EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS IN FOLLICLES AND CORPORA LUTEA IN THE BOVINE OVARY DURING DIFFERENT DEVELOPMENTAL AND FUNCTIONAL STAGES

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INTRODUCTION

The ovarian steroid hormones estrogen and progesterone fulfill a number of important functions related to reproduction by endocrine mechanisms of action. In addition to acting as hormones on structures remote from the ovary, the steroids produced by follicle or corpus luteum cells also act locally within the follicles or corpora lutea as paracrine/autocrine agents, acting on or within the cells in which they are produced.

Most of genomic actions of oestrogens are mediated by two oestrogen receptors ERα and ERβ, and for gestagens in ruminants by the progesterone receptor (PR). The aim of the present study was to evaluate the expression pattern of mRNA for ERα, ERβ and PR by block-RT-PCR and sensitive, quantitative real-time RT-PCR (LightCycler) in bovine antral follicles during final growth to the preovulatory stage, and in luteal tissue by collection of CL from different stages during the estrous cycle and pregnancy.

MATERIAL AND METHODS

The follicles and corpora lutea (CL) from mainly German Fleckvieh heifers and cows were collected from a local slaughter house within 10-20 min after slaughter. The stage of the cycle was defined by macroscopic observation of the ovaries (follicles and corpus luteum) and the uterus (size, colour, consistency, connective tissue and mucus). For RNA extraction, CL tissue, granulosa cells (GC), and the theca interna (TI) were separated, and RNA isolated by means of a guanidinium isothiocyanate/phenol extraction method. Follicles that were in the final growth stages (≥4-5 mm in diameter) were classified according to the estradiol (E_2) content in follicular fluid (FF) and were divided into five classes (E_2 < 0.5; 0.5-5; 5-20; 20-180, and >180 ng/ml FF). The CL were accordingly assigned to the following stages; Days 1-2, 3-4, 5-7, 8-12, 13-18, >18 (after regression) of estrous cycle and of early and late pregnancy (<4 and >4 month). The expression of mRNA for the ERα, ERβ and PR during follicle development and the corpus luteum was evaluated by means of block-reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time RT-PCR.

RESULTS AND DISCUSSION

The results of mRNA expression (LightCycler RT-PCR) of the steroid hormone receptors ERα, ERβ and PR in in bovine ovarian follicles during final growth and in CL during estrous cycle and pregnancy are shown in Fig. 1, Fig. 2 and Fig. 3. The mRNA expression of ERα and ERß mRNA in theca interna tissue (lower pg/µg RNA) increased continuously and significantly during final growth of follicles, with much higher levels for ERα (Fig. 2a). The mRNA expression of ERα and ERß in granulosa cells (fg/µg RNA) increased continuously during follicles growth but without any significant change (Fig. 2b) . The expression of mRNA for PR in follicles (lower fg/µg RNA) increased continuously to maximum level in pre-ovulatory follicle with significant change only in TI (Fig. 2c). The highest mRNA expression for ERα (fg/µg RNA) was detected in corpus luteum during the early luteal phase, following by a significant decrease of expression during the mid, late and regression phase (Fig. 3a). In contrast, ERβ mRNA expression (Fig. 3b) is relative high during the early stage, decreased during the late early and mid luteal phase and increased significantly again during late luteal phase and after CL regression. During pregnancy (>3 month), low levels of ERα and ERß mRNA expression (<25 fg/µg RNA) with no significant changes were measured. No significant change in PR mRNA expression (levels <13 fg/µg RNA) during the estrous cycle and pregnancy in bovine CL were found (Fig. 3c). The results suggest an autocrine/paracrine role of steroid receptors in the regulation of final follicle growth and corpus luteum formation and function.

