A138 OPU-IVP and ET

Long action progesterone application interferes with recovery and quality in oocyte production and embryos *in vitro* prepubertal Nelore heifers

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Keywords: bovine, oocytes, puberty.

Progesterone has been used for various purposes in the animal reproduction. P4 allows the follicle to be exposed for a longer period to short LH pulses, which may improve oocyte quality and thus the production of embryos. The aim of this study was to evaluate the influence of exogenous progesterone injection in the number of recovered oocyte, oocyte quality and embryo production of prepubertal Nellore heifers. We used 31 females aging from 16 to 18 months, non-pregnant and without corpus luteum (CL). Cows were divided into three groups in a cross-over design: Group P0 (n=11), in which animals received two placebo solution applications (1 mL), in a 7-day interval and starting 14 days (d-14) before the first aspiration (d-0); Group P7 (n=10), the treated group in which the animals received a placebo solution injection (1 mL) 14 days (d-14) and a progesterone injection (P4; 150 mg) 7 days (d-7) before aspiration; Group P7-14 (n = 10), in which animals received two P4 injections (150mμg) with an interval of 7 days, the first one 14 days (d-14) and the second one 7 days (d-7) before aspiration. We conducted three aspirations with 28-day intervals. At the first aspiration animals were divided so that all cows could pass through all treatments. The recovered oocytes were selected and submitted to the procedures of the *in vitro* embryo production (IVEP). After confirming the homocedasticity (BoxCox) and normality (Cramér-von Mises test) of the data, the analysis of variance (ANOVA) was carried out. Tukey's test was used to compare the means of the variables and the Pearson's correlation test was used for data correlations, considering significant when $p \leq 0.05$. There were no significant differences ($p > 0.05$) in the number of retrieved oocytes (total oocytes), viable oocytes (GI, II, III), between the animals treated (P7 and P7-14 groups) and animals that did not receive P4 (P0 group). The animals showed an average of 14.98 ± 10.82 oocytes collected by aspiration session. The mean and standard deviation of viable oocytes rates and rates of embryos produced did not differ ($p > 0.05$) between groups. The groups P0, P7, P7-14 had 76%, 80% and 68% of viable oocytes, and 36%, 42% and 43% of embryos produced, respectively. The groups P0, P7 and P7-14 had an average of 4.04, 5.03 and 4.43 embryos produced by aspiration, respectively. The use of progesterone therapy did not improve the oocytes' and embryonic variables analyzed.

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Evaluation off *in vitro* embryos production (IVP) of buffaloes using medium supplemented with essential oil lippia origanoides

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Keywords: antioxidant, buffaloes, embyos.

The *in vitro* embryo production (IVEP) in cattle is a widely used and quite successful biotechnology. On the other hand, in buffaloes the IVEP is still limited and provides low production rates. The whole process that has being done in buffalo is very similar to cattle. One hypothesis for the low production in buffaloes is related to the *in vitro* environment, which generally exposes the oocytes to high oxygen tension and visible light causing an increase in reactive oxygen species (ROS). A strategy to minimize these effects is the use of antioxidants in culture media. A powerful natural antioxidant already used in cell culture is extracted from the plant *Lippia origanoides*. Thus, the aim of this study was to evaluate the effect of supplementation on IVM medium with essential oil from *L. origanoides* (OELO) in different concentrations for buffaloes, using the bovine model as standard. For maturation, we used 2052 bovine oocytes and 1026 buffalo oocytes recovered from ovaries originating from slaughterhouse, divided into five treatments consisting of: T1 [Base Media (BM: TCM 199 + 10% FBS + 22µg / ml pyruvate + 5UI / mL LH + 0,05µg / mL FSH + 1µg / mL Estradiol + 83.4 mg / mL amikacin)], T2 (BM 50 uM / ml cysteamine), T3 (BM + 2,5µg / ml OELO), T4 (BM + 5 ug / ml OELO) and T5 (BM + 10 ug / ml OELO). The reagents used were purchased from Sigma-Aldrich®, St. Louis, USA. Oocytes were maturated at 5% CO2, 38.5 °C for 24 hours. IVF occurred in a period of 18-20 hours, using semen from the same batch. The zygotes were denuded and cultured in SOF medium + 2.5% FBS for 7 days. The cleavage rate was evaluated after 48 hours of culture and the rate of blastocysts production in days 7 and 8. For statistical analysis, the Shapiro-Wilk test was used to assess the normality of continuous variables. The mean comparison between buffaloes and the standard (cattle) was made with ANOVA and Tukey test. The level of significance was $p < 0.05$. The cleavage rates (mean ± standard deviation; %) were 39.9 ± 5.5; 35.6 ± 5.6; 44.1 ± 5.7; 45.2 ± 5.6 and 43.6 ± 6.9 for buffaloes and 64.0 ± 3.9; 62.6 ± 3.6; 64.7 ± 4.0; 53.3 ± 3.8 and 65.5 ± 4.9 for cattle. The blastocyst rates were 30.2 ± 6.5; 19.5 ± 5.9; 27.5 ± 5.1; 32.9 ± 6.5 and 23.3 ± 4.8 for buffaloes and 27.2 ± 2.9; 26.8 ± 3.9; 24.9 ± 3.5; 23.4 ± 2.6 and 27.1 ± 2.9 for cattle, for the treatments T1, T2, T3, T4 and T5, respectively. There was significant difference only in cleavage rates for treatments T1 and T2, and cattle had a best performance compared to buffaloes. The semen used was from a bull with proven fertility in IVEP, not influencing the difference in the production rates between the two species. However, when using the antioxidant essential oil from *L. origanoides*, regardless the concentration, the difference between the two species was eliminated. The use of essential oil of *L. origanoides* was effective to optimize the *in vitro* embryo production in buffaloes. Financial support - CAPES 096/10.

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Evaluation of *in vitro* embryo production according to the cyclicity of Nellore (*Bos indicus*) cows submitted to opu in different postpartum moments

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Keywords: corpus luteum, ovum pick up, postpartum.

The aim of the present study was to evaluate the effect of postpartum days and cyclicity at 45 days postpartum on *in vitro* embryo production. A total of 17 Nellore cows, presenting eutocic labor and physiological postpartum, were used and were maintained with calves along all experimental period. Females were submitted to ovum pickup (OPU; 20G; 90 mmHg; ALOKA SSDV 500), in the following postpartum moments: T1- 11.5 days; T2- 25.5 days; T3- 39.5 days; T4- 53.5 days; T5- 66,8 days and T6- 81.4 days. The means (±SEM) of body condition score (1-5) during the postpartum moments were: T1: 2.72±0.04; T2: 2.82±0.04; T3: 2.82±0.04; T4: 2.83±0.05; T5: 2.84±0.04 and T6: 2.88±0.06. Immediately before the OPU, all visible follicles (≥2mm) were quantified and classified according to the diameter [small follicles (SF = <6mm), medium follicles (MF = 6 to 10 mm) and large follicles (LF ≥ 10mm)]. The variables were analyzed using PROC GLIMMIX (SAS 9.2®). Cyclicity effects [presence (C) or absence (NC) of corpus luteum] at 45 days, days postpartum and the interaction of these factors were evaluated. There was no interaction in any variables analyzed. Ciclicity did not influence the number of aspirated follicles (NC:38.3±3.2; C:44.5±2.9; P=0.35), cleavage rate (NC=56.7% e C=60.0%; P=0.30) and blastocyst rate (NC=30.2% e C=34.3%; P=0.37). Concerning postpartum period, there was a higher number of aspirated follicles when the animals were aspirated 81.4 days postpartum compared to previous moments (T1:36.8±4,8c; T2:35.8±4,9c; T3:37.8±4, 4cb; T4:38.6±4,7cb; T5: 43.4±5,6b; T6: 54.2±6,9a; P<0.001). There were also differences between the periods in oocyte recovery rate (T1:88.6%a; T2:73.3%b; T3:73.3%b; T4:81.4%ab; T5:87.1%a; T6:79.4%ab; P=0.03). Regarding the blastocyst rate, animals aspirated 11.5 days after calving showed lower values compared to other periods (T1:23.3%b; T2:34.9%a; T3:38.9%a; T4:33.7%a; T5:32.5%a; T6:31.6%a, P=0.01). However, the number of blastocysts (T1:7.6±1.5; T2:9.2±2.2 ; T3:10.8±2.2; T4:10.6±2.1; T5:12.3±2.2; T6:13.6±2.7; P=0.06), cleavage rate (T1:60.4%; T2:49.0%; T3:61.7%; T4:64.7%; T5:59.8%; T6:54.1%; P=0.08) and the number of cleaved embryos (T1:19.7±3.2; T2:12.9±2.8; T3:17.1±3.0; T4:20.4±3.3; T5:22.6±4.2; T6:23.3±4.6; P=0.10) per OPU session did not differ between different postpartum periods. Thus, although the effect of cyclicity at 45 days postpartum had no effect on *in vitro* production of Nellore embryos, the postpartum period significantly affected the number of follicles aspirated, the oocyte recovery rate and the blastocyst rate.

Acknowledgments: Bioembryo and Institute of Animal Science (IZ - Sertãozinho).

A141 OPU-IVP and ET

Evaluation of *in vitro* embryo production in Nelore (*Bos indicus*) with high and low follicular counting submitted to OPU in different postpartum moments

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Keywords: beef cows, *In vitro* embryo, postpartum.

The present study aimed to evaluate the effect of follicular population, according to the postpartum period, on *in vitro* embryo production in Nellore donors. A total of 17 Nellore cows, presenting eutocic labor and physiological postpartum, were used and were maintained with calves along all experimental period. Females were submitted to ovum pickup (OPU; 20G; 90 mmHg; Aloka SSDV 500), in the following postpartum moments: T1- 11.5 days; T2- 25.5 days; T3- 39.5 days; T4- 53.5 days; T5- 66,8 days and T6- 81.4 days. The means (±SEM) of body condition score (1-5) during the postpartum moments were: T1: 2.72±0.04; T2: 2.82±0.04; T3: 2.82±0.04; T4: 2.83±0.05; T5: 2.84±0.04 and T6: 2.88±0.06. Immediately before the OPU, all visible follicles (≥2mm) were quantified and the animals were separated according to the counting of follicular population, into: High (HFP, 52.9 ± 5.5 antral follicles; n=9) and Low (LFP; 20.8 ± 2.5 antral follicles; n=8) groups. Variables were analyzed using the GLIMMIX procedure (SAS 9.3®). The percentages were calculated based on total number of oocytes. Animals with high follicular population had a greater number of total oocytes (HFP:44.7±3.1; LFP:20.9±1.9; P=0.004), and number of cleaved embryos (HFP:25.4±2.3; LFP:12.9±1.3; P=0.05) compared to low follicular count animals. Interaction between the effects of follicular population count and the period in which the cows were aspirated regarding the cleavage rate (HFP/T1:58.0%abc; HFP/T2:47.7%c; HFP/T3:61.8%ab; HFP/T4:65.0%ab; HFP/T5:62.9%ab; HFP/T6:47.2%c; LFP/T1:66.9%ab; LFP/T2:51.3%bc; LFP/T3:61.8%abc; LFP/T4:64.8%ab; LFP/T5:53.1%bc; LFP/T6:70.3%a; P=0.05) was observed, as well as number of blastocysts (HFP/T1:10.2±2.6ab; HFP/T2:12.9±3.9ab; HFP/T3:15.0±3.3a; HFP/T4:14.4±3.7a;HFP/T5:13.8±2.8a; HFP/T6:14.1±4.2ab; LFP/T1:5.0±1.0b; LFP/T2:5.4±1.6b; LFP/T3:6.7±2.5b; LFP/T4:6.8±1.2ab; LFP/T5:10.6±3.4ab; LFP/T6:13.0±3.7a; P=0.01) and blastocyst rate (HFP/T1:21.3%d; HFP/T2:34.8%abcd; HFP/T3:40.3%ab; HFP/T4:35.7%abc; HFP/T5:28.6%bcd; HFP/T6:24.6%cd; LFP/T1:29.1%bcd; LFP/T2:34.6%abcd; LFP/T3:37.0%abc; LFP/T4:30.0%bcd; LFP/T5:40.8%ab; LFP/T6:47.6%a; P=0.001). Regarding the time of postpartum in which cows were aspirated, regardless of their follicle population, there was difference on the number of total occytes according to the postpartum periods (T1:32.6±5.4bc; T2:26.3±4.6d; T3:27.7±4.5cd; T4:31.5±4.7bc; T5:37.8±6.1ab; T6:43.1±6.1a; P<0.0001) and oocyte recovery rate (T1:88.5%a; T2:73.3%b; T3:73.3%b; T4:81.4%ab; T5:87.1%a; T6:79.4%ab; P=0.03). Thus, it is concluded that the quantity of recovered oocytes was higher in animals with higher follicular count. Additionally, in cows with high follicular count, blastocyst rate was higher in the period between 25.5 and 53.5 days postpartum, while in low follicular count animals, the rate was higher from 25.5 to 39.5 days and between 66.8 and 81.4 days postpartum.

Acknowledgments: Bioembryo and Institute of Animal Science (IZ - Sertãozinho).

A142 OPU-IVP and ET

Use of microwave as an alternative method for sterilization of materials used in assisted animal reproduction

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Keywords: *Escherichia coli*, microbiology, *Staphylococcus aureus.*

The plastic material is routinely used in animal assisted reproduction, being disposable which increases the cost of embryos production. Ethylene oxide sterilization is effective, but the process is time consuming, expensive and has high carcinogenic potential. Thus, the use of microwaves as an alternative, is commonly applied by working professionals in this area. However, few reports relate sterilization efficacy. The study aimed to evaluate the microwave efficiency as a sterilization method. The methodology consisted of manual washing of plastic material (with detergent), oven drying, packaging and use of micro-waves (2450MHz) for 5 minutes, twice. A container with 500 mL of water was used, and the water was replaced by another at room temperature between stages. Two assays have been proposed; the first evaluated the efficacy of the process of eliminating two types of micro-organisms whilst the efficiency; in the second, filter embryo collectors sterilization. Two cryovials were contaminated with 10^8 UFC/mL of Staphylococcus aureus and two with the same charge of Escherichia coli, and were randomly divided into 4 groups, two of which were subjected to direct cultivation in Muller Hinton (MH) medium and the other two passed through the microwave before cultivation. The second stage consisted of bovine uterine fluid transcervical collection, female bovine was previously anesthetized. The urethral probe was positioned in the uterus body and a system coupled to "Y" with saline solution at one end and at the other an Erlenmeyer, whereof two liters of solution were drained. The content was homogenized and distributed random and equally in 12 embryo collectors filters. The liquid passed through the mesh and was despised. Four groups were outlined with 3 filters each. Group (G) 1: The filter did not suffered any type of cleaning and followed for cultivation; G2: Passed through microwave and further cultivation; G3: manual washing, drying and cultivation; G4: manual washing, drying, microwave and cultivation. The materials (cryotubes and filters) remained in MH culture for 48 hours at 37 °C. The evaluation criteria were turbidity of the medium after 24 and 48 hours of incubation compared to the negative control and spreading all on 5% sheep blood agar. In the first experiment, there was turbidity in 24 hours and bacterial growth of the cryovials was compatible with the inoculated bacteria. In the second experiment, all groups showed turbidity in 24 hours. Given the results, the use of microwave in the stipulated frequency and time, was not effective in sterilizing the selected materials

A143 OPU-IVP and ET

In vitro sperm capacitation with l-Arginine and heparin in the absence of cumulus-oocyte complexes and its impact on embryo production

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Keywords: l-arginine, embryos, sperm capacitation.

The aim of this study was to evaluate the effects of L-arginine (L-arg, a precursor of NO synthesis) in the quality of the *in vitro* capacitation of cryopreserved bovine sperm induced by heparin and its effects on *in vitro* embryo production. All reagents used in the capacitation and sperm tests were purchased from Sigma Chemical Co. Aldrick (St Louis, USA). The medium base used in the sperm tests was modified Tyrodes (TALP-sp). The wash medium used in sperm capacitation was Tyrodes (Chamberland, Theriogenology, vol. 55, p. 823-835, 2001). The medium used in the process of *in vitro* embryo production were obtained from Progest Biotechnology in Animal Reproduction Co. (Botucatu, Brazil). In experiment 1, the experimental groups were: control 0h without pre capacitation, and capacitated for 30 min in the absence of COCs with heparin (control 30 min); L-arg and L-arg + heparin. The sperm capacitation and acrosome reaction (AR) were evaluated by chlortetracycline test, and the integrity of the plasma membrane (PM) and acrosome (AM) by the association of three fluorescent probes (PI; Hoechst, FITC-PSA). The sperm capacitation with L-arg + heparin increased the percentage of capacitated sperm, compared to control 0h and the group capacitated with heparin (61,1 vs 18.24 e 47.01%, respectively), and decreased the AR (19.6 vs 25.20%) compared to the group capacitated with heparin ($P < 0.05$). There was no difference in the percentage of sperm with PM and AM integrity when compared with the control 0h ($P > 0.05$). In experiment 2, the sperm was incubated with COCs in the presence of heparin (control), or previously incubated in the absence of COCs for 30 min with heparin, L-Arg or L-Arg + heparin and then washed and transferred to the IVF droplets without heparin. The sperm quality was evaluated by the *in vitro* production rate of blastocysts. There was no significant difference in cleavage rate between treatments ($P > 0.05$), however the group capacitated with L-arg + heparin increased the blastocyst rate at 31.8% compared to the control group, capacitated with heparin in the presence of COCs (53.71 vs 40.76%, $P<0.05$). The results allow us to conclude that: 1) the addition of L-arg to the capacitation medium containing heparin increases the number of *in vitro* capacitated spermatozoa with 30 min of cultive; 2) maintains a low percentage of spermatozoa with damaged in the plasma membrane and acrosomal membrane and 3) the addition of L-arg to the capacitation medium with heparin, in the absence of COCs, was the most effective method in the blastocysts production.

