Comunicazioni orali Sira

DYNAMIC CHANGES OF LH RECEPTOR CONTENT IN CORPORA LUTEA DURING THE BOVINE ESTRUS CYCLE

Ostuni A.[1], Cuviello F.[1], Mascolo A.[1], Boni R.*[1]

[1]Dipartimento di Scienze ~ Potenza

Luteinizing hormone (LH) plays a crucial role in the development and maintenance of the corpus luteum (CL) in cattle. Upon binding to its receptor (LHR), LH stimulates adenylyl cyclase activity. The consequent production of cyclic adenosine 3',5'-monophosphate causes an increase of steroidogenesis, and, in particular, progesterone production (Davis et al., 1996). Based on this paradigm, it is possible to assume the amount of LHR in CL as an indicator of the progesterone secretory capacity and, hence, CL competence to support an adequate luteal phase.
CL life span in relation to the estrus cycle may represent the most important physiological source of variation of LHR content. Such variability has been previously evaluated in bovine LHR transcriptional expression (Kawate et al., 1998); however, to our knowledge, there are no information related to LHR protein.

For this study, bovine ovaries were collected from slaughterhouse and stored at 4°C. After a gross morphological in situ evaluation (Ireland et al., 1980), CLs were classified in relation to the estrus cycle stage (estrus= day 0); in particular, Stage I, II, III and IV included intervals between days 3-4, 8-11, 14-16 and 17-19, respectively.
Frozen/thawed CL samples were kept in lysis buffer on ice for 1 h, homogenized and centrifuged at 13000 rpm for 10 minutes. Proteins (100 µg) were resolved on SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. After blocking with non fat dry milk in phosphate buffered saline with 0.05% Tween 20 (PBS-T), membranes were incubated overnight at 4°C with 1:200 goat anti-LHR antibody (Santa Cruz Biotechnology) and with 1:500 mouse anti-α-tubulin antibody (Sigma-Aldrich) followed by incubation with 1:5000 donkey anti-goat IgG-HRP antibody (Santa Cruz Biotechnology) and with 1:3000 goat anti-mouse IgG-HRP antibody (Sigma-Aldrich), respectively. Proteins were detected with Pierce ECL Plus WB Substrate (Thermo Scientific) and CL-XPosure Film (Thermo Scientific). The films were scanned with GelDoc-It (UVP) using Vision Works LS software and proteins were quantified by ImageJ software. LHR/α-tubulin band ratios were analyzed by ANOVA (Systat 11.0).

A significant ($P< 0.001$) decrease of LHR protein content was found through the CL life span. In particular, LHR protein pattern showed the highest content at Stage I (93.7±19.3), a rapid decline at Stage II (28.2±6.6) and a smaller decline in the later stages (Stage III=26.5±9.0 and Stage IV=16.8±1.1).
This pattern may reflect a dependence of the CL to LH stimulus during its preliminary growth and a progressive refractoriness to such influence in later stages of CL life span.

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Kawate and Okuda. Mol Reprod Dev 1998, 51:66-75.
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Reproductive Biology
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