

developmental competence. Manipulation of the culture environment during this period of meiotic arrest could be a novel means of improving the quality of feline oocytes.

**P467 - Effect Of Physiological Heat Stress On HSP70 And CASPASE 3 Levels In Bovine Oocytes.** Luiz Camargo, Luiz Siqueira, Naiara Saraiva, Gilson Maia, Beatriz Nogueira, Carolina Quintao, Clara Oliveira

CASPASE 3 is an apoptosis downstream effector enzyme. HSP70 chaperones help to fold many proteins and are upregulated in response to stress. We wanted to evaluate if CASPASE 3 and HSP70 would be affected after physiological heat stress and could be used as markers of thermal injury in bovine oocytes. Girolando (Gir x Holstein crossbred) cows were housed for 23 days in controlled-climate chamber programmed with three climatic conditions along 24h: THI (Temperature and Humidity Index)=74.8 between 00:00 and 10:00h; THI=88.6 from 10:00 to 18:00h and THI=79.7 from 18:00 to 00:00h, with 12h light and 12h dark. Control group was cows housed in a free-stall barn located beside the climate chamber with an average THI=66.5. After the end of induced-heat stress, immature oocytes were collected by ultrasound-guided ovum pick-up (four replicates). Cumulus cells were removed and fixed in paraformaldehyde. Oocytes (n=197) were stained for immunofluorescence using primary antibodies mouse anti-HSP70 (SCBT, 1:50) and rabbit anti-active CASPASE 3 (Sigma, 1:750) and secondary antibodies Alexa 555-conjugated donkey anti-rabbit and Alexa 488-conjugated donkey anti-mouse. Images of each oocyte were recorded and analyzed for average fluorescence level in ImageJ software. Results are shown as mean $\pm$  SEM. Means were compared using the T-test. Levels of CASPASE 3 ( $14.9 \pm 1.2$  vs  $15.8 \pm 0.8$ , heat-stressed and controls, respectively;  $P=0.54$ ) and HSP70 ( $6.9 \pm 0.5$  vs  $7.7 \pm 0.6$ , heat-stressed and controls, respectively;  $P=0.31$ ) did not differ between groups. Our results suggest that HSP70 levels remain unaltered in bovine oocytes after short-term physiological heat stress. This highlights the importance of non-HSP70-based responses in bovine oocytes subjected to heat stress. Similarly, the apoptosis cascade was not affected by heat stress. In conclusion, CASPASE 3 and HSP70 may not be reliable markers of thermal injury in immature oocytes derived from heat-stressed Girolando cows. Financial support: CAPES, Fapemig and Faperj.

**P468 - Correlation analysis between C natriuretic peptide and pregnancy outcome.** Lidan Guo, Weina Yang, Donghui Huang

**Aim:** CNP was proved to played a key role in female reproduction, and be related to oocyte quality. This study were to found the relationship of CNP with pregnancy outcome, to provide a new indicator to access pregnancy outcome. **Methods:** Follicular fluid(FF) were collected from 158 patients undergoing IVF/ ICSI procedure at the Center for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology. CNP and cGMP levels in human FF were tested by ELISA. Then Distribution Patterns of CNP and NPR-B from GV oocyte to blastocyst in mouse were tested by Confocal microscopy. Finally CNP were added in the fertility or embryo development medium respectively, to observe the development of embryo. **Results:** CNP levels in FF from non-pregnancy patients is significant higher than that from pregnancy patients. a strong positive correlation between CNP and cGMP concentrations in