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A144 OPU-IVP and ET

Comparison of ovarian storage different solutions on qualitative-quantitative parameters evaluated by morphological criteria and brilliant cresyl test in bovine oocytes

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Keywords: bcb test, *In vitro* production, oocyte quality.

The objective of this study was to evaluate different solutions [Dulbecco Modified Eagle Medium (DMEM) *vs.* saline (NaCl) *vs.* phosphate buffered saline (PBS)] together with 10% fetal bovine serum (FBS) to refrigerated storage (5°C) of bovine ovaries during 24 h. Thus, ovaries derived from slaughterhouse were transported at 37°C for 15 min and in the laboratory divided in four groups: fresh (control) and cool (5°C) in DMEM/FBS, NaCl/FBS and PBS/FBS solutions. Immediately after ovary recovery (control) or storage (remaining treatments), were aspirated with a needle and syringe (21G/5 mL) follicles (2-8 mm). Then, recovered oocytes were classified in accordance with the *cumulus* cells (CCs) and homogeneity of the cytoplasm Grade I (≥3 layers of CCs and homogeneous cytoplasm), grade II (1-2 layers of CCs and homogeneous cytoplasm), Grade III (<1 layer of CCs and heterogeneous cytoplasm) and Grade IV (degenerated oocyte). Oocytes grade I and II were considered viable while grade III and IV non-viable. After conventional morphological classification, oocytes were submitted to the brilliant cresyl blue stain (Sigma, USA, BCB, 26 μM, 60 min) and classified in BCB^+ (cytoplasm blue/viable oocyte) and BCB^- (colorless cytoplasm/non-viable oocyte). All of the data were analyzed by the Fisher exact test ($P<0.05$). After five repetitions, a total of 120 ovaries resulted in 1047 recovered structures, obtaining an average of 8.8 oocytes/ovary and overall recovery rate (retrieved oocytes / aspirated follicles) of 51.9% (1047/2016). No difference ($P=0.12$) was observed in oocyte recovery rate between the DMEM/FBS [221/463 (47.7%)] and NaCl/FBS groups [157/372 (42.2%)], which were different and the smaller ($P<0.01$) that the control [322/559 (57.6%)] and PBS/FBS groups [347/622 (55.8%)]. As the oocyte quality by morphological criteria, a higher percentage of viable oocytes ($P<0.05$) was obtained from ovaries stored in DMEM/FBS [152/221 (68.8%)] and NaCl/SFB [111/157 (70.7%)] compared to the control [194/322 (60.2%)] and PBS/FBS groups [208/347 (59.9%)], which did not differentiate between them ($P=0.94$). As the oocyte quality by BCB, a percentage similar to control group [131/322 (40.7%)] of viable oocytes was obtained only from ovaries stored in NaCl/FBS [52/157 (33.1%), $P=0.13$]. Differences were observed among the groups; however, while PBS/FBS showed a greater number of recovery oocytes NaCl/FBS showed a higher number of viable oocytes for both oocyte quality assessments. Possibly, simple more solutions for the storage of cold ovaries for 24 h do not affect the quality of recovered structures. In conclusion, the use of NaCl/FBS may be used for storing ovaries at 5°C for 24 h. Further studies are needed to investigate the development of competence of these bovine oocytes.

A145 OPU-IVP and ET

Comparison of IVF embryo production and pregnancy, using conventional sperm and sex-sorted sperm in cattle

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Keywords: IVF, pregnancy, sex-sorted sperm.

The aim of this study was to evaluate the efficiency of sex-sorted sperm from 4 Nelore bulls on *in vitro* embryo production. For comparison, bovine embryos were also produced by IVF using unsorted sperm from the same bulls. Ovum pick up (OPU) -derived oocytes were matured and fertilized *in vitro* with either unsorted sperm (US) and sex-sorted sperm (SS). Subsequently, the presumptive zygotes were cultured *in vitro* for 7 days under standard conditions, to assess embryonic development rates. Development rates were documented on D7 and pregnancy rates were 35 days after embryo transfer. Our result demonstrated that embryo (Stage 7) production rate in SS Group was lower than US Group (29.0% -2709 blastocysts- vs 37.1% -3,167 blastocysts- respectively. P<0.001). However, the pregnancy rate in SS Group was higher than in the US Group (36.9% vs 30.1%, respectively. P<0.001). Comparing individual performance, from 4 bulls, just one showed higher embryo production rate when was used its sex-sorted semen than when was used its conventional semen (bull I, 26.6% vs 33.5%; bull ll, 40.0% vs 36.3%; bull III 22.3% vs 39.4%; and bull IV, 20.7% vs 35.9%, respectively). However, despite the lower embryo production rate with sex-sorted semen, the pregnancy rates were higher for 3 bulls in the Group SS comparing with Group US (bull I, 42.5% vs 24.1%; bull II 39.7% vs 34.8%; bull III 24.3% vs 36.0%; and bull IV 40.5% vs 33.3%, respectively). In conclusion, based on the results study, despite the embryo production rate was higher for unsorted sperm, the pregnacy rates was superior for sex-sorted sperm group.

A146 OPU-IVP and ET

Comparison between the number of recovered structures and viable embryos of ewes submitted to superovulation protocol supplemented with glycerol

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Keywords: embryo, energy flushing, reproduction.

The objective was to evaluate the influence of ultrashort energy flushing with glycerol on the amount of recovered structures and viable embryos from superovulated ewes. Sixty of Dorper ewes were submitted to a superovulation protocol that consisted of inserting an intravaginal device containing 0.33 g progesterone (CIDR - G®, Zoetis, São Paulo, Brazil) on Day 0, seven days after the device was replaced and 125µg administered IM sodium cloprostenol. (Ciosin®, MSD Animal Health, São Paulo, Brazil). Ovarian stimulation was started on D12 using 200mg of FSHp in 8 applications spaced 12h in decreasing doses IM (20, 20, 15, 15, 10, 10, 5, 5%). At 6 pm of D14, concomitant 6th application FSHp, IM eCG was administered 200IU (Novormon®, MSD Animal Health, São Paulo, Brazil). At 8 pm of the same day the CIDR - G was removed and the animals were divided into two groups, G-SUP (n = 24) consisting of ewes treated with 100ml glycerol, orally as glycogen energy source for flushing effect of ultrashort and G-CONTROL (n = 36) animals which received the compound for placebo effect 100ml water, orally. At 8 pm the D15 for induction of ovulation was applied 0.1mg IM gonadorelina (Fertagyl®, MSD Animal Health, São Paulo, Brazil). The ewes were inseminated two times (36 and 44h) after removal of the device containing P4, laparoscopic AI was performed using fresh semen and embryos were collected by surgical method on the morning of D21. Statistical analysis was performed using the Stastical Package for Social Sciences (SPSS) version 19, the variables were compared using the *Student's t test*, considering the significance level of 5%. The total average structures and viable embryos in the present study were 5.87 ± 5.97 and 3.73 ± 4.27 respectively. The average yield in total structures and viable embryos G-SUP were 5.83 ± 6.64 and 3.67 ± 4.08 respectively, statistically similar to that obtained in G-CONTROL which was 5.89 ± 5.58 and 3.78 ± 4.45 ($p>0.05$), respectively. In conclusion that supplementation with glycerol in the form of ultrashort energy flushing did not influence the amount of recovered structures and viable embryos in superovulated ewes.

A147 OPU-IVP and ET

Description of two new surgical techniques for the treatment of vaginal prolapse in zebuine cows: partial vaginectomy and dorsal vaginopexy

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Transfix Transplante de Embriões Ltda.

Keywords: prolapse; vaginectomy; vaginopexy.

Currently, the reduction of vaginal prolapse in non-pregnant zebu cows donors showed high incidence. Conventional techniques such as Caslick, Bühner or Flessa are not efficient and have significant recurrence of this affection. Thus this work proposes the evaluation of two new surgical techniques for correction of vaginal prolapse in female donors, in which terms: partial vaginectomy and dorsal vaginopexy. These techniques were applied in practice for a period of four years, providing up of adult zebu cows (n = 812) of the breeds: Nelore, Gir and Brahman, belonging to different properties. The vaginal prolapse was diagnosed by semiologic evaluation, and further determined the technique to be applied according to the severity and duration of the process. The partial vaginectomy and dorsal vaginopexy procedures demonstrated high percentage of recovery, 93.4% and 96.1%, and low rate of recurrence (6.3% and 3.7%) and low mortality (0.2% and 0.3%), respectively. Therefore, we suggest that the two technical proposals promote permanent reduction of vaginal prolapse in non-pregnant zebu donor females.

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Development of *in vitro* produced bovine embryos after cooling in medium 199 and Botuembryo®

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Keywords: cooling, culture media, embryo.

The *in vitro* production of bovine embryos obtained increased annually, but still is faced with a barrier, the lack of synchronized recipients for the time of embryo transfer (ET). Thus, freezing or vitrification of surplus embryos is an option. However, the pregnancy results to date are inconsistent. Recent research suggests the use of refrigerated embryos as a viable alternative and affordable economically. This study proposes the assessment of the effects for the environment and chilling time on the subsequent development of bovine embryos produced *in vitro*. The ovaries used for obtaining oocytes were originating from slaughterhouses. The oocytes were selected and rinsed in PBS with 10% FBS and then matured (IVM; groups 20-25/drop) in Medium 199 plus sodium bicarbonate, sodium pyruvate, penicillin, FCS, FSH and LH for 24 hours. The sperm select was performed by discontinuous gradient centrifugation (45 to 90%) of Percoll®; and the TALP medium supplemented with PHE and heparin for use in *in vitro* fertilization (IVF). The gametes were coincubated for 20 hours and the likely zygotes cultured (MIC) in SOF medium, maintained for seven days. The steps of IVM-IVF and IVC is effected in the incubator with 5% CO_2 in air, 38.7ºC and high humidity. After seven days of IVF, blastocysts were evaluated and randomly divided into five groups, namely blastocysts kept in SOF medium under the same cultivation conditions previously mentioned for the CIV for 6 hours (the control group); chilled blastocysts amid BotuEmbryo® (Botupharma, Botucatu, Brazil), 24 (B24 group) and 48 hours (B48 group); blastocysts and chilled in Medium 199 plus sodium bicarbonate, sodium pyruvate, HEPES (25 mM), FCS (50%) and penicillin, 24 (M24) and 48 hours (M48), in a polystyrene box (BotuFlex®; Botupharma) previously equilibrated at 5° C. After the cooling period, the blastocysts of refrigerated groups 24 and 48 h were transferred to SOF medium and incubated in CO_2 incubator for 6 hours for evaluation of subsequent development. ANOVA and Bonferroni t test were used to assess differences between groups, with $P<0.05$ indicating significance. There was no difference between the control group (100% vs 100%), B24 and M24 (85.6% vs. 65.8%) and B48 and M48 (58.4% vs. 43.8%) to rate blastocyst/expanded blastocyst (B/Bx). However, higher percentage ($P<0.05$) B/Bx was observed between the control group compared to B48 and M48 groups, with similar results between the groups, B24 and M24. Therefore, the BotuEmbryo® medium for 24 hours, proved to be efficient for cooling bovine embryos produced *in vitro*.

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Different embryo collection methods and superovulation protocols in crioula lanada ewes

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Keywords: hormone, reproduction, sheep.

The sheep breeds with specific environmental genetic adaptation provides economic benefits for the producer, which requires their preservation. In southern Brazil, the race Creole Lanada presents resistance to endoparasites and foot problems. The inclusion of other races reflected in intersections and spread the breed standard, requiring alternatives to facilitate the maintenance and preservation of genetic adaptations race. This paper proposes to determine the efficiency of different commercial formulations of FSH in superovulation protocol in different ways to collect embryos. Multiparous sheep females (n=8), from the in situ preservation of core Embrapa South Livestock (Bagé, RS), previously selected for cervical catheterization, were subjected to superovulation. It were formed with two groups (n = 4) in which the obtained synchronized estrus cycle, that superovulation occurs 60 hours post-estrus. One group received FSH (200mg, Foltropin V®) and the other FSH + LH (250 + 250, Pluset®) twice daily for four consecutive days. On the first day, it was deployed CIDR-G®; in which it was removed after 72 hours, by the oral application of hyperacute flushing glycerin and PGF2á IM application. The estrus was detected by ruffians and then the females were inseminated (12 and 24), by superficial cervical route with cooled semen (150 x 10^6 cells / mL) two males of the race. Two females from each group were selected for collecting embryos by laparotomy (LT) or via transcervical (TC). On the fifth day after estrus, females were submitted to water and fasting. The flock of the TC collection received an IM dose of BE and oxytocin, 12h and 15min before the procedure, respectively. In D6, the sheep of the LT group were anesthetized (ketamine + xylazine) and submitted to the collection and counting of corpora lutea (CLs). In the TC group, this count was later done by laparoscopy. Females group had FSH response averaged 5.5 LCs, in which the TC group of each animal recovery rate was 50% and 100%, and the rates LT group were 67% and 100%. In females FSH + LH group, the average was 11.2 LC, with recovery rate in the CT group 100% and 0%, and the LT group of 73% and 66%. Most of the collected structures were not fertilized. The collection by LT provided more consistent results, however, via TC provided an acceptable rate of embryo recovery. Because of the negative effects of LT, this result should be considered in races with limited number of copies. The number of LCs obtained with treatment FSH + LH was higher than that obtained with FSH, proposing new reproductive investigations of female sheep of the Creole race.

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Cervical dilatation in Santa Ines ewes induced with misoprostol, oxytocin and estradiol for development of non-surgical method for embryo recovery

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Keywords: embryo transfer, sheep, transcervical.

The objective of this study to develop a pharmacological protocol cervical dilatation in ewes Santa Ines aimed at collecting embryos transcervical. Multiparous ewes were used (n=30), cyclic, aged 24 and 48 months, with an average weight of 50.7 ± 5.4 kg and body scores between 2.75 and 3.5 (1-5) in design experimental model in "cross-over". The oestrus of the females was synchronized with short protocol and collection procedures were performed on the 14th day after estrus. Each animal received a dose of 0.5 ml of D-cloprostenol 12 hours before the procedure. All groups received epidural injections of 2.0 mg / kg ketamine (ketamine Agener® Agener Animal Health Union - São Paulo, Brazil) administered in the intervertebral space L7-S1. The experimental groups were: CG = control; GMI = misoprostol; GMiOE = misoprostol, estradiol and oxytocin; GOE = estradiol and oxytocin. The groups who used misoprostol received 5h before the procedure, 200µg of the drug (Prostokos®, Hebron Laboratory, Caruaru, Brazil) diluted in 1.5 mL of saline deposited directly on the cervical ostium. The estradiol benzoate (RIC-BE® Tecnopec, São Paulo, Brazil) was administered intravenously at a dose of 100 ug per animal, diluted in saline 2.5 mL + 2.5 mL of ethanol, 12 hours before the procedure. Oxytocin (Oxytocin Strong UCB®, Centrovet, Goiania, Brazil) was administered intravenously at a dose of 100 IU per animal, 15 minutes before the procedure. The animals were sedated with acepromazine association (0.1mg / kg Acepran®, Vetnil, Louveira, Brazil) and diazepam (0.2 mg / kg diazepam, Teuto Anapolis, Brazil) via IV and after ten minutes, received the injection epidural. The cervix was pinched, pulled to the vulvar commissure and fixed with two clamps to the side Pozzi cervical ostium. Cervical transposition attempts were made with a Hegar candle 10, 20 and 40 minutes after the epidural each trial persisted five minutes. The transposition was confirmed by injecting and recovering from 20 to 40 ml of saline. Data were analyzed in SAEG program (Statistical Analysis System, Version 9.1: Arthur Bernardes Foundation - UFV - Viçosa, 2007) and submitted to Fisher's exact test with 95% significance level. The cervical transposition rate varied between the groups, in which GOE introduced rate of 90% (27/30) did not differ from GMiOE, with a rate of 86.2% (25/30), but demonstrated superiority to other groups. In GMI group was possible to transpose 68.9% (20/29) of cervices equivalent value (P> 0.05) GMiOE and GC (62.1% - 18/29). The study proved the possibility of increasing the cervical transposition rate in ewes Santa Ines with the use of hormonal associations, enabling the collection of embryos transcervical in sheep, avoiding surgery.

A151 OPU-IVP and ET

Decreased lipid granules of *In vitro* produced bovine embryos with low concentrations of forskolin

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Keywords: Forskolin, IVP, lipids.

