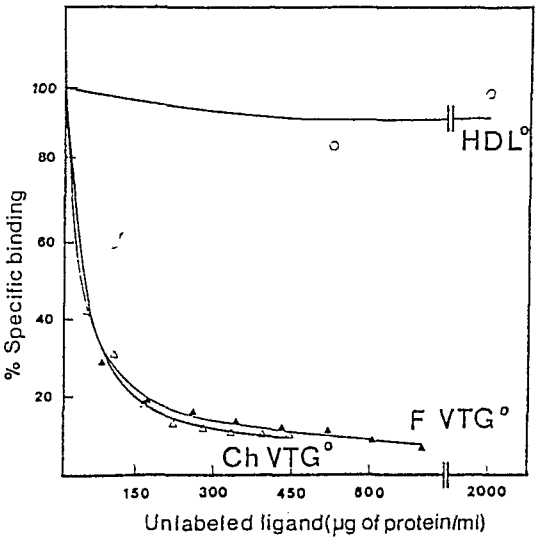

Fig. 2. Competition Binding.

c - Kinetics of vitellogenin receptor during vitellogenesis in trout.

In order to follow the kinetics of vitellogenin receptors in rainbow trout follicle we have tested different batches of oocyte membrane preparations during vitellogenesis of a winter strain. Seven samples were taken during type II vitellogenesis (exogenous vitellogenesis) from July to November just before spawning. Each time twenty five follicles were removed from the ovaries tested, carefully dissected out and measured. An average of oocyte diameters was taken to define the vitellogenesis stage : from 1.11 mm in July corresponding to gonadosomatic index of 0.57 to 3.97 mm just prior to ovulation corresponding to a gonadosomatic index of 9.84. The average ovule diameter is of 4.45 mm.

Binding of ^{125}I-trout vitellogenin to crude oocyte membrane preparations in presence (non specific binding) or in absence (total binding) of suramine (5 mM) allows the determination, using filter assay, of the affinity of the ligand for membrane preparations

If total binding is expressed as femtomoles of ligand per square millimeter surface unit versus

oocyte diameter, the number of receptors increases slightly during vitellogenesis but decreases dramatically at maturation. (Fig.3).

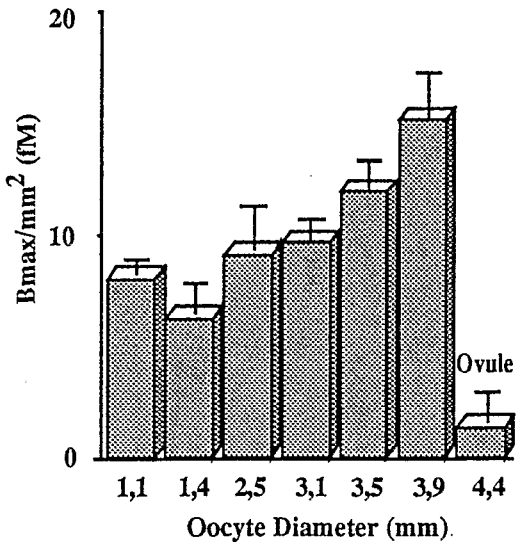

Fig.. 3. Maximum Binding per mm^2

The slope of linear regression of binding data per surface unit versus diameter indicates a two fold increase of the number of receptors between the beginning of vitellogenesis and the end, prior to maturation.

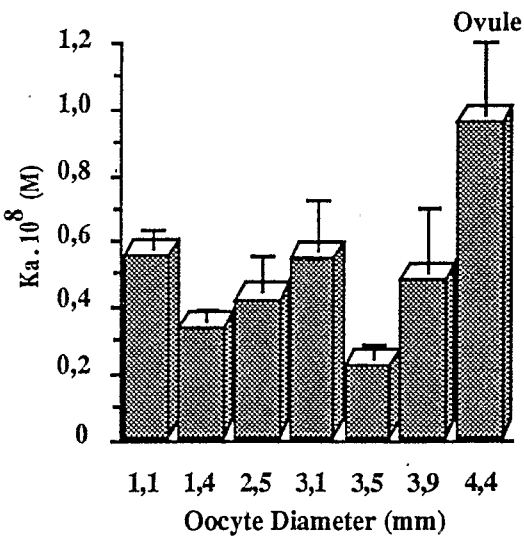

Fig. 4. Variations of Ka with oocyte diameter.

Ka expressed in fM of vitellogenin/mm^2 is almost constant (Fig.4).

Maximum binding expressed in fM of ligand per follicule is regularly increasing until maturation. Ovules exhibit a decrease of VTG binding (Fig.5).

Conclusion

Our experiments demonstrate the existence of vitellogenin receptors in fish oocytes characterized by low affinity and high capacity. These characteristics correspond to the definition of type II receptors.

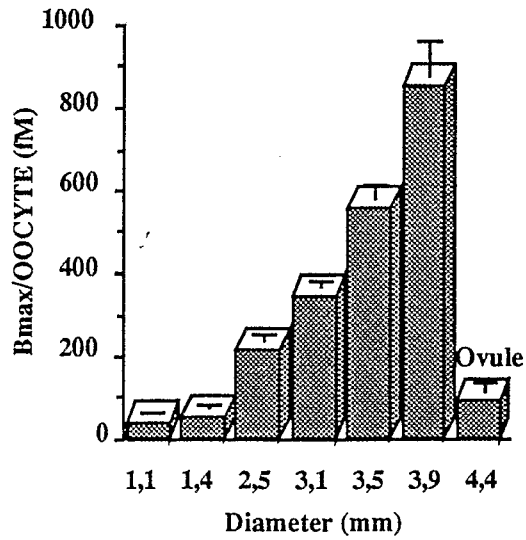

Fig. 5. Maximum Binding per follicle.

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