LH RECEPTOR CONTENT IN BOVINE LUTEAL CELLS DURING THE ESTRUS CYCLE

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Corpus luteum (CL) dysfunction represents one of the main causes of embryonic mortality in cattle. This may be related either to the origin of the gland or to the activity of other glands/tissues which are responsible for maintenance or regression of the CL. Luteinizing hormone (LH) is considered among the main factors in maintaining the CL in cattle. The dynamics of changes in the content of the LH receptor (LHR) in the bovine CL during the estrous cycle was evaluated in a previous study (Ostuni et al., 2014). The purpose of the present study was to evaluate the presence of LHR in CL cells obtained in different phases of the estrous cycle. Bovine ovaries were individually collected from slaughterhouse and stored at 4°C. After a gross morphological in situ evaluation (Ireland et al., 1980), the CLs found were classified in relation to the estrus cycle stage (estrus= day 0); in particular, Stage I, II, III and IV included intervals between days 3-5, 6-9, 10-15 and 16-19, respectively. CL slices were obtained by cutting along the sagittal axis of the gland; they were finely minced and shaked at 1500 rpm at 37°C for 90 min in PBS containing 0.1% PVA, Collagenase II, DNAse I, penicillin and streptomycin. The dissociated cells were filtered through a 50 μm nylon mesh to remove undigested tissue fragments. Cell suspensions were added with 20% FCS in PBS, centrifuged and then fixed with 2% paraformaldehyde in PBS for 60 min. After blocking for 30 min in 5% bovine serum albumin in PBS and double washing, cells were incubated for 90 min with 1:200 goat anti-LHR antibody (Santa Cruz Biotechnology) and then for 60 min with 1:100 donkey anti-goat IgG-FITC antibody (Santa Cruz Biotechnology) and DAPI. Cells were analyzed by either laser confocal or fluorescence microscope in order to test results by using two different and independent equipments. Background-subtracted fluorescence intensity (FI) data were analyzed by ANOVA (Systat 11.0) and related to the estimated estrus cycle stage and the donor age. The estrus cycle day played a significant role ($P<0.001$) on LHR content in CL cells In particular, FI increased from I to II stage and dropped down in cells belonging to CL older than 15 days. A significant difference of FI was also found between CL cells collected from cows and heifers ($P<0.01$). A very high correlation ($R=0.962$; $P<0.01$) was found between confocal and fluorescence microscopy evaluation. It was difficult to discriminate between large and small luteal cells, since dissociated LHR positive cells ranged from a small to a large size without any clear size discontinuity. Assuming that small and large CL cells represent 88.5% and 11.5% of the total steroidogenic cells, respectively (Wildbank, 1994) and considering the level of these threshold values, we did not found differences between these two cell categories. In conclusion, LHR content in luteal cells follows the same pattern already found in luteal tissue in toto; this further supports LHR as a reliable marker for evaluating CL quality.

Ostuni et al., Proc. SISVet 2014, 314.
Ireland et al. J Dairy Sci 1980, 63:155-160.
Wildtbank J Anim Sci 1994, 72:1873-1883.