This paper proposes the chemical induction of lipolysis addition of forskolin on the sixth day of embryo culture, in order to increase the rate of survival of IVP embryos. Eight replicates were performed using 1.519 oocytes, which were washed and transferred to drops of IVM in TCM 199 supplemented with 10% FCS and remained in an oven with 5% CO_2 in air, at a temperature of 38.5 ° C and humidity absolute for 24 h. Semen was selected by Percoll gradient obtaining a concentration adjusted to $2x10^6$ spermatozoa / mL. Oocytes were incubated for 24 h in incubator with 5% CO^2 in air, at a temperature of 38.5 ° C and absolute humidity. Presumptive zygotes were cultured in SOF and 2.5% FBS. The embryos were kept in an oven with 5% CO_2, 5% O_2 and 90% N_2 at a temperature of 38.5 ° C and absolute humidity D to 6 (0 = D FIV), which were divided time into four groups for the addition of Forskolin® (F-6886): Control (group cultured in the absence of forskolin for seven consecutive days); F 2,5µM (group cultivated with 2,5µM forskolin for 24 hours); F 5µM (group cultivated with 5µM forskolin for 24 hours); F 10µM (group cultivated with 10µM forskolin for 24 hours). Apoptosis was analyzed using the TUNEL technique (deoxinucleotil terminal transferase Uracil Nick End Labeling), and the lipid content analysis was performed with Sudan Black B (S-0395). Statistical analysis Data were analyzed using ANOVA using SAS PROC GLM (SAS Inst., Inc., Cary, NC, USA). Sources of variation in the model including treatment and replicas were regarded as fixed and random effects, respectively. Data are presented as mean and standard least squares error. For all analyzes was adopted the significance level of 5%. There was no difference in blastocyst formation rate: control (37.0 ± 4.0); 2,5µM F (38.6 ± 4.0); F 5µM (40.7 ± 4.0); F 10µM (31.4 ± 4.0). While all groups treated with forskolin showed differences on lipid measurement: control (50.6 ± $1,1^{ab}$); 2,5µM F (46.2 ± $1,1^{c}$); F 5µM (49.9 ± $1,2^{b}$); 10µM F (53.9 ± $1,2^{a}$); total number of whole cells: control (140.1 ± $10,7^{ac}$); F 2,5µM (173 ± $9,0^{b}$); F 5µM (120.6 ± $11,5^{c}$); F 10µM (157.0 ± $13,9^{ab}$) and cell apoptosis: control (12.1 ± 3.5^{a}); 2,5µM F (16.7 ± $4,1^{b}$); F 5µM (11.1 ± $6,5^{a}$); 10µM F (14.2 ± $4,6^{ab}$) ($P < 0.05$). It was concluded that forskolin is a lipolytic agent effective even at low concentrations (F 2.5 mM), leading to the formation of blastocysts with more cells, comparing it to the control group. However, the concentration of 5 uM forskolin induced programmed cell death at the lowest rate.

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Effect of addition of butaphosphan to the medium of oocyte maturation in the production of *in vitro* bovine embryos

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Keywords: butafosfan, IVM, IVP.

The quality of the oocyte maturation is directly related to the embryonic development rate, as it depends on the amount of oocyte mRNA stored during this period. During maturation, the oocyte undergoes different processes that are related to phosphorylation and dephosphorylation cascades. Butaphosphan, an organic phosphorus molecule, has been administrated in embryo or oocyte donor cows to improve the results of the assisted reproductive technology programs. Therefore, our hypothesis in this study was the one that the addition of butaphosphan to the *in vitro* maturation medium (IVM) could improve oocyte maturation, enlarging the production of embryos. Eight routines of IVF were performed, totalizing 800 cumulus/oocyte complex originated from slaughterhouses. Mediums of Cenatte® (Cenatte®, Pedro Leopoldo – MG – Brasil) were used to perform IVF. The oocytes were randomly divided, in each routine, into four groups of 25 oocytes and the IVM medium was supplemented with crescent doses of butaphosphan (Capot Chemical Company Limited, Hangzhou, Zhejang, China) (Gc = 0.0mg/L; G1 = 50mg/L; G2 = 100mg/L e G3 = 200mg/L). IVM took place for 24h in an incubator with 5% CO2 and temperature of 39°C. Spermatozoids were selected by the minipercoll method and 10µL with 1x106 spermatozoids/mL were used to perform IVF. After 18h, probable zygotes were transferred to the culture medium under controlled conditions (5% CO_2, 5% O_2, 90% N_2) at 39°C for 7 days. SAS (SAS, Cary, NC, EUA) was used to perform the statistical analysis, GLM was used to verify the linear, squared and cubic effect of the addition of the crescent doses of butaphosphan to the IVM medium over the embryos percentage in D7 in relation to the number of maturated oocytes. The production of embryos in D7 as of 34.7%, 28.5%, 33.5% e 31.6% in the groups Gc, G1, G2 and G3 respectively, there was no effect of the group ($P>0.05$). Our data showed that the addition of butaphosphan to the IVM medium does not interfere directly on oocyte maturation, as it did not affect the production of *in vitro* bovine embryos.

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Effect of estrus synchronyzation between embryo donors and recipients, embryo quality and state (fresh or frozen) on the pregnancy rate in enbryo transfer

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Keywords: embryo quality, embryo transfer, estrous synchronization.

The aim of this study was to evaluate the effects of estrous synchrony between donor and the recipient, the quality and the freezability status (fresh or frozen) of the embryo on the pregnancy rate in bovine recipients. The work was performed during two years in an embryo transfer (ET) center, located in the city of Santiago, Rio Grande do Sul, Brazil. Ninety Aberdeen Angus cows were submitted to superovulation (SOV) protocols, resulting in 1.097 ET. The SOV protocol was initiated ten days after the heat detection of donors and the embryos collection was performed, on average, seven days later. The heat of recipients was also observed and on the day of ET, the animals which showed closest synchrony with donors were selected. Thus, 11 groups were formed, with intervals of 6 h, from - 30 to + 30 h, regarding recipient versus donor heat detection. The evaluation of embryos quality followed the proposed by IETS (1998), as grade I- Excellent; II- Regular; III- Poor; IV- Dead or Degenerated. The analysis was performed with the GLM package of the R software, through logistic regression models. The occurrence or not of pregnancy was analyzed as binomial dependent variable. For all the analyses, the significance was considered when $P<0.05$. Also, the odds ratio for each unit increase in the explanatory variables was estimated. The total pregnancy rate was of 52%. There was no effect of synchrony between donor and recipient on pregnancy rate, ranging from 42% to 56% ($P>0.05$). The embryo qualityaffected the pregnancy rate: Grade I, 58%; Grade II, 56%; and, Grade III, 44% of pregnancy ($P<0.001$). As well as the embryo state affected the pregnancy rate, 84% for fresh embryo and 16% for frozen embryo ($P=0.04$). Embryo Grade III had 43% less likelihood of pregnancy than Grade I. Also, frozen embryos had 37% less chance of pregnancy than fresh embryo. The synchrony between donor and recipient, considering ± 30 hours interval did not affect the pregnancy rate. However, the embryo quality and state (fresh or frozen) affected the pregnancy rate.

A154 OPU-IVP and ET

Effects of rumen-protected methionine and choline supplementation on preimplantation embryo in Holstein cows

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Keywords: choline, embryo, methionine.

Our objective was to determine the effects of methionine and choline supplementation during the pre- and postpartum periods on preimplantation embryos of Holstein cows. Multiparous cows were assigned in a randomized complete block design into four treatments from 21 d before calving to 30 DIM. Treatments (TRT) were: CON (n=8, fed the close-up and fresh cow diets with a Lys:Met=3.5:1), MET (n=9, fed the basal diet + methionine, Smartamine® M to a Lys:Met=2.9:1), CHO (n=8, fed the basal diets + choline 60 g/d, Reashure®), and MIX (n=11, fed the basal diets plus Smartamine M® to a Lys:Met=2.9:1 and 60 g/d Reashure®). From 30 ± 1 to 72 ± 1 DIM the cows were randomly assigned to two groups (GRP); control (CTL; n=16, fed a basal diet with a 3.5:1 Lys:Met) and methionine (SMT; n=20, fed the basal diet + methionine to 2.9:1 Lys:Met). On d 60, dominant follicles greater than 5 mm were aspirated using an ultrasound-guided transvaginal approach. A CIDR® device was inserted in all cows after follicular aspiration and superovulation began at d 61.5 using FSH treatment equivalent to 400 mg of NIHFSH-P1 (Folltropin®) in 8 decreasing doses at 12 h intervals over a 4 d period. During the superovulatory period, all cows received two PGF2α injections at d 63 and d 64 (concomitant with the 5th and 7th FSH injections), and CIDR was withdrawn at d 65. Twenty-four hours after CIDR withdrawal, ovulation was induced with GnRH. Cows were artificially inseminated at 12 h and 24 h after GnRH. Embryos were flushed 6.5 d after artificial insemination. Global methylation of the embryos was assessed by immunofluorescent labeling with 5-methylcytosine, while lipid content was assessed by staining with Nile Red. Nuclear staining (propidium iodide or Hoescht 33342) was used to count the total number of cells/embryo. Statistical analysis was performed using the MIXED procedure of SAS. Methylation of the DNA did was not different (P>0.05) among treatments but there was a TRT × GRP interaction (P=0.03). Embryos from cows in CON (– 21 to 72 d) had greater (P = 0.04) methylation (0.87 ± 0.09) than the embryos from cows in MET and CTL (0.44 ± 0.07).Embryos from cows in SMT had greater lipid content (P=0.04; 7.02 ± 1.03) than CTL (3.61 ± 1.20). There was not difference (P>0.05) for cells/embryo, embryo recovery rate per flushing, number of embryos recovered, embryo quality, embryo stage, and numbers of CL at flushing (CTL: 61.48±5.12, 0.69 %, 7.69±1.39, 1.63±0.25, 3.92±0.12, 9.33±1.18; and SMT: 54.92±3.9, 0.80 %, 8.72±1.18, 1.99±0.20, 4.13±0.10, 11.47±0.99, respectively). In conclusion, supplementation of methionine, choline or both methionine and choline affect embryo methylation and lipid content.

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Effect of *in vitro* spom maturation system use on bovine embryos lipid score

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Keywords: bovine embryo, forskolin, Lipidic score.

Oocyte quality determines the proportion of oocytes that will develop to blastocyst stage, and although the lipid content is important in oocyte development, the elevated number of lipid droplets have been associated with reduced cryosurvival, which is a relevant issue for embryo IVP system. The *in vitro* maturation system (IVM) Simulated Physiological Oocyte Maturation (SPOM) mimics the physiological maturation events by using AMPc modulators that promote the increase of oocyte competence. Forskolin is an example of AMPc modulator and this molecule has a lipolytic action. The aim of this study was to evaluate the effect of the SPOM system (Albuz, Hum. Reprod, v25, p12; 2010) on embryonic lipid score (relation between the lipid content and the total number of cells,TNC). In four replicates oocytes were obtained from slaughterhouse ovaries, selected and randomly divided into three groups: SPOM, CONTROL 1 (C1) e CONTROL 2 (C2). The MIV occurred during 24 h in C1 (TCM199 medium without FBS) and C2 (commercial medium Bioklone® Animal Reproduction, Sao Paulo, Brazil/ with FBS) in culture incubator at 38.5° C, 5% CO_2 in atmospheric air and high humidity. In SPOM group, oocytes were incubated in pre-IVM (TCM 199 medium with 100µM Forskolin and 500µM IBMX) for 2 h followed by an extended IVM (TCM 199 medium + 20µM cilostamide) period (28 h) under the same conditions as described for other groups. After IVM, oocytes were fertilized, and transfered to culture droplets, where they remained for seven (n=25-46 per group) or 9 (n=6-9 per group) days. The lipid content analysis and TNC meansure were performed using Oil Red and HOECHST 33342 staining, respectively. The lipid score was obtained by the stained lipid area divided by the TNC of each embryo and the averages were compared according to the days (D7 or D9), for each treatment, by the Kruskal-Wallis test in the Instat GraphPad program, with significance level of 5%. There was no difference ($P<0.05$) between groups (SPOM: 298.5 ± 139.9a ; C1: 226.0 ± 75.7; C2: 211.3 ± 1003a) in D7, suggesting that the time of exposure to Forskolin was not enough to ensure lipolytic action. At D9, only the C2 showed increase compared to others (SPOM: 154.0 ± 27.1a; C1: 135.7 ± 26.2a ; C2: 291.8 ± 71.4b); possibly due to the FBS effect on lipid accumulation. Between D7 and D9, there was a reduction ($P<0.05$) in the lipid score at SPOM (298.5± 139.9 vs 154.0± 27.1) and C1 (226.0 ±75.7 vs 135.7± 26.2) groups, which can be explained by the increase of embryonic metabolic consumption with the advance of embryonic development. However, the C2 showed no difference ($P>0.05$) between day 7 and 9 (211.3 ± 100.3 vs 291.8 ± 71.4), suggesting that the FCS effect on lipid accumulation was greater than the embryos metabolic activity. It was concluded that SPOM system had no effect in lipid score of *in vitro* produced embryos.

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The effect of diets with different levels of degradable and undegradable protein in rumen on apoptosis in oocyte and in cumulus-oophurus cells of Girolando cows

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Keywords: nutrition, reproduction, urea.

The ammonia produced during rumen protein degradation and not used in microbial protein synthesis, is absorbed by the rumen wall and converted to urea in the liver. High serum concentrations of ammonia and urea cause increase of both in tissue and reproductive fluids, interfering negatively in the fertility and *in vitro* embryos production. This study evaluated the effect of diets with different levels of degradable protein (RDP) and undegradable protein in rumen (RUP) on apoptosis of oocytes and cumulus-oophorus cells in crossbred Girolando cows. 22 Girolando cows were evaluated (n=10 3/4 HG; n=12 7/8 HG) with average weight 475.8 ± 7.75 kg, BCS 3.22 ± 0.03 and 105.33 ± 23.15 days of postpartum. The animals were distributed in four experimental groups, fed twice daily for 68 days with a total diet based on corn silage and concentrate. The diet of each group varied in the relation between RDP:RUP, with maintenance of metabolizable protein (1888g/day) and reduction of crude protein (CP) (RDP:RUP1.68= 15.4% CP, 62.7% RDP, 37.3% RUP; RDP:RUP 1.31= 13.6% CP, 56.7% RDP, 43.3% RUP; RDP:RUP 1.08= 13% CP, 52% RDP, 48% RUP; RDP:RUP0.83= 12.4% CP, 45.4% RDP, 54.6% RUP). OPU's were done on days 33 and 63 after initiation of treatment. The follicular waves were synchronized at 72 hours before by puncture of all follicles present in the ovaries. The cumulus-oocyte complex (COC) were recovered in PBS medium with 20 UI/mL of heparin, classified as viable (grades 1, 2 and 3) and non-viable. The COC viable were fixed in formalin 10%, alcohol PVA, grouped by treatment, collection day and stained with DAPI and TUNEL (Promega, Wisconsin, USA). The structures were photographed in fluorescence confocal microscope Leica TCS SP5II (Leica Microsystems®, Wetzlar, Germany) at 40x magnification. Images obtained at each 16µm were evaluated in Leica LAS AF Lite software. Getting the number and ratesof the cumulus apoptotic cells and the percentage of apoptotic oocytes. The variables were submitted to analysis of variance, using a generalized linear model (PROC GLM) and means were compared using the Student t test ($P<0.05$). Were recovered 78 COC (RDP:RUP1.68= 3; RDP:RUP1.31= 19; RDP:RUP1.08= 21; RDP:RUP0.83= 35). No significant effect was found in the number of apoptotic cumulus cells (RDP:RUP1,68= 9.33±15.3; RDP:RUP1.31= 40.94±9876; RDP:RUP1.08= 11.12 ±20.0; RDP:RUP0.83= 4.35 ±10.11) and rates cumulus cell apoptotic (RDP:RUP1.68= 0.027±0.02; RDP:RUP1.31= 0.1±0.16; RDP:RUP1.08= 0.12±0.14; RDP:RUP0.83= 0.03±0.1), but there were effect on the percentage of apoptotic oocytes (RDP:RUP1.68= 0%ab; RDP:RUP1.31= 26.32%a; RDP:RUP1.08= 4.76%ab; RDP:RUP0.83= 0%b). The variation in relation between RDP:RUP in diet interfered in apoptosis of oocytes, however, the highest ratio RDP:RUP not different from the two smaller relations.

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Effect of the treatment with progesterone and FSH on follicular aspiration of the first follicular wave in ewes

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Keywords: follicle, oocyte, progesterone.

The objective was to determine how progesterone (P4) and FSH of slow release influences the quantity and quality of the oocytes collected by aspiration of the first follicular wave. The experiment was conducted in breeding season (33 º S, Uruguay) in ewes Merino Australian using the Day 0 Protocol for superstimulation of the first follicular wave (Menchaca et al., 2010, Reproduction Fertility and Development, Volume 22, pp 113-118). Intravaginal sponges of medroxiprogesterone acetate (Progespon, Syntex, Argentina) were used for 6 days associated with 125 ug of sodium cloprostenol (Ciclase DL, Syntex) and 200 UI eCG (Novormon, Syntex) given at sponges withdrawal and one dose of a GnRH analogue (8.4 mg of buserelin acetate, Receptal; Hoechst, Germany) was given 36 h after sponges withdrawal. This treatment ensure ovulation (Day 0) and the emergence of the first follicular wave around 72-84h after sponges withdrawal. The ewes were assigned to four experimental groups: Control Group (n=21); P4 Group (n=24) that received an intravaginal device with P4 (0.3 g, DICO, Syntex); FSH Group (n=16) treated with one dose of 80 mg FSHp (Folltropin, Bioniche Animal Health, Canada) reconstituted in sodium hyaluronic acid for slow release (10 ml, MAP-5, Bioniche Animal Health); and P4+FSH Group (n=14) that received the intravaginal device with P4 plus one dose of 80 mg FSHp in MAP-5 solvent. In both progesterone treated groups, the intravaginal devices were kept in place until laparoscopic follicular aspiration that was performed for all the females 72h after Day 0. Statistical analysis was performed by using ANOVA, Kruskall Wallis nonparametric test or chi square test. The treatment with P4 itself analysed as main factor did not affect the number of aspirated follicles (4.1 ± 0.7 vs. 3.8 ± 0.6) neither the number of collected oocytes (2.1 ± 0.7 vs. 1.9 ± 0.4) in comparison with no P4 treated females (P=NS). The treatment with FSH analysed as main effect increased the number of aspirated follicles (6.5±0.7 vs. 4.1±0.7; P<0.05) and the number of collected oocytes (3.4 ± 0.7 vs. 2.1 ± 0.7) in comparison with no FSH treated ewes. An interaction between treatments was observed (P<0,05), and the higher results were obtained in the P4+FSH group. The recovery rate and oocyte quality were not affected in any of the treatments (P=NS). In conclusion, the treatment with intravaginal P4 associated with one im dose of FSH in MAP-5 slow release solvent increases the follicular response and oocyte recovery when aspirating sheep at the first follicular wave.

