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A100E Folliculogenesis, oogenesis, and superovulation

### **PGE2 concentration of the follicular fluid as a measure of heterogeneity of the response to hormonal stimulation of the bovine ovarian follicle**

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The LH surge promotes prostaglandin E2 (PGE2) production within the preovulatory follicle. Oocyte microenvironment levels of PGE2 affect the developmental competence after fertilization. This study aimed to characterize the follicular fluid PGE2 enrichment during superovulation treatment. Six heifers (Holstein, 20.3 +/-0.85 months old) received FSH (Stimufol®, Reprobiol, Belgium): half dose, ie, 250 µg of porcine follitropin (pFSH), combined with 50 µg of porcine lutropin (pLH). At the rate of 8 injections, in degressive dose, spread over 4 days. PGF2α (Estrumate®, MSD Santé Animale, France) was injected at the same time as the 5th injection of Stimufol®. LH peak was assumed to occur between 35 and 40 hours after the PGF2α injection. Individual sampling of fluid from antral follicles was performed by ovum pick up 12 hours before PGF2α injection and 60 hours after PGF2α injection. This protocol was designed to allow the collection of fluids from ovarian follicle containing either a pre-matured or a matured oocyte. Each heifer was his own control as we took the "pre-matured" follicular fluid on a first ovary and the "matured" follicular fluid on the 2nd ovary, 3 days later. The punctures were repeated twice and were cross-checked for the next repetition to evaluate the impact of the ipsi or contralateral side of the corpus luteum (CL) on the follicular fluid composition. The volume of fluid was measured for each punctured follicle. The PGE2 concentration of the follicular fluid was measured by Elisa (Cayman Chemical) to determine the progress of terminal follicular differentiation. An average of 13 +/- 5.06 and 28 +/-13.9 follicles were punctured per session/heifer for respectively pre-matured (n=78) and matured (n=169) follicles. The mean collected volume differed between the two groups (pre-matured: 0.229 +/- 0.213 ml/follicle; matured: 0.575 +/- 0.379 ml/follicle; two samples t-test, pval<0.0001). No effect of the side of CL on fluid volume was detected (2-way Anova, p=0.397). The PGE2 concentration was determined in 25 pre-matured follicles and 127 matured follicles. The mean PGE2 concentration significantly differed between the two groups of follicular fluids (pre-matured: 7.2 +/-7.5 ng/ml; matured: 60.2 +/-58.6 ng/ml) No effect of the side of CL was detected (p=0.278). Surprisingly, there was no linear relationship between fluid volume and PGE2 concentration (adjusted R-squared: -0.0002, p-value=0.327). PGE2 concentrations were very spread out within the matured group. This important dispersion (Interquartile range=58.6 ng/ml) indicated that despite follicle growth in response to hormonal stimulation (FSH/LH) the ability of follicular granulosa and cumulus cells to synthesize PGE2 was imperfectly achieved. Only 48% of the follicular fluids in the mature group had higher PGE2 levels than those in the premature follicle group. In conclusion, despite the ability of the stimulation treatment to promote growth of many follicles, there was a great heterogeneity in terms of PGE2 synthesis. This alteration could represent defective signaling mechanisms that could impact the developmental competence of the oocyte.

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