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The effect of lipopolysaccharides on the expression of genes involved in steroidogenesis and inflammatory response in bovine granulosa cells

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The lipopolysaccharides (LPS) induce inflammatory response and have been detected in follicular fluid (FF), uterine fluid and plasma of cows with metritis and with infectious mastitis. LPS acts in the hypothalamus or pituitary gland, inhibiting the release of gonadotropins. However, LPS can have also a direct effect in the ovary, especially in the theca and granulosa cells, affecting the steroidogenesis. The objective of this study was to evaluate the effect of a LPS challenge on the expression of genes involved in steroidogenesis and activation of the inflammatory response in granulosa cells of the dominant follicle in cows. For this, 20 Jersey cows were randomly assigned to 2 groups: control group (n = 10) and LPS group (n = 10). The follicular wave in both groups was synchronized using an intravaginal progesterone releasing device (CIDR; Eazi-Breed CIDR®, Zoetis Animal Health, NJ, USA) and 2 mg of estradiol benzoate (Hertape Calier, MG, Brazil) on day 0. Twelve hours before CIDR removal (day 8), cows received an IM injection of PGF2α (25 mg of dinoprost tromethamine, 5 ml of Lutalyse, Zoetis Animal Health). Two hours after the CIDR removal a single dose of intramuscular LPS (2.5 ug / kg BW, Sigma-Aldrich® Inc., MO, USA) was administered in the cows of LPS group and a placebo IV solution (2 mL NaCl 0, 9%) in the control group. Six hours after LPS challenge, the dominant follicle of each animal was identified by ultrasound, and aspirated. The granulosa cells were recovered by FF centrifugation. Rectal temperature was measured five hours after the LPS challenge. Total RNA from granulosa cells was extracted using Trizol® and mRNA expression of target genes was carried out using qRT-PCR (Applied Biosystems, Foster City, CA, USA) using the H2α gene as endogenous control. For statistical analysis the Mann-Whitney test was used (GraphPad Software Inc., La Jolla, CA, USA). The cows receiving LPS had a systemic inflammatory response, supported by higher rectal temperature (40.4 ± 0.1°C) compared to the control group (38.8 ± 0.1°C) ($P < 0.05$). Furthermore, there was a 91% reduction in the expression of *TLR4* ($P = 0.002$) and 89% reduction in the expression of TNF ($P = 0.001$) in cows challenged with LPS, both genes are important regulators of the immune response. Moreover, mRNA expression of *STAR*, an important regulator of steroidogenesis, was decreased 93% ($P = 0.01$) in cows receiving LPS. No difference was observed in the expression of the genes *NF-kB*, *CYP19A1*, *P450C17*, *LHCGR*, *CYP11A1* and *HSD3B1* between groups ($P > 0.05$). Intrafollicular estradiol concentration was not different between groups ($P > 0.05$). In conclusion, LPS induced an inflammatory response in the granulosa cells of dominant follicle, mediated by the *TLR4*, but through mechanisms that should be further investigated. Moreover, LPS can affect the production of steroid hormones in the long term, since it drastically reduced *STAR* expression.