A158 OPU-IVP and ET

Effect of camp modulators on *in vitro* pre maturation in production rate and lipid content of crossbred bovine embryos

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Keywords: bovine, forskolin, IVP.

Simulated Physiological Oocyte Maturation system (SPOM; Albuz, Hum Reprod, vol 25, p 12, 2010) was developed to improve the oocytes quality by using cAMP modulators, among which forskolin, which also has delipidant action - desirable for structures directed for cryopreservation. In this system, two stages are proposed: Pre-IVM and extended IVM. The aim of this study was to evaluate the effects of only the pre-IVM step (2h culture in the presence of forskolin and IBMX) on blastocyst rates and lipid content of *in vitro* produced bovine embryos. COCs obtained from slaughterhouse ovaries in three replicates, were selected based on the number of cumulus cells and homogeneous cytoplasm and randomly distributed into two groups: control [C, n = 84; Standard IVM for 24 hours in the commercial medium (Bioklone® Animal Reproduction, Brazil)] and pre-IVM [PM, n = 99; pre-IVM for 2 hours in pre-IVM medium (TCM 199-Hepes, BSA 1,6mg/mL, sodium pyruvate 100mM, ITS 100x, penicillin 10.000UI, streptomycin 10mg/mL, forskolin 100µM and IBMX 500µM) followed by standard IVM]. After IVM, the groups underwent at the same time IVF in TALP and IVC in SOF, both using Bioklone® medium. The cleavage and blastocyst rates were evaluated in D3 and D7, respectively. The blastocysts obtained were fixed in 4% PFA in D7 and stored at 4 ° C until subjected to Oil Red staining technique for lipid content evaluation by analyzing stained area fraction using ImageJ software. The cleavage and blastocyst rates were compared using Fisher's exact test (different averages identified with distinct superscript letters), and the mean lipid stained area fraction compared by Mann Whitney test. Statistical analyzes were performed using GraphPad INSTAT, at a 5% significance level. There was no difference (P>0.05) between the cleavage rates of C and PM (80.95%a - 68 vs 82.83%a - 82), blastocyst rate/total oocytes (39.28%a - 33 vs 28.28%a - 28) and blastocyst rate/cleaved (p = 0.0951; 48.53%a vs 34.14%a) respectively. The mean stained area fraction for lipid to the C was 30.60 ± 5.48 vs 40.23 ± 4.01 for the PM (p>0.05). These results suggest that isolated from SPOM system, usage of pre-IVM step before comercial IVM resulted in no improvement in blastocyst rates, nor the delipidant effect of forskolin was observed in resulting blastocysts.

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A159 OPU-IVP and ET

Effects of sperm selection in single layer Percoll® before freezing on the kinetic characteristics of bovine sperm after thawing

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Keywords: percoll gradient, sperm kinematics, sperm selection.

The aim of this study was to investigate the effects of sperm selection through single layer centrifugation in Percoll® at 90% (SLCP) before freezing, evaluating the spermatic kinetics after thawing through computer-assisted sperm analysis. Semen of 3 Nelore bulls (3 ejaculates of each one), kept in collecting and processing semen station, was collected by artificial vagina. On the day of each collection, after routine sperm evaluation, the ejaculate from each bull was mixed and divided equally at concentration of 1,000 x 106 of sperm (regardless of volume) between the control group (P0, without SLCP) and P9 and P6 groups, which were subjected to centrifugation in 15 ml tubes with 9 ml and 6 ml of Percoll®, respectively. After centrifugation (700 x g/13 min) the supernatant was removed and the pellet of spermatozoa was diluted in freezing medium (tris, fructose, citric acid, egg yolk, glycerol, and antibiotics). Afterwards, 0.5 ml straws were filled with 25 x 10^6 of spermatozoa, cooled for five hours in a cold chamber at 4° C and then frozen in a programmable freezer (Digitcool, IMV, France). After 30 days of storage, four straws from each group and different collection were thawed in water bath at 46º C/20 s and immediately evaluated for spermatic kinetics (CASA, Hamilton Thorne®, Beverly, USA). The following parameters were analyzed: progressive motility (PM; %), curvilinear velocity (VCL; µm/s), average path velocity (VAP; µm/s), straight line velocity (VSL; µm/s), amplitude of lateral head displacement (ALH; µm), beat cross frequency (BCF, Hz), straightness (STR; %), linearity (LIN; %), and rapid sperm movement (RAP; %). ANOVA and Tukey test were used for analysis of results, with values expressed as mean ± standard deviation, and $P<0.05$ taken as significant. Significant difference ($P<0.05$) was observed between P0 and P9 groups, respectively, for the variables ALH (5.67 ± 0.24 vs. 5.1 ± 0.26), BCF (26.7 ± 0.72 vs. 30.2 ± 1.19), LIN (52.0 ± 2.16 vs. 62.5 ± 2.88) and STR (81.7 ± 2.63 vs. 88.8 ± 1.6). However, no difference was found among all groups for PM, VCL, VSL, VAP and RAP. Results of P6 were similar to those found in groups P0 and P9. Based on the results, SLCP was effective in identifying sperm subpopulations with different kinetics characteristics, which can determine an increase on the rate of fertilized oocytes in *in vitro* embryo production programs. Further analysis in progress, including integrity of acrosome membrane, mitochondrial function, determination of intracellular ROS, membrane lipid peroxidation, and IVF, will provide a better understanding of the proposed protocol.

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A160 OPU-IVP and ET

Cellular and molecular effects of follicular fluid's exosomes from Nelore cows submitted to ovarian superstimulation

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Keywords: exosomes, *in vitro* maturation, ovarian superestimulation.

Cell-cell communication within the ovarian follicle involves many signaling molecules, and this process may be mediated by secretion and uptake of exosomes (Exo) that contain several bioactive molecules. Thus, to gain insight into the effects of superstimulatory treatments in bovine ovary, Exo of follicular fluid from Nelore cows (*Bos taurus indicus*) submitted to ovarian superstimulation by FSH (P36 protocol) and Exo from cows only synchronized were added during *in vitro* maturation (IVM) to assess nuclear maturation, DNA fragmentation and mRNA abundance of bovine cumulus-oocyte complexes (COCs). Nelore cows were slaughtered, fluid from pre-ovulatory follicle aspirated and the Exo were obtained by ultracentrifugation. Five replicates of COCs (20 COCs/group) were matured *in vitro* for 22-24h in TCM 199 supplemented with, BSA, FSH, estradiol, amikacin, pyruvate and: 10% Exo from Non-Superstimulated (Exo-NS group) cows; 10% Exo from P-36 cows (Exo-P36 group) or without Exo (control group). In experiment 1 (Exp.1), after IVM, oocytes were submitted to the TUNEL assay and stained with Hoesch-33342. In experiment 2 (Exp.2), the oocytes and its cumulus cells were submitted to total RNA extraction and reverse transcribed with random primer, separately. The mRNA abundance of H2AFZ and PDE3 in oocytes; GREM1 and COX2 in cumulus cells; and GDF9, BMP15 in both cells types was measured by RT-qPCR using SYBR green system and normalized by the expression of more stable endogenous gene; cyclophilin A (PPIA). The mRNA abundance (target gene/PPIA) was calculated using ΔΔCt method corrected by Pffafl's equation. Effects of the Exo addition on the meiosis progression (%), apoptosis rates (%) and on the mRNA abundance of target genes were tested by ANOVA ($p \leq 0.05$: significant difference and $0.05 < p < 0.10$: considered tendency). In Exp.1, addition of Exo-P36 did not affect apoptosis rates in matured oocytes, nevertheless, Exo-P36 group tended to higher percentage of oocytes in meiosis II (74 ± 3.2) when compared with Exo-NS group (60.2 ± 4.5; p=0.09). In Exp.2, no differences ($p > 0.10$) on the GDF9, BMP15, H2AFZ, PDE3 mRNA abundance were demonstrated in oocytes, however, GDF9 mRNA abundance tended to be higher in cumulus cells from Exo-P36 group (20.3 ± 13.16) when compared with control and Exo-NS groups (2.2 ± 0.8; 4.1 ± 3.2; p=0.06). Even as, BMP15 mRNA abundance also tended to be higher in cumulus cells from Exo-P36 group (5.7 ± 3.2) when compared with control and Exo-NS groups (0.8 ± 0.5; 1.2 ± 1.2; p=0.06). In conclusion, the effects of Exo, from cows submitted to P36 protocol, during IVM showed that ovarian superstimulation using FSH seems to modulate the bovine follicular fluid content and consequently it could be used to improve *in vitro* oocyte competence by increasing of meiosis progression and GDF9 and BMP15 gene expression.

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A161 OPU-IVP and ET

Effect of *in vitro* culture on size and sex ratio of bovine embryos on d14 of development

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Keywords: embryos, *in vitro* production, sex determination.

Several studies have been conducted to evaluate the culture effect of in a quality of *in vitro* produced embryos. Most of these studies use as an evaluation method producing blastocyst until the day 7 (D7) or day 8 (D8) of development. Few studies have focused on more advanced stages of embryo, or even if the *in vitro* culture affect embryo development after the embryo transfer into the recipient. Within this perspective, assessment of embryos on day 14 (D14) development allows a more accurate assessment of their quality, produced by different assisted reproduction techniques or those that have undergone different treatments and manipulations. The objective was to evaluate the effects of vitro-produced (IVP) in size and sex of embryos in an advanced stage of development (D14).. For IVP, oocytes obtained from slaughterhouse ovaries were matured, fertilized (D0) and cultured *in vitro* to D7. On D7 culture, blastocysts grade one were selected and transferred in number from 15 to the uterine horn of previously synchronized recipients (group vitro/vivo). As a control, collected embryos on day 7 post-insemination were used of donors overstimulated. After the uterine washing the blastocyst stage and in similar quality *in vitro* were transferred in number from 12 to synchronized recipients (live group vivo/vivo). Embryos from both groups were collected in D14 and measured individually. Later, biopsy of each embryo trophoblast was stored for genomic DNA extraction and determination of sex. The recovery rate of the embryos and sex ratio data male: female from embryos produced *in vivo* or *in vitro* were analyzed by chi-square test ($P < 0.05$). Measurement of embryos was compared using the Kruskal-Wallis test ($P < 0.05$) by Prophet 5.0. We observed a higher rate of embryo recovery for the group vitro/vivo (45.5% ± 55.8) compared to the vivo/vivo group (26.7 ± 55.1). However, when analyzing the proportion of male: female embryos, vitro/vivo (28:19) and vivo/vivo group (12:12), no difference was detected. Relative to the size of the collected embryos in D14, there was no difference between male or female embryos produced *in vitro* (722.6 ± 116.8 and 608.2 ± 121.3, respectively) or *in vivo* (1194.9 ± 244.3 and 1181.5 214.7 ± respectively). Furthermore, the size of the male and female embryos from the same groups was also similar. These results suggest that the developing embryos of D14 are not affected by *in vitro* culture because they suffer no change in the male/female ratio, or the size compared to those produced *in vivo*.

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A162 OPU-IVP and ET

Effect of angiotensin-converting enzyme inhibitor on bovine blastocyst rate

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Keywords: angiotensin, captopril, IVP.

The reproductive performance of herds requires in-depth knowledge of the endocrinology of ovarian reproduction in vivo and *in vitro*. The renin-angiotensin system (RAS) is widely known as regulator of blood pressure, and has been described in several organs and tissues. Captopril is an inhibitor of the angiotensin-converting enzyme (ACE) that prevents angiotensin I (Ang. I) from being converted to angiotensin II (Ang. II). Ang II is capable of reversing the inhibitory effect of follicular cells on the *in vitro* nuclear maturation of bovine oocytes and improves the cytoplasmic maturation of cumulus-oocyte complexes (COC). The aim of this study was to evaluate the effect of captopril on the bovine blastocyst rate. COCs from female cattle originating from slaughterhouses of the municipality of Teresina-PI, Brazil, were used. A total of 472 COCs were distributed into four experimental groups: G1 (n=58) - 0 μM captopril; G2 (n=83) - 5 μM captopril; G3 (n=81) - 10 μM captopril; and G4 (n=78) 15 μM captopril in the maturation medium. After maturation, 300 COCs were co-incubated with spermatozoa at a temperature of 38.5 ºC for 20 h, at 5% CO2. The presumptive zygotes were isolated from the cumulus cells by successive aspirations and washed three times in SOF medium (Nutricell®) supplemented with 5% FBS. Subsequently, they were transferred to a 60 × 15 mm Petri dish containing microdroplets of 100 μL SOF medium (Nutricell®) supplemented with 5% FBS and kept in an oven at 38.5 ºC, at 5% CO2, for seven days. Feeding (exchange of 50% of the SOF medium for a previously-stabilized new one) was performed on the fifth day of growth. The blastocyst rate was evaluated after 168 h (D7) of growth, by the nonparametric chi-square test (X^2). The use of different concentrations of captopril in the growth medium did not change the blastocyst rate, as there was no statistical difference between the experimental groups (G1=37%, G2=48%, G3=53%, and G4=62%). In conclusion, supplementation of the maturation medium with captopril did not improve the blastocyst rate.

A163 OPU-IVP and ET

Effect of resveratrol on bovine sperm viability in *in vitro* fertilization medium

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Keywords: acrossome, antioxidant, spermatozoa.

Despite a large number of researches about the protector effect of antioxidants on sperm cryopreservation, few studies report their effects on sperm cells for *in vitro* fertilization. The resveratrol (3, 5, 4′-trihydroxystilbene) is a compound found in many species of plants and has several pharmacological activities including antioxidant effect. This study aimed to evaluate the effect of different concentrations of resveratrol diluted in *in vitro* fertilization medium on sperm viability and acrosome integrity. Motile frozen-thawed sperm was thawed at a temperature between 35 °-37 ° C and separated by Percoll gradient method (90 e 45%) and distributed to the following treatments, accordingly to resveratrol concentration in FERT medium: R0 (control – 0µg/mL), R1 (7.5µg/mL), R2 (15µg/mL) and R3 (30µg/mL), and then incubated for 24h at 38.5°C with 5% CO_2 and 95% humidity at concentration of $10x10^6$ spermatozoa/mL. The proportion of live and dead spermatozoa with an intact or reacted acrosome was evaluated by trypan blue-giemsa staining at 6h, 12h e 24h of incubation. Experiments were repeated three times and in triplicate. Data were analyzed by ANOVA and means compared by Student-Newman-Keuls. Values are shown as mean±SEM. The rate of live spermatozoa with decreased from 6 to 24h of incubation in all evaluated concentrations of resveratrol and control, with an accentuated ($P<0.05$) decline between 6h (R0=23.8 ± 1.7%; R1=27.9 ± 2.5%; R2=28.3 ± 1.7% and R3=24.7 ±2.8%) and 12h (R0=7.5 ± 0.7%; R1=9.8 ± 1.4%; R2=9.0 ± 0.6% and R3=7.8 ± 0.7). However, there was no significant difference ($P<0.05$) in the proportion of live sperm among different concentration of resveratrol and the control at 6h, 12h and 24h of incubation. Resveratrol at all concentrations tested increased ($P<0.05$) the percentage of live sperm with reacted acrosome when compared to control, mainly at 6h (R0 = 15.2 ± 1.3%; R1 = 22.9% ± 1.8; R2 = 23.7% ± 1.8; R 3 = 20.9 ± 2.8%) and 12h (R0 = 3.1 ± 0.4%; 6 = R 1, 1 ± 0.6%, R2 = 4.5 ± 0.6%; R3 = 4.8 ± 0.5%) of incubation. In conclusion, the resveratrol concentrations evaluated do not prevent the decrease of sperm viability within 6h to 24h of incubation *in vitro* fertilization medium and increase the number of live sperm with reacted acrosome, showing to have an effect on the integrity of sperm acrosome.

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A164 OPU-IVP and ET

Effect of the length of FSH treatment (4d vs 7d) on the superovulatory response of lactaing Holstein cows

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Keywords: dairy cow, FSH; superovulation.

