



Characterization of *Felis catus* oviductin expression in dependency of ovarian cycle

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Introduction

During the oestrus cycle, the mammalian oviduct undergoes significant changes leading to a microenvironment for maturation and transport of gametes, capacitation of spermatozoa, fertilization and early embryonic development. The high molecular weight glycoprotein oviductin, synthesized and released by the non-ciliated epithelium, is known to associate with various structures of the oocyte, spermatozoa and early embryo, indicating a potential biological role of this protein during fertilization and development.

Recently, we detected oviductin in feline oviducts and identified the full-length cDNA sequence. The objective of the current study was to investigate expression of oviductin mRNA and protein within the ampulla and isthmus during the oestrus cycle in cats.

Materials & Methods

Immunohistochemistry

Oviducts were obtained from domestic cats, classified according to the ovarian state and prepared for immunohistochemical investigation. For labelling we used the human anti-oviductin N-20 antibody (Santa Cruz, 1:50 dilution in 1% BSA) and HRP-conjugated mouse anti-goat IgG (1:50). The peroxidase colour reaction was developed by incubating in diaminobenzidine (DAB⁺) substrate chromogen solution (Dako).

Semiquantitative RT-PCR Analysis

Total RNA was isolated from oviductal tissue using the Precellys Tissue RNA kit (Peqlab), reverse-transcribed and the cDNA was amplified using polymerase chain reaction (PCR).

After gel electrophoresis, the visualized PCR products were analyzed with the ImageJ (National Institutes of Health) processing program. ImageJ detects the mean grey value of the bands. Using β -actin as a standard, the relative intensity was calculated.

Results

Highest oviductin expression was found in the late follicular stage. During the luteal or inactive phase, mRNA is weakly expressed and protein, synthesized in the non-ciliated secretory cells, is almost undetectable. The glycoprotein was present both in the ampulla and isthmus compartment (Fig. 3) and there were no differences on the mRNA level between the two oviductal regions (Fig. 1, 2).

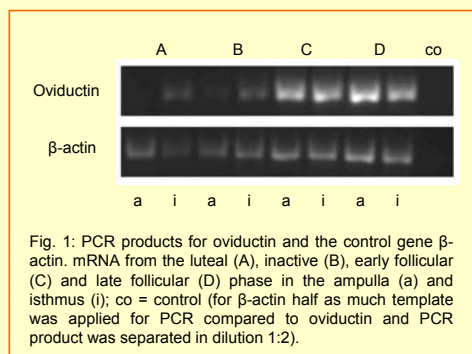


Fig. 1: PCR products for oviductin and the control gene β -actin. mRNA from the luteal (A), inactive (B), early follicular (C) and late follicular (D) phase in the ampulla (a) and isthmus (i); co = control (for β -actin half as much template was applied for PCR compared to oviductin and PCR product was separated in dilution 1:2).

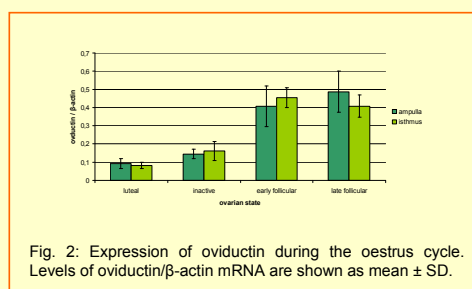


Fig. 2: Expression of oviductin during the oestrus cycle. Levels of oviductin/ β -actin mRNA are shown as mean \pm SD.

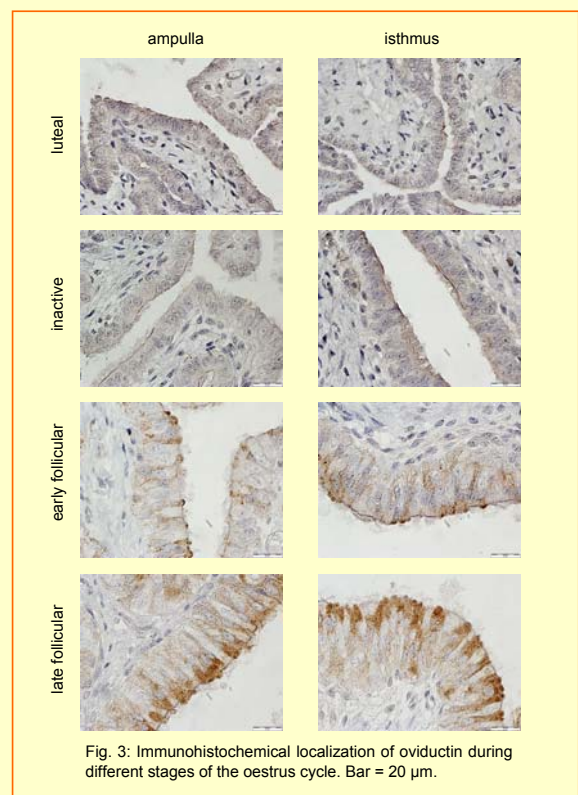


Fig. 3: Immunohistochemical localization of oviductin during different stages of the oestrus cycle. Bar = 20 μ m.

Summary

The present study demonstrate a cycle dependent oviductin expression, suggesting an oestrogen-induced upregulation of this protein in cats. Moreover, the results confirm the potential role of oviductin during reproductive events since the highest expression was detected in late follicular samples.

Additional studies utilizing purified native or recombinant oviductal glycoprotein will be used to supplement *in-vitro*-fertilization protocols and to identify the binding partner(s) of oviductin in the feline oviduct.