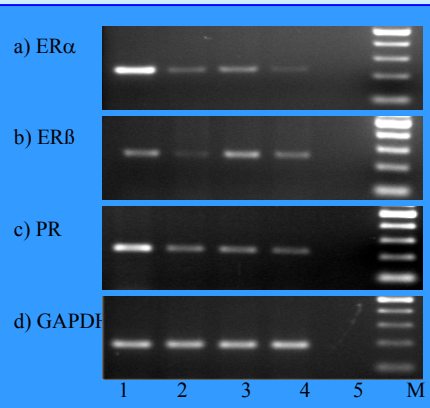

Figure 1: *Representative sample of specific RT-PCR products for a) ERα (234 bp); b) ERβ (262 bp); c) PR (227 bp) and d) GAPDH (197 bp) from: 1) bovine endometrium; 2) corpus luteum; 3) theca interna; 4) granulosa cells 5) no template control and M) DNA mass ladder (100-500 bp), separated by agarose gel electrophoresis.*

CONCLUSIONS

In conclusion, the presented results give evidence for the presence and possible function of ERα, ERβ and PR in bovine follicle and corpus luteum. The results support the hypothesis that ERα is the dominating and positive acting (stimulation and mitogenic activity) factor for follicle maturation and corpus luteum formation and function.

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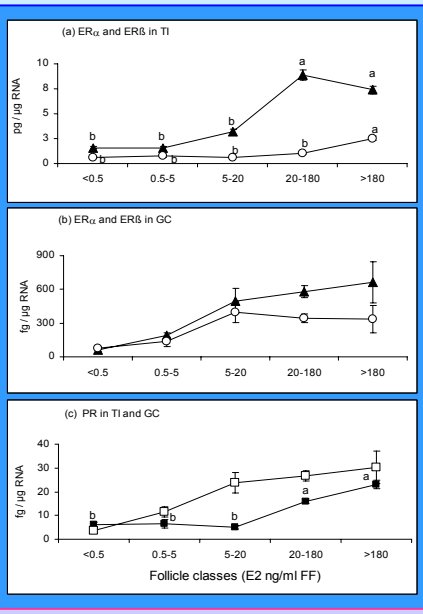

Figure 2: *Tissue-specific ERα, ERβ and PR mRNA expression (LightCycler real-time RT-PCR) in different bovine follicle classes: (a) ERα in TI (▲) and ERβ in TI (○); b) ERα in GC (▲) and ERβ in GC (○); c) PR in TI (■) and PR in GC (□). Results (specific mRNA / µg total RNA) represent means ± SEM from 4 – 5 follicles / class. Different superscript letters indicate significant differences between groups (P<0.05).*

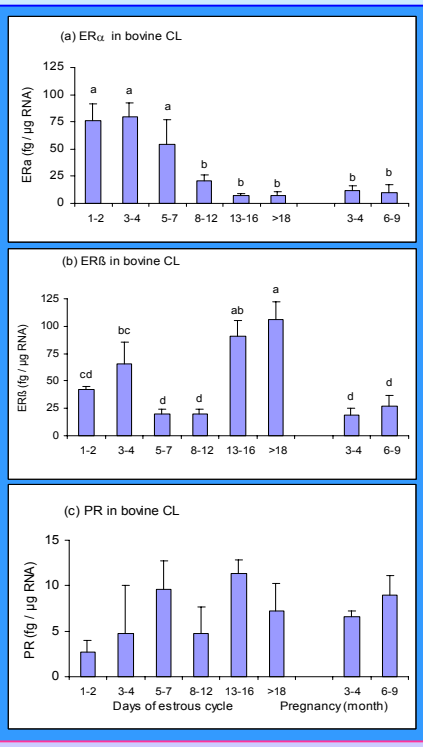

Figure 3*: Steroid receptor mRNA expression (LightCycler real-time RT-PCR) in bovine corpus luteum during the estrous cycle and pregnancy: (a) ERα; (b) ERβ and (c) PR. Results (specific mRNA / µg total RNA) represent means ± SEM from 4 - 6 CL / stage. Different superscript letters indicate significant differences between groups (P<0.05).*