The aim of the study was to evaluate whether long-term treatment with FSH improves superovulatory response of dairy cows.. Holstein cows (n = 89) producing on average 40.2 ± 5.2 kg/day were randomized to two superovulation protocols at 63 ± 3d DIM, as follows: 1) Short protocol (FSH4d): D-1 = Follicle ablation, D1 to D4 = CIDR insertion and 400 mg of FSH (Folltropin®) applied AM/PM, D3 and D4 AM = PGF2a and CIDR removal together with 2nd PGF2a treatment and 12h before last FSH, D5 AM = GnRH and AI 12 and 24h after GnRH with conventional non-sexed semen from same sire and a single ejaculate. The long protocol (FSH7d) followed a similar sequence of treatments but the 400 mg dose of FSH was extended over 7 days instead of 4. All cows were inseminated by the same AI technician and nonsurgical collection of ova/embryos took place 7 days after GnRH by one of three technicians. Embryo grading was performed by a single technician (blindly) according to IETS guidelines. Data was analyzed by the Glimmix procedure of SAS (version 9.3), the Poisson distribution was assumed for most embryo-related variables and cow was interpreted as the experimental unit. Results for FSH4d and FSH7d were, respectively: total number of CL (FSH4d = 12.0 ± 0.8 vs FSH7d = 12.9 ± 0.8; P=0.22); total structures (FSH4d = 4.8 ± 0.5 vs FSH7d = 4.5 ± 0.6; P=0.49); fertilization rate (FSH4d = 83.8 ± 5.0 vs FSH7d = 92.3 ± 4.0; P=0.18); number of transferable embryos (FSH4d = 1.8 ± 0.4 vs FSH7d = 2.4 ± 0.6; P=0.43); number of freezable embryos (FSH4d = 1.3 ± 0.3 vs FSH7d = 2.1 ± 0.5; P=0.43); percent of degenerated embryos out of fertilized (FSH4d = 41.4 ± 6.2 vs FSH7d = 43.6 ± 6.4; P=0.79). These results suggest that the 7-day (long) FSH protocol failed to improve embryo production in high producing Holstein cows.

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A165 OPU-IVP and ET

Estabelecimento e acompanhamento da g establishment and monitoring of pregnancy in Holstein cows after embryo transfer, produced *in vivo* or *in vitro*

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Keywords: dairy cows, pregnancy, pregnancy loss.

The aim of this study was to evaluate the establishment and maintenance of pregnancy, until parturition, of embryos produced in vivo (superestimulation and uterine flush, SOV-UF) or *in vitro* (ovum pick-up and *in vitro* production, OPU- IVP), derived from donor Holstein cows (black and white Holstein cows, BWH) lactating (LAC) or not (NLAC) and transferred to recipient BWH multiparous (MULT) or primiparous (PRIM). The data was obtained from a commercial dairy farm (Agrindus S/A, Descalvado-SP) during the year 2013. 2225 embryo transfers were performed, and 1337 embryos were obtained from the SOV-UF and transferred to fresh (SOV-UF-F); 474 embryos were obtained from SOV-UF and transferred thawed (SOV-UF-T) and 444 embryos were obtained from OPU-IVP and transferred to fresh (IVP). The pregnancy diagnosis was performed by ultrasonography exams at 30 days of gestation (DG30) and 30 days later the pregnancy was confirmed by rectal palpation (DG60). Pregnancy losses between 30 and 60 days and between 60 days and parturition were also recorded. Data were analyzed using the GLIMMIX procedure of SAS 9.2®, considering category (LAC and NLAC), parity (MULT and PRIM) and embryo production technics (SOV-UF-F, SOV-UF-T, IVP) in the mathematical model, and presented as means of least squares (mean adjusted by the model). The recipients that received embryos derived SOV-UF-F showed higher conception rate at 30 days of gestation than the recipients that received embryos derived SOV-UF-D and IVP [SOV-UF-F: 48.0% (632/1337), SOV-UF-D: 14.3% (64/474), IVP: 31.6% (138/444), P <0.0001], as well at 60 days of gestation [SOV-UF-F: 36.8% (488/1337), SOV-UF-D: 10.7 % (50/474), IVP: 21.8% (99/444), P<0.001]. No significant effects were observed on pregnancy loss between 30 and 60 days of gestation [SOV-UF-F: 21.2% (141/632); SOV-UF-D: 20.8% (14/64); IVP: 30.2 % (39/138); P=0.11] and pregnancy loss between 60 days of gestation and until parturition [SOV-UF-F: 23.7% (120/488); SOV-UF-D: 17.0% (9/50); IVP: 24.9% (25/99); P=0.54]. Additionally, the recipients MULT showed decreased rate of pregnancy at 30 days than PRIM [MULT: 28.9 % (519/1472); PRIM: 33.8% (315/783); P=0.015] and higher pregnancy loss between 60 days of gestation and parturition [MULT: 25.3% (105/392); PRIM: 18.4% (49/245); P=0.049] than the PRIM. Lastly, embryos that were derived from donors LAC showed increased rate of pregnancy loss between 30 and 60 days when compared the embryos derived of donors NLAC [27.5% (122/490) vs. 20.7% (71/343), respectively; P=0.037]. Therefore, it is concluded that pregnancy loss rate was not affected by the type of embryo (in vivo or *in vitro*), however the establishment of pregnancy at 30 and 60 days was higher in embryos produced in vivo and transferred fresh. The number of births affected the pregnancy at 30 days and losses between 60 days and parturition, and lactation status of the donor influenced the pregnancy loss between 30 and 60 days.

A166 OPU-IVP and ET

Gene expression of AREG and EREG in bovine cumulus cells after *in vitro* maturation in medium supplemented with FCS, BSA or PVA

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Keywords: amphiregulin, epiregulin, transcripts.

The analysis of gene expression of Amphiregulin (AREG) and Epiregulin (EREG) in cumulus cells (CC) showed the importance of these two genes in the regulation of ovulation and CC expansion. This study was carried out to quantify the transcripts of AREG and EREG in bovine oocyte cumulus cells cultured in *in vitro* maturation (IVM) medium, supplemented with FCS, BSA or PVA, as well as in CC of immature oocytes. Follicles (2 to 7 mm in diameter) were punctured from ovaries obtained at slaughterhouse; then, oocytes were divided in two groups, as follows: immature oocytes and *in vitro* matured oocytes. All reagents were purchased from Sigma-Aldrich (St. Louis, USA), unless otherwise stated. Oocytes were washed and selected in PBS medium plus 10% FCS (Nutricell®, Campinas, Brazil), but for IVM, groups of 20-25 oocytes were cultured in Medium 199, supplemented with sodium bicarbonate, sodium pyruvate, penicillin, FSH and LH (Lutropin®, Bioniche Inc., Canada), estradiol, cisteamine and different macromolecules (10% FCS, 4 mg/ml BSA or 1 mg/ml PVA), at 38.8º C, under humid atmosphere, 5% CO2, in air, for 24 h. The CC were removed by several pipetting in PBS medium with 0.1% hyaluronidase. Then, the droplets containing only CC were centrifuged (3,355 x g/10 min) and frozen in total RNA extraction medium. Gene expression was investigated by quantitative RT-PCR, normalized by GAPDH constitutive gene; five repetitions were performed for each group. The results were analyzed using ANOVA and Tukey test, with $P<0.05$ taken as significant. The abundance of transcripts for AREG was higher ($P<0.05$) in the CC from *in vitro* matured oocytes, in the presence of FCS, in comparison with immature oocytes and *in vitro* matured oocytes supplemented with PVA or BSA. The CC of immature oocytes had lower expression of EREG compared to oocytes matured *in vitro*. In conclusion, the addition of FCS to IVM medium positively influenced the gene expression of AREG in the CC probably due to its complex composition, acting alone and/or interacting with certain compounds found in the IVM medium.

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A167 OPU-IVP and ET

Identification of arthritis encephalitis caprine (CAEV) in flushing media and embryos from naturally infected herd goats

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Keywords: CAEV transmission, dairy goats, *in vivo* embryos.

The goat production in Minas Gerais state has been growing in recent years, especially with intensive dairy production systems. The artificial reproduction biotechnologies are a powerful mechanism for genetics dissemination. However, the caprine arthritis encephalitis virus (CAEV) is present in most of the dairy goats farms, which represent a risk for disease transmission. The aim of this study is to identify the presence of pro-viral and viral CAEV genome in uterine lavage fluid and embryos samples collected by the non cirurgical transcervical assay. Eight goats were selected with 56.0 ± 13,4kg and a body condition score of 3.5 ± 0.75. The animals were all positive for CAEV in "Western Blotting" diagnosis. Oestrus sycronisation and ovarian superovulation was performed from adapted protocol from Fonseca e*t al,* 2006 (Acta Scientiae Veterinariae, v. 34, sul. 1). After transcervical embryo flushing, embryos that had intact ZP, had their inner cell mass aspirated by a micromanipulator, and the product (zona pellucida and inner cell mass) was stored in RNA later. Centrifugation was performed in uterine liquid washings, until the identification of a pellet (pellet 1) and subsequently viral concentration of uterine flushings (pellet 2) was performed. The material obtained (pellets 1 and pellets 2) was submitted to viral and pro-viral genome extraction with Cador Pathogen® mini kit (Qiagen, Germany) assay. Embryonic samples (zona pellucida and inner cell mass) cDNA synthesis was performed from the viral genome with the use of M MLV enzyme according to protocol. The samples were submitted to nested PCR (Fieni, 2010,). The presence of viral and pro-viral genome in pellet (samples 2), but not in the pellet 1 or embryonic samples (zona pellucida and inner cell mass) was identified. Results shows viral replication could happen in the reproductive system from naturally infected goats, what have never been described before. However, embryos recovery from the flushing media did not show to be permissive to virus. This might indicate that the non cirurgical technique can provide virus dilution, since previous studies in embryos collected surgically revealed the presence of the virus pellet in 1, what did not happen in present work.

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Genetics and climatics influence in pregnancy rate in cattle embryos recipients

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Keywords: biotechnology, bovine, pregnancy rate.

Bovine embryo biotechnologies have been widely used in Brazil, mainly in the *in vitro* embryo production (IVEP), and have increased for dairy cattle and European genetic donors (*Bos taurus taurus*; Viana JHV, Embrião, 51:6-10, 2012). However, pregnancy rates in recipients exhibit seasonal variations and in accordance to IVP embryos genetics. The aim of this study was to evaluate the genetic effects of the bull, the donor and the embryo in the pregnancy rates at 30 and 60 days in different seasons. We evaluated data from 2,254 inovulations of IVP embryos generated in the same laboratory, performed over a period of 36 months, in the same property. Crossbred recipients were kept in Brachiaria grass pasture, water and mineral salt ad libitum. Breeders and donors from different breeds were grouped by genetic characteristics, forming the groups Bos taurus taurus (TAU), Bos taurus indicus (IND) and Bos taurus taurus x Bos taurus indicus (MEST). It was considered as summer (VE) months from November to April and winter (IN) months from May to October. The data was plotted in spreadsheets and the pregnancy rates were compared by $\chi 2$ test, considering 5% of probability, using SAS software, version 9.2. Embryos produced with IND bulls semen demonstrated superior results compared to TAU at 60 days (42.0 vs 36.9%; $p<0.05$), however no differences were observed at 30 days. The donor genetic influenced pregnancy rates in both diagnostic periods, whereas the donor TAU showed inferior results (35.8a, 43.2b and 43.7b at 30 days and 31.2a, 41.1b and 40.6b at 60 days in TAU, IND and MEST, respectively; $p<0.05$). In relation to embryo genetics, lowest pregnancy rates ($p<0.05$) were observed in TAU embryos for both diagnostics (32.9a, 43.3b and 43.5b at 30 days and 27.4a, 41.2b and 41.1b at 60 days in TAU, IND and MEST embryos, respectively). The seasons, VE or IN respectively, did not influence pregnancy rates at 30 and 60 days (41.4 vs 42.2 and 38.5 vs 39.6; $p> 0.05$) if considered all embryos. No effects of embryo genetics groups were observed in pregnancy rate on IN months ($p> 0.05$). In VE, TAU embryos showed decreased rates in both diagnostics (34.0a, 44.0b and 43.9b at 30 days and 29.6a, 41.2b and 40.1b at 60 days for TAU, IND and MEST, respectively; $p<0.05$). There was no difference in the occurrence of pregnancy loss between 30 and 60 days in different groups or season ($P> 0.05$). In conclusion, the genetics of TAU animals, regardless of origin from the donor, semen or both, produced embryos with less probability of pregnancy. In general, the seasons of year did not influence the pregnancy rate at 30 and 60 days.

Acknowledgments: Fapemig, Capes e CNPq.

A169 OPU-IVP and ET

Influence of ovarian status on bovine *in vitro* embryo production

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Keywords: corpus luteum, *in vitro* fertilization, ovaries.

Despite the advances on *in vitro* embryo production, its efficiency remains low. There are evidences indicating that late blastocyst rate depends on the quality of oocytes and sperm cells used as raw material for the process [Dieleman et al., Theriogenology, 57(1), 5-20, 2002]. Regarding oocytes, the source has been considered more relevant for quality than culture conditions in the laboratory (Moussa et al., Anim Reprod Sci, 155, 11-27, 2015). The aim of this study was to evaluate the effect of bovine ovarian status on fertilization and embryo production rates after *in vitro* fertilization. Ovaries were obtained from local slaughterhouse in pairs belonging to the same animal and assigned into three experimental groups according the following criteria: 1) Ovaries with corpus luteum (CL+); 2) ovaries without corpus luteum from cows with cyclic sexual activity (CL-); 3) ovaries from cows in anestrus (NCL) (ovaries without corpus luteum and no visible follicle at divergence or dominance phase in both ovaries). Briefly, after follicular aspiration, groups of 20 cumulus-oocytes complexes (COCs) (420/experimental group) were cultivated for 24 hours at 38.5 °C, 5% CO_2 and saturated humidity in 100 µL of maturation medium (TCM-199/10% of FCS). Afterwards, the COCs were co-cultured with spermatozoa for 18 hours in fertilization medium (SOF/BSA). Finally, presumed zygotes were placed in co-culture for 7 days with cumulus cells under same atmospheric conditions aforementioned. After 18 hours through *in vitro* fertilization process, pronuclei formation was assessed by lacmoid stain. The cleavage rate and blastocyst development were evaluated at 3 and 7 days of *in vitro* culture, respectively. Results were analyzed by Kruskal-Wallis test, using confidence interval of 95%. There was no difference in pronuclei formation between experimental groups. Cleavage rate of embryos derived from oocytes obtained from ovaries of cows in anestrus (NCL) was lower [49.5% ± 3.6, (p=0.029)] compared with CL- group (62.2% ± 3.9). Nevertheless, there was no difference in the cleavage rate of embryos derived from oocytes collected from ovaries with (CL+) and without (CL-) corpus luteum from cows with cyclic activity. In addition, there was no difference on blastocyst production rate between NCL, CL- and CL+ groups (20.3% ± 3.3, 20.9% ± 3.2 and 16.4% ± 2.9, mean ± SEM, respectively). In conclusion, the source of oocytes could influence the rate of cleavage after *in vitro* fertilization. However, under the culture conditions assessed, there was no effect on blastocyst production.

A170 OPU-IVP and ET

The animal temperament influence on responsive rates to hormonal protocol and pregnancy in recipient cows of embryos

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Keywords: hormonal protocol, pregnancy, temperament.

The aim of this research was to study the influence of animal temperament in the response to hormonal synchronization protocol for embryo transfer in fixed-time (TETF) and in pregnancy in embryos recipient cows. It were used (n = 235) embryos recipient cows, native from Acre, Brazil, whit uni or multiples pregnancies, 03 to 06 years old, not-lactating, extensive raising, with ad libitum water and mineral salt. The hormonal synchronization procedure was based on simplifying P36 TETF protocol, as follow: on a random day of the estrous cycle (D0), each cow received 1g P4 intravaginal progesterone device and 2.5 mg estradiol benzoate (BE) intramuscular (IM). On D8, P4 implant was removed and 150μg D-cloprostenol (PGF2α), 400 IU eCG and 1mg BE were administered IM. On D16, one embryo (blastocyst grade 1 or 2) was transferred to each cow after the detection of one corpus luteum (CL) in one of the ovaries (US - Aloka SSD 500, Aloka, Japan). Embryos were obtained by *in vitro* production technique (PIV) originating from dairy Gir donors and bulls. Temperaments were classified in three levels: 1 - docility, 2 - slightly aggressive temperament and 3 - very aggressive temperament, according to cow's behavior during the synchronization process until embryo transfer'day (D16). Chi-square test was used in this study. From 235 cows submitted to hormonal protocol (TETF), 77 (33%) showed no CL in the ovaries by ultrasound examination at D16, therefore classified as unresponsive to hormonal protocol. From those, 53 cows (69%) were level 1, 16 (21%) level 2 and 8 (10%) level 3. On the other hand, 158 (67%) cows responded to the protocol, presenting a CL and been submitted to TETF. From 158 transferred embryos, 54 (34%) resulted in pregnancies. The response or not to hormonal protocol showed no statistical difference in relation to temperament levels. However, in relation to pregnancy rate, group 1 animals (docility) had higher levels (41%) than groups 2 and 3 (21% and 28% slightly and very aggressive, respectively). Therefore the recipient temperament didn't influence significantly the hormonal protocols response ($P > 0.05$), but influenced the pregnancy rate ($P < 0.05$).

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Inhibition of methylation with 5-aza-2'-deoxycytidine interferes on development of bovine embryos derived from heat-shocked oocytes

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Keywords: IVEP, methylation, stress.

