Title: Association of catalase enzymatic activity in bovine follicular fluid with both the phases of folliculogenesis and the stages of the estrus cycle

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Objective: Oxidative metabolism is essential for gamete and embryo energy production and is unavoidably associated with the generation of reactive oxygen species (ROS). Catalase (CAT) is an enzymatic antioxidant expressed in the mammalian oocytes which scavenges the damaging oxygen products. The objective of this study was to correlate the CAT enzyme activity in follicular fluid with phases of folliculogenesis and stages of the estrus cycle.

Design: Prospective study.

Materials and Methods: Bovine ovaries were collected from the slaughterhouse (Champlain Beef Co., Whitehall, NY) within 30 minutes of death of the animal and placed in room temperature saline solution with antibiotic. These samples were obtained from naturally cycling cows (11/2-3 yrs). The follicular fluid collected from antral follicles of different sizes ranging from 2-25 mm was spun (7000g for 3 min) to remove all cellular contaminants, aliquoted, and frozen at -80°C. After processing of the follicular fluid, the antioxidant CAT was measured with a chemiluminescence method. The phase of folliculogenesis was assessed by examining the range of follicle sizes along with the presence of a corpus luteum (CL). Folliculogenesis was divided into four phases: 1) early (all follicles ≤8mm), 2) middle (all follicles between 8 and 15 mm), 3) late (largest follicle ≤15mm), and 4) luteal (all follicles ≤8mm with the presence of one CL). Stages of the estrus cycle were estimated based on the presence and features of the corpus luteum, with stage I corresponding to days 1-4, stage II days 5-10, stage III days 11-17 and stage IV days 18-20 of estrus cycle.

Results: The average CAT level was 22.98 ±17.97 μM. There was a statistically significant change in the levels of catalase activity throughout the different stages of the estrus cycle (p<0.001). The dynamics of the CAT activity change were especially significant between stage 1 vs. 2 (p<0.001), stage 1 vs. 3 (p<0.004) and stage 1 vs. 4 (p<0.039) by a pair wise sub group analysis with Kruskal-Wallis test. Significant differences were seen between corpus luteal stage 2 vs. 3 (p<0.007).

Conclusions: The above study showed significant changes in the follicular fluid levels of CAT enzyme according to the different stages of the estrus cycle and within stage II and III corpus luteal stages. Characterization of the antioxidant profiles in different compartments of follicles at stages of folliculogenesis may help characterize the antioxidant requirements needed to optimize in-vitro culture media.

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