P 142 | Study of advanced DNA fragmentation parameters in fertile and infertile stallions

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Diminished male fertility has been related to DNA fragmentation, which can be assessed by flow cytometry by Sperm Chromatin Structure Assay (SCSA). SCSA yields a DNA fragmentation index (%DFI) and distinguishes between high DNA (%hDFI) and moderate DNA (%mDFI) fragmentation; also a High DNA Stainability (%HDS) population due to lack of full protamination can be detected. Although these parameters are readily used in human sperm to predict fertility, in the horse, these values are still under investigation. In the present work we compared the differences existing between 2 fertile stallions (IP and JC) with >65% pregnancies per cycle and an infertile sire (MB; 0% pregnancy per cycle). Ejaculates (2 per stallion) were collected, diluted in TNE buffer and frozen at −80°C until analyzed. %DFI showed the highest value in the infertile stallion among all the studied (3.77% and 3.23% for MB; 0.52% and 0.61% for IM and 0.86% and 2.25% for JC); %hDFI varied from 0.06% to 0.19% in the fertile stallions, while for MB the values were 0.95% and 2.51%. %mDFI ranged from 0.33 to 2.06% in the fertile stallions and reached 0.72% and 2.82% in the infertile male. %HDS varied from 1.03% to 2.95% in all the stallions and did not discriminate between fertile and infertile stallions. Our preliminary data show that %HDS did not vary between fertile and infertile stallions, while other variables related to DNA fragmentation could discriminate between fertile and infertile stallions.

P 143 | Calcium Sensing Receptor (CaSR) regulates in vitro bovine embryo development but not bovine oocyte maturation

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Calcium Sensing Receptor (CaSR) is a ubiquitous G-protein coupled receptor present in different somatic cell types that modulates calcium homeostasis. The aim of this work was to study the role of CaSR in bovine oocyte maturation and embryo development. First, the presence of CaSR was demonstrated by immunofluorescence; then, bovine oocytes were matured in vitro in the presence or absence of 15 μM NPS2143 (a specific CaSR inhibitor) and chromatin conformation was assessed by fluorescence microscopy. To study the role of CaSR on embryo development, bovine oocytes were fertilized by conventional in vitro fertilization and subsequently incubated in TCM-199 in presence or absence of 15 μM NPS2143. On days 2, 6 and 7 after insemination cleavage, morula and blastocyst rates were evaluated. Maturation rate (mean % ± SEM; 72.5 ± 1.8; vs. 78 ± 5.4; $p > 0.05$), and cleavage rate (79.1 ± 6.8 vs. 73.7 ± 5.3; $p > 0.05$) were not modified by 15 μM NPS2143 addition compared to the control group (control vs. inhibitor respectively). Conversely, development to the morula stage (46.6 ± 7.3 vs. 24.3 ± 4.3; $p < 0.05$) and blastocyst rate (29.9 ± 9.0 vs. 9.9 ± 3.6; $p < 0.05$) decreased significantly in presence of NPS2143 (control vs. inhibitor, respectively). These results demonstrate the presence of CaSR in bovine oocytes and a core role in embryo development. In addition, CaSR does not seem to play a significant role during oocyte in vitro maturation in the bovine species.

P 144 | Effect of the FSH dose on superovulatory response in Santa Inês ewes

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This study aimed to evaluate the superovulatory response under effect of different FSH doses in superovulatory protocols. Santa Inês ewes (n = 24) received an intravaginal progesterone device (CIDR®) on Day 0, which remains until the Day 8. On Day 0 and 8 were also administered 0.125 mg of a synthetic analogue of $PGF_{2\alpha}$ (Sincrocio®). Gonadotrophic treatment started on Day 6 when females were divided into three groups according to the total dose of exogenous pFSH (Folltropin®): G1 (n − 8) – 100 mg; G2 (n = 8) – 133 mg; G3 (n = 8) – 200 mg. FSH total doses were administered in eight injections given twice a day in descending order. On Day 6, all ewes also received 300 IU of eCG (Novormon®). Ultrasonography was performed to assess the follicular growth of the wave. Six days after the onset of estrus, a videolaparoscopy for counting the number of corpora lutea and anovulatory follicles was performed. Data were compared using Kruskal Wallis test and posttest Dunns ($p < 0.05$) using the software R®. Rates were compared between treatments by Chi-square test. There were no differences ($p > 0.05$) among groups for ovulations (12.46 ± 5.93) and anovulatory follicles (0.96 ± 1.23) numbers and score of superovulatory response (1.5 ± 0.59). However, G1 showed higher ovulation rate (96.85 ± 3.53) compared to the G2 (86.30 ± 21.75) ($p = 0.016$) but similar to G3 (93.74 ± 6.85). G1 showed the lowest anovulatory failures (3.13 ± 3.51) compared to the G2 (13.70 ± 21.75) ($p = 0.016$) but similar to G3 (6.26 ± 6.85). In conclusion, best results in superovulation treatment in ewes were obtained using FSH dose of 100 mg. Higher doses of FSH (133 mg and 200 mg) are not indicated. (Financial support: Fapesp no 2014/04614-6, EMBRAPA no 02.13.06.026.00.00, PROPE no TC1288/2015)