Epigenetic modifications are required during pre-implantation embryo development but little is known about the effects of heat shock. Chemical agents that modulate epigenetic events can contribute to understand the effect of IMV and heat shock on embryo development. This study evaluated the effect of 5-aza-2'-deoxycytidine (5-aza; Sigma, St. Louis, USA), a DNA methylation inhibitor, on development of bovine embryos derived from oocytes submitted or not to heat shock during IVM. Experiment 1 (NHS – non-heat-shock) evaluated the effect of 5-aza on development of embryos derived from oocytes matured at 38.5°C and 5% CO_2 for 24h, and the Experiment 2 (HS - heat shock) evaluated the effect of 5-aza on development of embryos derived from oocytes matured at 41.5ºC for 12h in 6.5% CO_2 followed by 38.5°C for 12h in 5% CO2. After IVM and IVF, denuded presumptive zygotes were cultured with 0 or 10 nM of 5-aza for 24h or 48h in CR2aa plus 2.5% FBS at 38.5°C with 5% CO_2, 5% O_2 and 90% N_2. After that, embryos were cultured in CR2aa plus 2.5% FBS until day eight post-fertilization. The experiments were composed by the following treatments: Exp. 1: NHS (control, without 5-aza; n=391); NHS24h (5-aza for 24h, n=380) and NHS48h (5-aza 48h, n=379); Exp. 2: HS (control without 5-aza; n=329); HS24h (5-aza 24h, n=320) and HS48h (5-aza 48h, n=381). The proportion of embryos that reached the 8-cell stage on day three (D3) post-fertilization was analyzed by chi-square. Total cleavage rate on D3 and blastocyst rate on day eight (D8) were analyzed by ANOVA and means compared by SNK. Values are shown as mean±SEM. The proportion of embryos with 8-cell in the Exp 1 was lower ($P<0.05$) in NHS24h (28.1%) and NHS48h (33.4%) than in the control (NHS: 42,3%). In Exp. 2 HS48h (23.0%) had lower ($P<0.05$) proportion of embryos at 8-cell stage than the control (HS: 34.0%), with no significant difference with HS24h (27.1%). There was no ($P>0.05$) difference in the cleavage rate among treatments in the Exp. 1 and in the Exp. 2. In the Exp. 1 blastocyst rate was lower ($P<0.05$) for NHS48h (15.7 ± 2.9%) than for NHS (32.2 ± 3.4%) and NHS24h (25.8 ± 3.9%) treatments, and in Exp. 2 blastocyst rate was lower ($P<0.05$) for HS24h (9.5 ± 2.2%) and HS48h (11.1 ± 2.4%) than for HS (21.6 ± 3.4%). In conclusion, inhibition of DNA methylation for 48h in embryos derived from non-heat-shocked oocytes influences the production of blastocysts (Exp. 1) whereas that same effect is found with shorter time of embryo exposure to DNA methylation inhibitor (24h) for embryos derived from heat-shocked oocytes (Exp. 2). Those data suggest that embryos derived from heat-shocked oocytes are susceptible to epigenetic modulation but in a different time-dependent manner from those derived from non-heat-shocked oocytes.

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A172 OPU-IVP and ET

Hormonal stimulation in Nelore calves for *in vitro* embryo production

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Keywords: hormonal stimulation, IVF, prepubertal females, OPU.

The study of reproductive management on prepubertal bovine females for including these animals in the IVF could be an alternative for accelerating genetic gain by reducing the generation interval. This work aimed to evaluate a hormonal treatment response on the ovarian characteristics and on the OPU (Laparoscope Storz Xenon300W. Tuttlingen, Germany)/IVF in 9 Nelore calves (cross-over design) 4-7 months old. In the control group (CG, n=9) transrectal ultrasonography (US) was performed daily (MyLab 30VetGold,Esaote, 5-7,5MHz transducer. Génova, Italy) during 8 days (D0 to OPU) and ablation of larger follicles was performed on D2 (5 days before OPU). In the treated group (TG, n=9) US was also performed during 8 days, which D0 represented the start of treatment (Intravaginal device. Progesterone-P4 0.33g. Eazi-Breed-CIDR, Pfizer Animal Health, Brazil) including an injection of estradiol benzoate (im. 2mg. Ric-BE, Tecnopec-Brazil). From the D4 on, 6 FSH injections were administered, during 3 days (im. 12/12h: 40mg+5 20mg doses. Total:140mg; Folltropin, Bioniche Animal Health, Belleville-Ontario, Canada). On the last FSH dose, it was administered 2.5mg of LH (im. Lutropin, Bioniche Animal Health, Belleville-Ontario, Canada). The OPU was performed 20-24h after the last FSH injection (D7). The P4 devices were removed right after the OPU. Aspirated COCs were selected, matured, fertilized and cultured *in vitro* until D7. As IVF control, COCs obtained from slaughterhouse ovaries were used (Slaughterhouse Group-SG). The US or OPU grouped data were simultaneously compared between CG vs TG on D0 and D7 and analyzed according to their distribution, by t and Man-Whitney tests. The IVF results were compared among CG, TG and SG by Chi-square test and Kruskal Wallis (PROPHET 5.0, BBN-Systems&Technologies, Cambridge-Massachusetts, USA). The TG had increased ($p<0.05$) total follicular population (20.0 ± 4.95, D0 vs 26.66 ± 4.24, D7), follicles ≥2,5mm (4.11 ± 1.96, D0 vs 11.55 ± 4.09, D7), ovarian diameter (13.08 ± 1.0 mm, D0 vs 14.81 ± 1.38mm, D7) and the number of aspirated follicles (95 vs. 152, respectively CG vs TG). The TG had more grade I and II oocytes (59% vs 25% in TG vs CG, respectively) and, conversely, the CG had more grade III and IV oocytes (53.3% vs 37.1% in CG vs TG), $p<0.05$. However, it did not increase ($p>0.05$) the cleavage rate (49.33% vs 51.42%, respectively CG vs TG), the rate and the number of blastocysts in D7 (1.33% vs 8.57% or 3 vs 9, respectively for rate or number in CG vs TG. SG blastocysts rate: 24.41%) nor the number of embryos/female donor (0.33 vs 1.0, respectively CG vs TG). It was concluded that this hormonal treatment increased the number of observed and aspirated follicles, grade I and II oocytes, but it did not increase embryo production on these animals.

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Postpartum spontaneous ovulation in quarter horses embryo recipients mares

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Keywords: ET, follicles, ultrasonography.

The seasonal polyestrous is characteristic of the equine species, which enters in heat regularly in the period of greatest brightness. In spring and fall transitional period, the follicular growth is still uneven and determines failures in ovulation, by the negative effect of melatonin on the neuroendocrine system. In embryo transfer (ET) programs, the inclusion of mares, as recipients, in early spring determining limited success. Therefore, this study evaluated the influence of the foaling period on first estrus post partum, time to first ovulation and follicle diameter of embryo recipients. Quarter Horse mares (n = 34) with 11 y.o. in average, and 500-600kg were evaluated from February to March 2015 in a farm located in Weatherford, Texas/USA. The mares received alfalfa and sweet feed, and were kept in paddocks after the second day postpartum (d.p.p.). The mares were distributed according to the birth date on A (Feb 1st to 28th), B (March 1st to 15th) and C (after March 15th). After the 8th d.p.p., the mares were evaluated (every other day) by transrectal palpation and ultrasonography for the following parameters: time of foaling, follicular development, diameter of the pre-ovulatory follicle, ovulation distribution in right or left ovary, d.p.p. after first ovulation, and the follicles growth rate. Once detected, a follicle with diameter ≥30mm was followed until ovulation. The data were submitted to PROC GLIMMIX of SAS® (SAS 9.3, USA, 2003). The total number of ovulations on the left and right ovary were 21:13, respectively. On the left ovary the growth rate was 5.62 ± 0.69mm/day, the ovulation occurred with follicular diameter of 43.57mm ± 0.99 to 12.19 ± 0.43 d.p.p. On the right ovary, the growth rate was 3.36±0.4 5mm/day and ovulation occurred at 13.07 ± 0.8 d.p.p. with a diameter of 43.00 ± 1.13mm. All mares (n=8) at group A ovulated in the left ovary with a growth rate of 6.7 ± 1.07mm a day and in average ovulated at 11.2 ± 0.65 d.p.p. with a follicle diameter of 43.1 ± 1.6mm. Group B, showed 7 ovulations in the left ovary, and daily growth rate was 4.00 ± 1.30mm/day. Ovulation occurred 12 ± 0.61 d.p.p., and the average follicular diameter was 42.33 ± 1.45mm. In the right ovary 6 ovulations occurred, the growth rate was 3.00 ± 0.57mm/day, ovulation occurred after 12 ± 1.03 d.p.p., and the mean follicular diameter was 40.00 ± 1,87mm. Group C showed 6 ovulations in the left ovary with a growth rate of 5.75 ± 1.03mm/day, ovulating on day 13.66±0.8 with a diameter of 45.33 ± 2.17mm. Seven ovulations occurred in the right ovary; the growth rate was 3.57 ± 0.64mm/day, until ovulation at 14 ± 1.15 d.p.p. with a diameter of 45.14 ± 0.73mm. The analysis of time of foaling revealed no difference in time to p.p. ovulation (P=0.717), daily growth rate (P=0.741), and the follicle diameter at ovulation (P=1.00). The time of foaling did not influence the follicular growth rate, postpartum days to ovulation or follicular diameter of recipient mares prepared for ET that foaled at different dates in the season.

A174 OPU-IVP and ET

In vitro production parameters in Sindhi breed bovine females (*Bos taurus indicus)*

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Keywords: *Bos indicus*, IVF, OPU.

Ultrasound-guided follicle aspiration (OPU) related to IVP of embryos is a technique of great economic interest that have been growing rapidly in the industry due to the efficiency of sex-sorted semen, and zebu cattle (Bos indicus) have been largely used for dairy purposes (Viana et al., Theriogenology, 73, 966-972, 2010). Therefore, the aim of this study was to evaluate the effect of the donor age, season of the year, sire and type of semen (sexed and non sexed) on IVP of Sindhi embryos. Data were provided by Sexing Technologies do Brazil (Sertãozinho, São Paulo, Brazil) regarding to 499 OPU sessions performed from January 2008 to January 2015 on 150 Sindhi oocyte donors raised at different farms from São Paulo state. Conventional and sex-sorted Sindhi semen from 22 different bulls were used. The effects of the donor (150 animals), age of the donor (up to 6 years vs. over 6 years old), season of the year (rainy vs. dry), sire (22 animals) and type of semen (conventional vs. sex-sorted) on variables like number of oocytes collected, number of viable oocytes (in grades I, II and III), number of degenerated oocytes, cleavage rate and blastocysts rate in D7 were analyzed. Data were analyzed by ANOVA using the GLIMMIX procedure of SAS. All OPU sessions resulted in 7.971 COCs recovered. The season of the year (rainy vs. dry) did not affect the number of oocytes collected per OPU (23.4 vs. 24.0 ± 1.43; P=0.74). However, in the rainy season the number of viable oocytes was greater (67.8 vs. 64.0 ± 0.01%; P=0.02), and the number of degenerated oocytes smaller (32.2 vs. 36.0 ± 0.01%; P=0.02). However, the cleavage and blastocyst rates did not differ (P>0.45) in OPU performed between the different seasons and they were in average 72.0 ± 0.03 and 26.8 ± 0.01%, respectively. The age of the donor at the time of OPU affected the results of PIV. Donors with up to 6 years old had greater number of oocytes collected (26.9 vs. 20.8 ± 1.33; P<0.01), as well as greater number of viable oocytes (68.5 vs. 64.4 ± 0.01; P<0.01) and smaller number of degenerated oocytes (31.5 vs. 35.6 ± 0.01; P<0.01). However, there was no difference on cleavage rate between young and old cows (74.4 vs. 70.0 ± 0.03%; P=0.31), nonetheless there was a tendency for young cows to had smaller blastocyst rate in D7 (25.0 vs. 28.2 ± 0.01%; P=0.07). The conventional semen resulted in better cleavage rate (76.4 vs. 58.9±0.04%; P<0.01) and blastocyst rate than the sex-sorted (27.5 vs. 23.1 ± 0.01%, respectively; P=0.02). There was an effect of sire on the number of oocytes collected (P<0.03) and on blastocyst rate (P<0.02). In addition, there was an effect of donor on viable oocytes (P<0.02), in the number of degenerated oocytes (P<0.02), and on blastocyst rate in D7 (P<0.01). In conclusion, in donors with less than 6 years old, the number of oocytes collected and embryo produced is greater. The sexed semen is less efficient than the conventional one in IVP programs in this breed. Females Sindhi produce a greater number of viable oocytes during the rainy season, when the availability of forages is greater.

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Plasma FSH profile following single injection of pFSH combined with hyaluronan

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Keywords: FSH, Holstein heifers, plasma profile.

We evaluated the plasma FSH profile after injection of different doses of porcine FSH (pFSH; Folltropin®, Bioniche) associated with hyaluronic acid (HA; MAP-5®, Bioniche). A total of 23 Holstein heifers were allocated in one of four groups: Control (no superstimulatory treatment; n = 4), F200 (200 mg of pFSH, n = 6), F200HA (n = 6) and F300HA (n = 6; 200 or 300 mg of pFSH diluted in HA 0,5%). On a random stage of the estrous cycle, all heifers received a Norgestomet ear implant (Crestar® MSD), 2.0 mg IM of estradiol benzoate (RIC-BE®, Tecnopec) and 0.150 mg of cloprostenol (ESTRON®, Tecnopec). On Day 5, all females received 0,150 mg of cloprostenol and on Day 7 they were submitted to an ultrasonographic evaluation to verify the diameter of the largest follicle. On Day 8 and Day 9 the F200 group received 200 mg of pFSH (14.3 mg/mL) administered in 4 decreasing doses (57.1, 57.1, 42.9 and 42.9 mg) every 12 h. The F200HA and F300HA groups received a single dose of pFSH (IM) on Day 8 AM, 5.0 ml (F200AH) and 7.5 ml (F300HA; 40mg/ml of FSH). The control group received no additional treatment. On Day 12 AM, the implant was removed. Blood sampling started on Day 8, immediately before the first pFSH administration (0h). The samples were collected by jugular vein every 6 h until 96 h to evaluate the plasma FSH concentration. The variables were analyzed by orthogonal contrast using the GLIMMIX procedure of SAS. The established contrasts were: C1 (superstimulation effect): control vs. (F200 + F200HA + F300HA); C2 (HA effect): F200 vs. (F200HA + F300HA); C3 (dose effect): F200HA vs. F300HA. The area under the curve was calculated by the trapezoid method. The total period with elevated FSH concentration (greater than two standard deviations) was determined by the interval between the treatment and FSH return to basal levels. Heifers submitted to superstimulatory treatment (F200: 75.8 ± 5.6; F200HA: 64.8 ± 11.5; F300HA: 97.8 ± 5,3 ng*h/mL) presented higher area under the curve when compared to the control group (53.8 ± 5.1 ng*hr/mL; C1, P = 0.002). The F200 group did not differ (C2; P = 0.56) from females treated with HA and; F300HA group animals presented higher area under the curve in comparison to F200HA group (C3; P = 0.006). Still, F200 group presented an extended period with high plasma FSH concentration (55.0 ± 4.5h) compared to groups receiving single dose of pFSH in HA (C2; F200HA: 32.0 ± 8.2 and F300HA : 37.0 ± 4.5h, P < 0.0001), being this period similar among the groups treated with HA (C3, P = 0.17). Therefore, the treatment with a single dose of pFSH diluted in HA increased plasma FSH concentrations compared to the Control group, being similar to the treatment with two daily injections. However, treatment with pFSH diluted in HA presented shorter period with high plasma FSH concentration compared to the group with pFSH daily injections.

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A176 OPU-IVP and ET

GnRH potential to synchronize follicular emergence and ovulation prior to superovulatory day 0 protocol in sheep

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Keywords: lecirelin, MOET, superovulation.

The role of GnRH to synchronize ovulation and follicular emergence previous to superovulatory protocol, started on the first day of the estrous cycle (Day 0), was assessed in Santa Inês ewes. For estrus synchronization, 60 mg medroxyprogesterone acetate sponges were used for 6 d plus 37.5 µg d-cloprostenol and 300 IU eCG at fifth day. After sponge removal, ewes were assigned to three treatments: GControl – saline at 12 h (n = 10); G24h – GnRH at 24 h (n = 10); or G36h – GnRH at 36 h (n = 9). Ovarian ultrasonography was conducted every 12 hours, after sponge removal, to assess the occurrence of ovulation and emergence of follicular waves until the fifth day of the estrous cycle. Ewes from G24h and G36h had earlier ovulation (48.0 ± 3.2 and 56.7± 1.9 h) compared to GControl (64.1 ± 3.0 h – $P < 0.05$). It is reasonable to affirm that G36h was more effective in synchronizing ovulation compared to G24h probably due to the lower SEM obtained. The follicular growth in the post-ovulatory day was affected by day of the estrous cycle ($P < 0.05$) as well as by interaction treatment x day of the estrous cycle ($P < 0.05$). There was a greater population of medium follicles during the first 24 h post-ovulatory period in G24h compared to GControl and absence of large follicles in G36h between 36 and 72 h after ovulation. The greatest population of medium follicles in G24h compared to GControl may arise from the previous ovulatory follicular wave, but due to anticipation of the LH surge, it was not able to promote the growth and maturation of these follicles. After 60 h, the medium follicles from wave emergence of GControl and G36h stabilized compared to the follicles from G24h. The greater number of dominant follicles 12 h after ovulation in G36h compared to G24h has correlation with the largest number of ovulated follicles in this group. It is important to highlight that during the first 96 h of the estrous cycle, G36h presented no dominant follicle between 36 and 72 h after the ovulatory period. In conclusion, considering the beneficial effects of G36h in synchronizing ovulation and to promote the absence of dominant follicles in the first days of estrous cycle. According to data obtained, the best time to start the superovulatory treatment, known as "Day 0", could be 80 h after sponge removal (56 h for the occurrence of ovulation plus 24 h to reset dominant follicles), in the induction of synchronized estrus, for Santa Inês ewes.

