

# Inducible and endothelial nitric oxide synthases (NOS) mRNA in the bovine ovary



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## Introduction

Nitric oxide (NO) is a highly reactive molecule, which is able to pass membranes by diffusion. It is known to act as a paracrine mediator during various processes associated with female reproduction by generating NO from L-arginine by nitric oxide synthases (NOS). The role of NO in the regulation of folliculogenesis is substantiated by its involvement in the control of ovulation rate, ovarian apoptotic cell death and granulosa-luteal cell steroidogenic activity (1). In the present study, the mRNA expression of the inducible (iNOS) and endothelial (eNOS) NO-synthases were examined in the bovine ovary focusing on the follicular development and the corpus luteum (CL) during the estrous cycle and pregnancy. Additionally, an *in vitro* approach using isolated granulosa cells (GC) the effects of exogenous applied gonadotropins LH and FSH were analysed.

## Materials and Methods

Bovine ovaries were collected at the local slaughterhouse and grouped depending on the stage of the cycle. A classification of follicles (<0.5; >0.5-5; >5-20; >20-180; >180 ng/ml) was performed according to the estradiol-17 $\beta$  (E) content of follicular fluid (FF). The corresponding size of follicles was 5-7, 8-10, 10-13, 12-14 and >14 mm, respectively. Follicular tissue was separated in theca interna (TI) and GC. CL were assigned to the following stages; Days 1-2, 3-4, 5-7, 8-12, 14-17, >18 of estrous cycle and of early and late pregnancy (<4 and >4 month). Tissues were dissected in small pieces and snap-frozen in liquid nitrogen. For *in vitro* investigation, GC were recovered by rinsing antral follicles with PBS. After 1.5 days of preculture, the GC were treated with FSH, LH and FSH+LH, respectively (0.01 IU/mL of bovine FSH and/or LH) for 4 or 24h. Total RNA of both tissues and cells was extracted and reverse transcribed. Resulting cDNAs were amplified by conventional PCR and quantitative real-time PCR (LightCycler®) using specific primers for iNOS and eNOS.

## Results and Discussion

During follicular growth the mRNA expression of eNOS did not vary ( $p>0.05$ ) in TI, while iNOS was upregulated ( $p<0.05$ ) in large follicles (Fig.1). In GC, iNOS expression tendentially increased whereas eNOS decreased with follicular size and increasing content of E2 in the FF (Fig.1). iNOS mRNA concentration in CL tended to increase during the late luteal phase (Days 13-18) and regression (Days >18), but markedly decreased ( $p<0.05$ ) during pregnancy (Fig.2). eNOS showed highest ( $p<0.05$ ) mRNA expression during the early luteal phase compared to the late luteal phase, regression and pregnancy. *In vitro*, iNOS responded to gonadotropin stimulation with strongest ( $p<0.05$ ) mRNA expression after exposure to LH followed by FSH ( $p<0.05$ ) (Fig.3). The physiological functions of NO/NOS in follicular development have not yet been clarified. A positive correlation between follicular size and follicular NOS concentration has been reported (2). One may speculate that iNOS derived NO in GC and TI may act in an paracrine/autocrine fashion to cause local vasodilatation and mediate, in part, the increase in blood flow to the developing follicle. Data from the CL suggest that iNOS may be the main NOS isoform involved in the luteolytic process while eNOS may enhance the formation of the CL.

## Conclusion

In conclusion, these data demonstrate the presence of an intraovarian NO-generating system. eNOS and iNOS mRNA were differently expressed in the bovine ovary in a cell-specific manner and iNOS transcripts were stimulated by gonadotropins *in vitro*. Distinct expression patterns of both NOS suggest a participation in the final growth of the preovulatory follicle and in the regulation of CL function.

## References

- (1) Jablonka-Shariff A. and Olson L. (1997). *Endocrinology* 138, 460-468
- (2) Anteby et al. (1996). *Human Reproduction* 11, 1947-1951

## NO production by NO-synthases

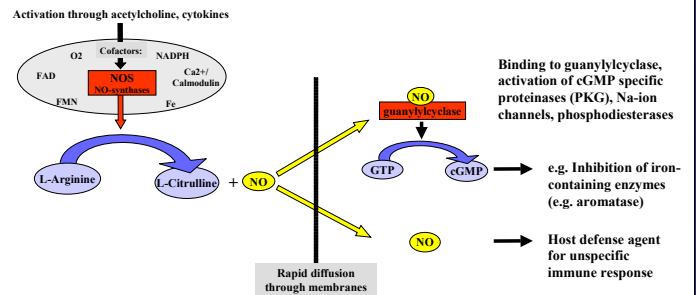


Fig.1: iNOS and eNOS mRNA during follicular development *in vivo*

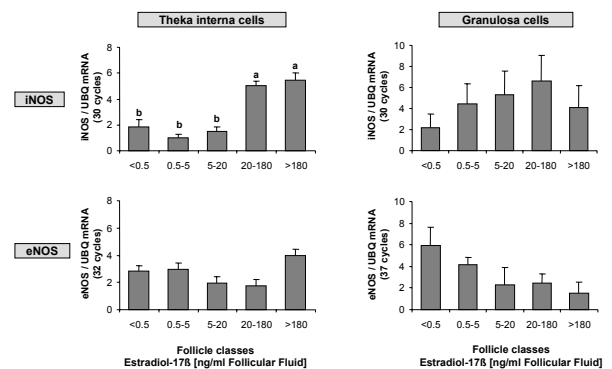


Fig.2: iNOS and eNOS mRNA during luteal growth *in vivo*

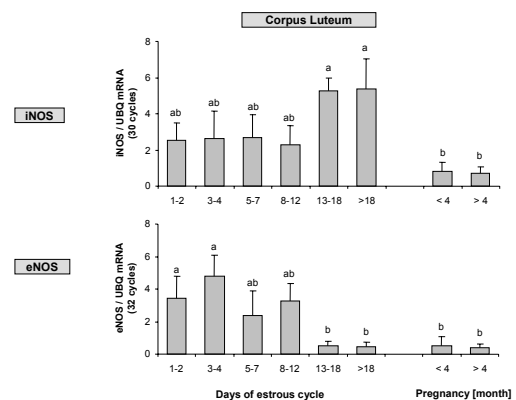
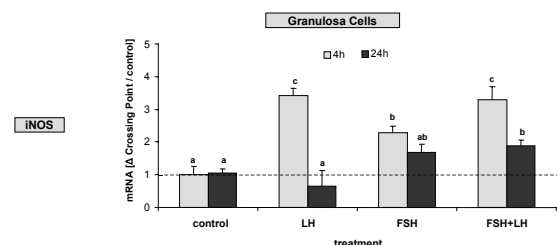


Fig.3: iNOS mRNA in GC after gonadotropin treatment *in vitro*



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