Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

**Folliculogenesis, oogenesis and superovulation**

# Follicular fluid progesterone variation due the proximity to the corpus luteum can affect bovine cumulus cells molecular pattern

Marina Port Lamellas [1], Paola Maria da Silva Rosa [1], Giuliana de Ávila Ferronato [1], Natália Marins Bastos [1], Alessandra Bridi [1], Gislaine dos Santos [1], Juliana Germano Ferst [1], Felipe Perecin [1], Flávio Vieira Meirelles [1], Juliano Coelho da Silveira [1]

[1] FZEA USP - Faculdade de Zootecnia e Engenharia de Alimentos - Universidade de São Paulo (R. Duque de Caxias, 225 - Jardim Elite, Pirassununga - SP, 13635-900)

## Resumo

Progesterone (P4) is a well-known hormone due to its association with oocyte functions and its role in establishment and the maintenance of pregnancy. In this study, we hypothesized that the follicular environment is exposed to different P4 levels, according to the proximity of the corpus luteum (CL), leading to molecular differences within the intrafollicular environment. To test this hypothesis, ovaries from local slaughterhouse were collected in pairs and separated in groups ipsilateral and contralateral to the CL, according to morphological characteristics associated with stage 3 of the estrous cycle (Ireland et al. 1980. J Dairy Sci. 63:155–160). Small follicles (3-6 mm in diameter) were aspirated from each group and cumulus oocyte complexes (COCs) were collected. Additionally, follicular fluid was used to measure the P4 levels by immune assay. Next, the cumulus cells (CCs) from immature COCs were collected and frozen (-80°C) for gene expression analysis. The CCs were submitted to total RNA extraction according to Trizol® (Thermo Fisher Scientific) protocol, with an RNA co-precipitant (GlycoBlue®; Thermo Fisher Scientific), treated with DNase Amplification Grade (Invitrogen, Brazil) followed by the reverse transcription using the High-Capacity Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). The RT-qPCR analysis of FSHR, LHR, CYP17A1, 3b-HSD, CYP19A1, ADAMTS1, PGR, PAQR8, PAQR5, PGRMC1, PGRMC2, ESR1, ESR2, GPER1, BAX and BCL2 transcripts were performed using the Power SYBR Green PCR Master Mix kit (Thermo Fisher Scientific), according to the manufacturer's instructions. The relative expression of progesterone-related genes involved in oocyte maturation (PGR, PAQR8, PAQR5, PGRMC1, PGRMC2, ESR1, ESR2, and GPER1), steroidogenesis (FSHR, LHR, CYP17A1, 3b-HSD, CYP19A1 and ADAMTS1) and apoptosis (BAX and BCL2) on CCs from ipsilateral and contralateral follicles were analyzed in 6 and 4 replicates/group, respectively. Expression levels were calculated using the 2ˆ-ΔCt method, and normalized by the geometric mean of PPIA and YWHAZ as reference genes. P4 concentration and relative gene expression data (mean ± SEM) were tested for outliers' presence, normality (Shapiro-Wilk test) and were analyzed by Student's t-test ($p<0.05$) (GraphPad Prism; Software). The intrafollicular P4 concentration in ipsilateral and contralateral was 348.38 ng/mL and 91.06 ng/mL, respectively ($p=0.0018$). The intra and inter-assay were 13.42 ng/mL and 30.02 ng/mL for progesterone, respectively. The results demonstrated that ADAMTS1 relative expression increased in CC from ipsilateral follicles compared to the contralateral ($p=0.01$). ADAMTS-1 is a secreted protease that is involved in several biological functions important for oocyte competence and ovulation. In conclusion, different P4 levels, due corpus luteum proximity, modulates CCs at molecular levels which can impact oocyte quality.

**Funding:** FAPESP 2021/06645-0 and CAPES finance code 001.