A177 OPU-IVP and ET

Bovine and buffalo *in vitro* embryo production with the addition of lippia origanoides essential oil in the maturation medium

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Keywords: antioxidant, buffalo, *in vitro* production of embryos.

The use of embryo *in vitro* production (IVP) has grown in recent years, however, the efficiency of the process is low with unsatisfactory embryo production levels. It is believed that many factors can influence this process, including, reactive oxygen species (ROS) produced as a result of artificial maturation and culture conditions. Thus, antioxidants have been incorporated into the IVM and IVC medium in attempt to reduce the formation of ROS and to improve the embryo production rates. A natural antioxidant already used in cell culture, whose active ingredient were carvacrol and thymol, extracted from Lippia origanoides plant, represents a promising potential to be used in embryo culture (Vicuña, et al., Fitoterapia, v. 81, p.343-349, 2010). Thus, the aim of this study was to evaluate the effect of supplementation of maturation medium for buffaloes and cattle IVP with essential oil of Lippia origanoides (EOLO) at different concentrations. Were used 2052 bovine oocytes and 1026 buffalo oocytes recovered from slaughterhouse ovaries, divided into 5 treatments consisted of: T1 (Base Media (BM): TCM 199 + 10% FBS + 22μg/ml pyruvate + 5UI /mL LH + 0,05μg/ml FSH + 1μg/ml estradiol + 83.4 μg/ml amikacin), T2 (BM + 50 μM/ml cysteamine), T3 (BM + 2,5μg/ml EOLO), T4 (BM + 5 μg/ml EOLO) and T5 (BM + 10 μg/ml EOLO). The reagents used were acquired from Sigma-Aldrich®, St. Louis, USA. Oocytes were maturated at 5% CO_2, 38.5 °C for 24 hours. IVF occurred for a period of 18-20 hours and then the zygotes were denuded and cultured in SOF medium + 2.5% FBS for 7 days. It was evaluated the rate of cleavage after 48 hours of cultivation and the production of blastocysts on 7 and 8. For statistical analysis we used the ANOVA test and considered statistically significant $p < 0.05$. The cleavage rate (mean ± standard error) did not differ ($P>0.05$) between treatments for buffaloes (39.87 ± 5.54; 35.64 ± 5.60; 44.16 ± 17.04; 45.19 ± 5.56 and 43.57 ± 6.86; respectively for treatments T1, T2, T3, T4 and T5). The blastocyst rate for buffaloes was also similar ($P>0.05$) between treatments (30.21 ± 6.49; 19.52 ± 5.92; 27.56 ± 5.11; 32.87 ± 6.49 and 23.35 ± 4.77; respectively for treatments T1, T2, T3, T4 and T5). For cattle, cleavage rates did not differ ($P>0.05$) between treatments (64.06 ± 3.98; 62.65 ± 3.59; 64.72 ± 4.04; 53.32 ± 3.84 and 65.51 ± 4.90; respectively for treatments T1, T2, T3, T4 and T5). The cattle blastocyst rate did not differ $P>0.05$ between treatments and was 27.25 ± 2.98; 26.82 ± 3.94; 24.97 ± 3.52; 23.36 ± 2.57 and 27.14 ± 2.87 for T1, T2, T3, T4 and T5 ($P> 0.05$), respectively. The use of Lippia origanoides essential oil did not affect cleavage and development capacity of cattle and buffaloes in vitro produced embryos.

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A178 OPU-IVP and ET

In vitro production of bovine embryos and buffalo during two periods of the year

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Keywords: *Bos taurus,* buffalos, *in vitro* production of embryos.

The PIVE aims to maximize the genetic gain by increasing the number of female offspring during the reproductive life and optimizing the use of semen dose. Despite the considerable importance of this biotech, the rates found in the literature are variable, especially when it comes to bovine and buffalo. It has been reported that the blastocyst production rate in buffalo ranges around 20%while in the bovine it is around 48% (Elamaranetal, Reprod. Dom. Anim, V.47, p. 1027 to 1036.2012). One of the possible reasons for the low production of embryos in buffaloes is the influence of seasonality on the reproduction because they are considerate short days animals. In view of this, the present study aimed to evaluate the *in vitro* production of bovine embryos and buffalo during two periods of the year, considered favorable and unfavorable to buffalo. The experimental period was divided into favorable season (1 March to August 31) and unfavorable (1 September to February 28) according to the seasonality characterized for buffaloes. Altogether 174 and 566 buffaloes and cattle oocytes were used, respectively. They were placed in wash medium comprising TCM 199, 10 % fetal bovine serum, 22µg / ml sodium pyruvate and 83µg / ml of amikacin sulfate. Oocytes were matured in an incubator (38.5 ° C , 5 % CO_2 , 95 % humidity) for 24 hours in solution (TCM 199 + 10 % FCS + 22µg / ml + Pyruvate 5UI / ml LH + 0.05 mg / ml FSH + 1 / ml estradiol + 83.4 ug / ml amikacin + 50 mcg / ml cysteamine). They were fertilized for 18 to 22 hours and the sperm used was from the same bull with fertility proven in PIVE. The reagents used were from Sigma - Aldrich®, St. Louis , USA . The presumptive zygotes were denuded and then cultured in SOF medium (Synthetic oviduct fluid) + 2.5 % FBS for 7 days. Cleavage rate was assessed 48 hours after fertilization. The production of blastocysts was evaluated on D7 and D8. For statistical analysis, the Shapiro -Wilk test was used to assess the normality of continuous variables. For the mean comparison we used the Student T- test test and the level of significance was $p < 0.05$. Regarding the time of year (favorable and unfavorable) there was no difference between the cumulus expansion rates (89.49 ± 6.61 vs 92.00 ± 2.83) cleavage (55.87 ± 4.22 vs 62, 83 ± 4.20) and blastocyst formation in cattle (20.12 ± 6.33 vs 27.68 ± 4.18) ($p> 0.05$). In Buffalo there was no difference between the cumulus expansion rate (78.73 ± 4.44 vs 77.67 ± 7.64) , cleavage (36.29 ± 4.11 vs 35.13 ± 10.12) and blastocyst formation (22.79 ± 7.14 vs 16.90 ± 9.58), in both favorable and unfavorable time, respectively. In the favorable time there was no difference in cumulus expansion and blastocyst production among cattle and buffaloes ($p> 0.05$). However the cleavage rate was differed between bovine and buffalo ($p <0.05$) in unfavorable time.

A179 OPU-IVP and ET

In vitro embryo production of Nelore (*Bos indicus*) donors submitted to a commercial program during 12 months

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Keywords: bovine, embryo, IVF.

The aim of this study was to evaluate the effect of the number of OPU sessions (OPU), bull and season (summer vs. winter) on the *in vitro* embryo production (IVEP) of Nelore (Bos indicus) donors submitted to a commercial IVEP program during 12 months. Data of 36 cycling cows were analyzed during 12 consecutive follicular aspirations (OPU), with a 30 days interval at an experimental farm (Instituto de Zootecnia Sertãozinho, APTA). All cows were aspirated without previous synchronization of the follicular wave. A total of three Nelore bulls were used for *in vitro* fertilization of retrieved oocytes. Data were analyzed as repeated measures using the GLIMMIX procedure, SAS 9.3. The total number of oocytes [20.3 ± 2.7 (1st session) vs. 13.5 ± 1.21 (12th session); P = 0.0003] and number of viable oocytes [17.0 ± 2.4 (1st session) vs 10.0 ± 1.0 (12th session); P = 0.001] decreased over time according to the number of aspiration sessions. It was observed interaction (P = 0.01) between the number of OPU sessions and season for rate of viable oocytes, which was greater during the winter (P = 0.002) in sessions 1, 2, 3, 6, 10, 11 and 12 (Winter: 86.4, 83.3, 77.1, 76.5, 74.8, 76.3, 75.5, 74.0, 69.8, 77.6, and 80.1 78.4% and Summer: 64.7, 67.3, 72.0, 77.9, 76.7, 67.5, 67.2, 76.4, 71.8, 74.7, 68.8 and 69.4%, aspiration sessions 1 to 12, respectively). There was no effect of aspiration session on the number of embryos produced by OPU session (P = 0.97) and blastocyst rate (P = 0.30). The total number of oocytes (P = 0.29) and number of viable oocytes (P = 0.11) did not differ between season. However, the number of embryos per OPU session (6.1 ± 0.4 vs 4.5 ± 0.3; P = 0.06) and blastocyst rate (34.86 vs 31.86%, P = 0.009) was greater in winter compared to summer. The number of embryos per OPU session was not different among the diferent bulls (P = 0.14). However, a bull effect was observed on blastocyst rate (Bull A: 31.0%b; Bull B: 30.3% b and Bull C: 40.4% a; P = 0.0004). In conclusion, the number of total and viable oocytes decreased over time, according to the consecutive OPU procedures. Still, the efficiency of in vitro embryo production in Bos indicus donors can be compromised during the hot season of the year. Finally, the efficiency on in vitro embryo production can be affected according to the bull used for IVF.

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A180 OPU-IVP and ET

Acrosome reaction induced by theophylline associated or not to heparin in bovine semen

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Keywords: capacitation, fertilization, sperm.

The sperm capacitation consists in biochemical and physiological changes during which the sperm becomes hyperactivated and able to undergo the acrosome reaction to penetrate the zona pellucida of the mature oocytes. The aim of this study was to evaluate theophylline as a capacitation inducing agent in replacement or in combination with heparin for sperm cells acrosome reaction. Theophylline (T1633 - Sigma-Aldrich®) was added to fertilization medium provided by Biodux® company (Campinas, SP, Brazil). The experiment was performed with 4 Gir breed bulls and 3 treatments, in a total of 12 experimental groups. Each bull was evaluated using the following treatments: Treatment 1 (T1): Heparin - 10µg/mL; Treatment 2 (T2): Theophylline – 5 mM; Treatment 3 (T3): Heparin (10µg/mL) + Theophylline (5mM). Heparin is used as capacitation agent in most of embryo production laboratories, and for this reason was chosen as the control group. The acrosome integrity was analyzed by double staining technique (Trypan blue/Giemsa – TBG) described by Didionet al. (Gamete Res, v.22, p. 51-57, 1989). The semen was thawed and submitted to Percoll gradient separation (45 and 90%). Tubes containing fertilization T1, T2 and T3 medium were inseminated with 2 x 10^6 sperm/mL and kept on incubator at 38.8°C and 5% CO_2, in the absence of oocytes. The semen was incubated in capacitation medium for 0, 6, 12 and 18h then stained with Trypan blue / Giemsa and analyzed by bright field microscopy with 1000x magnification to evaluate the acrosome reaction. The analyzed characteristics in sperm cells were true acrosome reaction - Acrosome and post-acrosome region unstained; False acrosome reaction - Acrosome unstained and post-acrosome region stained; Dead - Stained post-acrosomal region and acrosome staineds. The data were submitted to analysis of variance followed by Tukey Kramer test ($p < 0.05$). Treatment did not affect the acrossome reaction analysis. The same was observed to bulls. However, the true acrosome reaction rate was higher ($p < 0.05$) at time 0h (61.50 ± 6.78) compared to 6h (19.63 ± 12.71), 12h (7.21 ± 4 40) and 18h (7.04 ± 2.66). For the dead sperm, we observed a higher rate ($p < 0.05$) at time of 12h (84.46 ± 5.82) and 18h (86.75 ± 4.19). It was observed that the incidence of true acrosome reaction was relatively lower than the dead sperm rate, which may be due to the total incubation time (18 hours). This suggests that the incubation conditions in the absence of COCs and essential growth factors for the acrosome reaction stimulation impaired sperm viability in vitro. However, theophylline was as effective as heparin in the induction of acrosome reaction.

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A181 OPU-IVP and ET

Efficient recovery of oocytes from ''onça parda'' (*Puma Concolor*) by laparoscopic ovum pick-up of gonadotropin-stimulated females

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Keywords: laparoscopic ovum pick-up, oocytes, *Puma concolor*.

Developing of an optimized procedure for oocyte collection would be critical for allowing the use of *in vitro* embryo production technologies (IVEP) and somatic cell nuclear transfer (SCNT) as tools for rebuilding balance in animal numbers for such endangered species. One young adult puma female of 2.5 years and two sister juvenile females of 1.5 years of age, were injected with 750 UI eCG five days prior to laparoscopic ovum pick-up (LOPU). The puma adult and one of the young females were also injected with 500 IU of hCG 24-30h prior to LOPU to promote *in vivo* maturation of oocytes, while the other sister female could not be injected and served as control. All injections were conducted using blow darting technique. Animals were deprived from food (24h) and water (12h) in preparation for surgery.The LOPU procedure was similar to the one previously described in small ruminants. Briefly, the females were restrained on a laparoscopy table in a 45° angle and then, using a 5 mm laparoscope and an atraumatic grasping forceps to uncover the ovaries, all follicles ≥ 2 mm diameter were aspirated using a 20G needle mounted in a plastic pipette connected to a collection tube and vacuum line. A grand total of 98 oocytes were recovered, representing >90% of the number of follicles aspirated and an average of 32.6 oocytes/donor. Interestingly, 42/43 oocytes recovered from the 2 females that received hCG were showing expanded cumulus which has been shown to be an accurate sign of *in vivo* maturation and readiness for IVF, while from the female that was not injected with hCG all oocytes had compact cumulus and required *in vitro* maturation prior to further processing. IVM was performed in 50 µL drops of maturation medium under mineral oil, at 38.5°C in humidified atmosphere with 5% CO2 in air for 24 h. The maturation medium consisted of M199 supplemented with bLH (0.02 U/ml), bFSH (0.02 U/ml), 17β-estradiol (1 µg/ml), sodium pyruvate (0.2 mM), gentamycin (50 µg/ml) and 10% heat-inactivated fetal bovine serum. Unfortunately, we were unable to obtain semen to conduct IVF as originally planned, so we resorted into vitrifying the oocytes in order to store the oocytes until semen becomes available. The *in vivo* matured oocytes were vitrified shortly after collection while the immature oocytes were vitrified after completing 24h in the above-described IVM conditions. In all cases, prior to vitrifiation, the oocytes were completely stripped of the cumulus cells by pipetting in handling medium containing 0.1% hyaluronidase. Oocyte vitrification was conducted following the procedure by Vajta et al. Briefly, oocytes were sequentially passed through holding medium (HM, TCM199 supplemented with 20% FBS), HV1 medium (HM with 7.5% DMSO and 7.5% ethylene glycol, v/v), HV2 medium (HM with 1M sucrose plus 16% DMSO and 16% ethylene glycol, v/v) and finally placed in homemade cryotops (longitudinally hemi-sectioned 0.25 straws) prior to immersion in liquid nitrogen.

A182 OPU-IVP and ET

Reduction in volume of Percoll gradients improves the quality and recovery of bovine sex-sorting for IVP

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Keywords: discontinuous percoll gradient, sperm selection for IVF, volumes.

The discontinuous Percoll gradient technique is one of the most used for animal sperm selection. However, in order to obtain a better use for conventional and/or sex-sorting semen pallets, besides reducing the costs of IVP and the IVF execution time, changes have been studied in the technic, such as different forces and time of centrifugation, number of Percoll gradients and volumes. This study aimed to evaluate the effect of different volumes of Percoll on the recovery membrane integrity and movement of bovine sexed sperm. Five replicates were conducted from a pool of sexed semen from two *Bos taurus taurus* bulls. The sperm selection was performed by discontinuous gradients (30, 60, 90%) Percoll (Folchini et al., Rev. Bras. Reprod. Anim., V.36, p.239-244, 2012), with volumes adjusted as treatments: Control: 300µL; Treatment 1 (T1): 100 µL and Treatment 2 (T2): 200µL for each gradient. At the first and second centrifugation a force of 2200 x g was used during 5' and 1', respectively. The samples were evaluated by a computerized system (SCA®, Sperm Class Analyzer, New Route, Barcelona, Spain) immediately after treatment concerning curvilinear velocity (VCL, µm/s) straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), amplitude of lateral head displacement (ALH, µm), beat cross-frequency (BCF, Hz), straightness (STR, %) and linearity (LIN, %), membrane integrity (staining with propidium iodide and 6-carboxyfluorescein diacetate) and sperm recovery rate obtained by the formula considering volumes and initial and final sperm concentrations (Machado et al., Theriogenology, v.71, p.1289-97, 2009). Data were analyzed by ANOVA ($p<0.05$). Reducing the volume of discontinuous Percoll gradients to 100 uL increased curvilinear velocity compared to the control and T2 (control: 34.4 um / s, T1: 70.2 um / s; T2: 43.9 um / s) and it was similar to T2 and higher than control as amplitude of lateral head displacement (Control: 1,3µm; T1: 3,5µm; T2: 1,8µm). About membrane integrity, it was observed that the Control (65.2%) and T2 (72.2%) were superior to T1 (55.8%). Evaluating the recovery rate, it was found that reducing the volume to 100 uL increased recovery rate (control: 28.3%; T1: 46.3%; T2: 28.8%) of sexed semen. These results demonstrate it is possible to obtain a higher recovery rate and selecting sperm motility with higher motility with the use of gradients 100 uL volumes.

A183 OPU-IVP and ET

Embryonic survival and birth rate after the transfer of *in vitro* produced ovine embryos cryopreserved by slow freezing or vitrification with minimum volume methods

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Keywords: birth rate,cryopreservation, IVP ovine.

Recently has been reported in sheep IVP embryos at different stages of development a new vitrification methodologies with minimum volume method (Cryotop and Spatula MVD) (dos Santos Neto et al., 70, 17-22, 2015). The objective of this study was to evaluate the survival rate of embryos at day 30, birth and survival rate after the 1st week of life of *in vitro* produced sheep embryos cryopreserved by slow freezing with ethylene glycol and vitrified with Cryotop method or Spatula MVD method. A total of 240 embryos cryopreserved at morulae and blastocyst stage were transferred to recipient ewes divided into 3 experimental groups: Slow freezing (N = 68); Cryotop (N = 93); Spatula MVD (N = 79). The oocytes were matured, fertilized and cultured *in vitro* until day 6 of embryonic development following the protocol routinely used at the laboratory. On day 6 embryos were frozen with ethylene glycol or vitrified by both methods. The embryos were cryopreserved by slow freezing and vitrified with Cryotop method developed for humans and mice by Kuwayama et al., Theriogenology, 67, 73-80, (2007) or Spatula MVD method reported by our laboratory. The synchronization of the recipients was performed using a short term protocol and 6 days after heat detection (Day 0) cryopreserved IVP embryos were transferred. We evaluated embryo survival (number of embryos at 30 days / total embryos transferred) performed by trans-rectal ultrasonography (5MHz, Well-D, China), birth rate (born lambs / total lambs at 150 days), and survival rate the 1st week (live lambs the 1st week / total lambs born). Statistical analysis was performed by logistic regression with significance level P <0.05. The results demonstrated that embryos in vitro produced and vitrified with Cryotop method had a higher embryonic survival at day 30 when compared to those frozen with ethylene glycol as compared to vitrified with Spatula method MVD (Slow freezing 7.3% 5/68; Cryotop 38.7% 36/93; Spatula MVD 11.4%, 9/79; P <0.05), respectively. No differences were found in survival rates when the embryos were frozen with ethylene glycol or vitrified with Spatula method (Slow freezing 7.3% 5/68; Spatula MVD 11.4%, 9/79; P=NS). No differences were found in any of the groups assessed on birth rate (85.0% 34/40; P=NS) and survival of the first week (72.5% 29/40; P=NS). These results suggest that the achievement of acceptable survival rates at 30 days as well as the birth and survival of lambs the 1st week allow us to consider vitrification with Cryotop method as a possible tool to embryo transfer programs in sheep.

A184 OPU-IVP and ET

Superovulation and non-surgical embryo recovery in Santa Inês ewes

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Keywords: sheep, superovulation, transcervical collection.

The objective of this study was to test the efficiency of two superovulatory protocolos and non-surgical embryo recovery in Santa Inês sheep. Sixteen pluriparous ewes were allocated to two experimental groups. G1 ewes (n=07) received intravaginal devices (60mg MAP; Progespon®, Syntex, Buenos Aires, Argentina) for six days, 37.5µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina) laterovulvar at sponge insertion and 24hs before its removal, 200IU eCG (Novormon 5000®, Syntex, Buenos Aires, Argentina) i.m. at device removal, and 200mg pFSH (Folltropin®, Bioniche, Canada) in eight decreasing doses (40, 40, 30, 30, 20, 20, 10, and 10mg) at 12h interval beginning two days before sponge removal. G2 (n=09) ewes received intravaginal devices for 15 days, 200IU eCG i.m. at sponge removal, and 200mg of pFSH i.m. in six decreasing doses (50, 50, 25, 25, 10, and 10 mg) at 12h interval beginning two days before sponge removal. Ewes were checked for estrous twice daily and natural mated by fertile rams while in estrus. In both groups, ewes received 0.025mg de GnRH (Gestran Plus®, ARSA S.R.L., Buenos Aires, Argentina) i.m. and 250IU hCG (Vetecor®, Hertape Calier, Barcelona, Espanha) i.m., 24 and 84h after estrous onset, respectively. All ewes received 37.5mg d-cloprostenol and 1mg estradiol benzoate (Estrogin®, São Paulo, Brasil) i.m. 16h plus 50IU oxytocin (Ocitocina forte ucb®, São Paulo, Brasil) i.v 20min before uterine flushing. Embryo collection was performed with ewes in standing position at day 7 estrous cycle by transcervical technique (Fonseca et al., Small Ruimin. Res., 111:96-99, 2013). Qualitative data were tested by qui-square test, and quantitative data were evaluated by ANOVA and t-test at 5% significance (SAEG®). The parameters were similar (P>0.05) for G1 and G2 ewes. Estrous response was 100%. The interval to estrus was 22.3 ± 4.5 and 16.0 ± 6.0h to G1 and G2 ewes, respectively. The percentage of ewes flushed was 85.7% (6/7) and 77.8% (7/9) G1 and G2 ewes, respectively. Overall flushing successful rate (liquid injected/liquid recovered) was 96.2%. The total (6.4 ± 7.9 and 7.4 ± 5.6) and viable (3.3 ± 4.7 and 2.7 ± 2.8) structures recovered was similar (P>0.05) to G1 and G2 ewes, respectively. Results of this study suggest that superovulated Santa Inês ewes can be subjected to non-surgical transcervical embryo recovery with satisfactory flushing efficiency and structure recovering.

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A185 OPU-IVP and ET

Nasal swab as pre test for herpesvirus seropositive oocyte donnors

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Keywords: BHV-1, nasal swab, oocytes.

Bovine herpesvirus is the infectious bovine rhinotracheitis disease (IBR) agent; this disease has a high prevalence in Brazil and is considered restrictive for international commercials trades of gametes and embryos. However, neutralization in microplates test is recommended by the OIE for diagnosis, but positive samples cannot differentiate vaccinated animals from non - vaccinated. The present study aimed to identify viral DNA in COCs and nasal mucosae from non-vaccinated and serologically BoHV positive cows as a method of pre selection of donors. Nine cows (n = 9) were separated into two experimental groups, six animals receive 0.1 mg / kg / bw / iv of dexamethasone (CORTVET, UCB saúde animal, Jaboticabal, Brasil) for five consecutive days (treated group – GT; n= 6), and in three animals (non treated group - GNT; n=3) were administered saline solution (0,05 mL/kg/ bw / iv) as the GT. The OPU and the nasal swab were performed on day 8 and 12 after the protocol began. The material was sent to PCR (Gasparini et al., Biologico, São Paulo, v.73, Suplemento 2, p.25-81, 2011). All nasal swabs and COCs samples were considered positive on PCR. The results show that PCR of nasal swab mighty have applicability as a tool for pre-selection of COCs donors and further studies are needed to validate this methodology for donors selection.

A186 OPU-IVP and ET

Conception rates of biopsied IFV bovine embryos either transferred fresh or after cryopreservation

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Keywords: biopsy, IVF embryos, vitrification.

In Brazil, among animal reproduction biotechnologies, in vitro fertilization (IVF) is a tool that is being increasingly incorporated into the routine of beef and dairy cattle herds. One of the advantages of this technique is the use of sexed semen, which allows the producer to choose the sex of the embryo and therefore increase their productivity. However, there are limitations, due to the absence of sexed semen for some desired bulls. A solution to identify the sex of the embryo before implantation is embryonic biopsy. The objective of this work was to evaluate the rates of development of in vitro produced bovine embryos either intact or biopsied and transferred either fresh or cryopreserved. Embryos were produced on a commercial scale from aspirations of oocytes from Angus donors (FSL Angus; Itu, SP). On the sixth day of culture, embryos were submitted to biopsy of cells through the microaspiration technique, using two mechanical micromanipulators coupled to two borosilicate micropipettes (holding and biopsy). Approximately 10% of the embryo mass from the trophoblast was removed for sex identification by PCR technique. After micromanipulation, embryos were cultured individually and identified. After 8-12 hours, viable embryos were prepared for transfer (fresh) or subjected to vitrification. The transfer was performed into previously synchronized recipients. Pregnancy rates were analyzed by logistic regression with data considered significant with $P<0.05$. Day 60 conception rates were significantly lower ($P<0.02$) for biopsied, vitrified embryos (33.75%; 27/80) compared to biopsied, fresh (53.33%; 32/60), non-biopsied, fresh embryos (50.58%; 131/259) and non-biopsied, vitrified (54.78%; 63/115) embryos. The results of this preliminary survey show that the association between the two technologies, biopsy and vitrification, led to a significant loss of embryonic viability compared to these techniques individually. However, considering the possibility of obtaining a duly identified embryo bank according to the needs of the producer, and the flexibility that these techniques adds to IVF, further studies are necessary in order to preserve viability and increase conception rates of biopsied embryos after vitrification.

A187 OPU-IVP and ET

Theophylline associated or not to heparin as a capacitation inducing agent to the *in vitro* production of bovine embryos

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Keywords: bulls, cleavage, fertilization.

The theophylline mechanism of action is similar to the caffeine mechanism. However theophylline is more efficient to increase the intracytoplasmic levels of adenosine 3',5'-cyclic monophosphate (cAMP), a nucleotide involved in the sperm capacitation process. The aim of this study was to evaluate theophylline as a capacitation inducer agent in replacement or in combination with heparin in the development of bovine embryos produced *in vitro*. The culture media used to produce the embryos were provided by Biodux® company (Campinas, SP, Brazil). Theophylline (T1633 - Sigma-Aldrich®) was added to the fertilization medium. The experiment was carried out with 4 Gir breed bulls and 3 treatments, in a total of 12 experimental groups. Each bull was evaluated according to the following treatments: Treatment 1 (T1): Heparin - 10mg / mL; Treatment 2 (T2): Theophylline - 5 mM; Treatment 3 (T3): Heparin (10mg / ml) + Theophylline (5mM). The cumulus-oocyte complexes (COCs) recovered from slaughtered cow ovaries were incubated in the maturation medium at 38.8ºC in atmosphere of 5% CO2 for 24 hours. In IVF step, the capacitating agents were added to the medium, composing the treatments T1, T2 and T3. The fertilization day was considered as day 0 (D0). The cleavage rate, embryo production and hatching were evaluated after 2, 7 and 10 days of fertilization, respectively. The data were submitted to variance analysis using generalized linear models. The averages were compared by Tukey Kramer test ($p < 0.05$). The embryo production rate was higher ($p < 0.05$) for the T1 (37.97 ± 13) relative to the T2 and T3 (10 ± 28.55, 27.60 ± 11.0, respectively). The same was observed in the hatching rate ($p < 0.05$) in the T1 (33.50 ± 14) compared to T2 and T3 (22.81 ± 11; 23.08 ± 10, respectively). Bull did not influence cleavage rates ($p > 0.05$) in the cleavage rates, produced embryos and hatching. Apparently, theophylline alone or associated with heparin did not induce a good sperm capacitation, which could be the reason of reduced fertilization rate. However, this fact was not evidenced by the cleavage rate, which was similar between treatments. This provides evidence that, although the rate of cleavage did not differ between treatments, not all of cleaved structures were competent to sustain the embryo development. These findings may be due the increased incidence of parthenogenesis or polyspermy and consequently blocking of embryonic development (Ramos et al., Brazilian Journal of Veterinary Medicine and Animal Science, V.52, 2000, online). Further investigations are necessary in order to explain the possible factors that led to theophylline alone or associated with heparin to reduce embryo production rates in in vitro fertilization.

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A188 OPU-IVP and ET

Methyl beta cyclodextrin use as agent of sperm capacitacion on the *in vitro* production embryos buffaloes (preliminary results)

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Keywords: buffalo embryo, cyclodextrin, sperm capacitation.

Cyclodextrins are cyclic oligosaccharides which have the ability of lipids such as cholesterol incorporation and can be used to alter the cell membrane cholesterol content (Visconti et al., 1999). Studies show their use in cholesterol removal from seminal membranes and in the induction of bovine sperm capacitation (Purdy et al, 2004, BiolReprod, 71, 522-527;Kato et al, 2010, Zygote, 19, 21. 30). Thus, the aim of this work is to evaluate the use of cyclodextrin as sperm capacitation agent, analyzing their influence on IVPE in buffalo. Buffalo ovaries were collected from slaughterhouse and the cumulus-oocyte complexes (COCs) were matured *in vitro* in TCM-199 medium supplemented with 10% FBS, FSH and LH, for 22 hours at 38.5 ° C in 5% CO2. COCs were fertilized in TALP - FERT medium supplemented with penicillamine, hypotaurine and epinephrine, modified according to the experimental groups: Negative Control - NC (without BSA, heparin or cyclodextrin), Positive Control - PC (with BSA and heparin as capacitation agent) and groups with different Methyl Beta Ciclodextrin (MBCD) concentrations (Sigma, St Louis, USA) (MBCD-0.5mM, MBCD-0.75mM and -MBCD-1.5mM) and incubated under the same conditions mentioned for IVM. 24 hours after fertilization, the zygotes were placed on SOF medium drops supplemented with BSA (6 mg /mL), 10% FBS, aminoacid, pyruvate, gentamicin and antioxidant. The cleavage and blastocyst rates were evaluated on the 2nd, 6th and 7th cultivation day, respectively, and the results were analyzed by ANOVA and Tukey post-test, adopting the significance level of 5%. The cleavage rate there was no significant difference between PC and MBCD-0.5 mM (0 vs 39.23 ± 4.06 and 32.15 ± 17.25), but NC differed MBCD-0.75mM and MBCD-1.5mM (0 vs 46.94 ± 17.00 and 46.45 ± 17.70, respectively), but it had no significant difference between the groups with MBCD. In the production of blastocysts at 6th day of culture, the PC groups (22.25 ± 5.75), MBCD- 0.75mM (18.18 ± 8.27) and MBCD-1, 5mM (17.77 ± 6.74) did not differ significantly ($p>0.05$), however they differ from NC groups and MBCD 0.5mM (0 vs. 10.74 ± 6.24, respectively) ($p<0.05$). And at 7th day of culture PC groups (33.97 ± 3.38) and MBCD- 1.5mM (25.90 ± 10.68) are not different, but differed from NC (0), MBCD-5mM (11.36 ± 10.54) and MBCD-0.75mM (18.18 ± 8.27). Thus, more repetitions are needed in order to confirm that the use of buffaloes MBCD in concentration of 1.5mM shows similar results to the positive control, thus demonstrating that MBCD can be used as a heparin substitute.

A189 OPU-IVP and ET

Viability of transcervical embryo transfer in goats

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Keywords: cervical via, goat, non-surgical embryo transfer.

Embryo transfer consists in the deposition of the embryo in the recipient uterus. The results of recipient's fertility vary widely depending on the origin of these embryos: fresh or cryopreserved and produced in vivo or in vitro. Typically, pregnancy rates ranging from 40 to 80% are reported. The objective of this study was to test the feasibility of non-surgical embryo transfer of goat fresh embryos. Pluriparous goats (n=28) received intravaginal sponges containing 60 mg medroxy acetate progesterone (MAP; ProgesponÒ, Syntex, Buenos Aires, Argentina) for six days, 30mg d-cloprostenol (ProliseÒ, ARSA S.R.L., Buenos Aires, Argentina) latero-vulvar and 200 IU eCG (NovormonÒ 5000; Syntex, Buenos Aires, Argentina) i.m., both at 24 h before sponge removal. After sponge removal, females were teased individually every 12 h to identify the onset and end of estrus. All animals were evaluated by transrectal ultrasound one day before transfer for identification of corpora lutea. At the seventh day of estrous cycle, the females received embryos by transcervical via, through the use of Collin speculum, Allis forceps and urethral catheter (Arq. Bras. Med. Vet. Zoo., 66(2):613-616, 2014). As the technique is minimally invasive, similar to routine procedures as Artificial Insemination, anesthesia was not used. The following end points were evaluated: type (morula/blastocyst) and classification (grade I, II and III) of embryos, the number of embryos transferred per recipient, number of corpora lutea presented per recipient and uterine horn receiving the embryo (right/left). For data analysis, logistic regression models (univaried and multivariate) were performed using the Epi Info version 3.5.3 software. All synchronized females presented estrus. The pregnancy rate was superior ($P<0.05$) to blastocysts (45.5%; 5/11) when compared with morulae (16.0%; 4/25) and for Grade 1 embryos (55.6%; 5/9) compared to Grade 2 (23.1; 3/13) or Grade 3 (7.1%; 1/14). There was no effect ($P>0.05$) in the number of embryos transferred per recipient or uterine horn to which the embryos were transferred on the pregnancy rate. Recipients presented from one to three corpora lutea per ovary and the number of corpora lutea did not affect the pregnancy rate ($P>0.05$). At parturition, 14 kids were born representing 38.9% of birth (14/36). The results of this study suggest that embryo transfer may be successfully performed by the non-surgical via in goats, representing an alternative to the surgical traditional procedure.

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A190E OPU-IVP and ET

Fertility effects of performing ovum pick up at young age

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Keywords: fertility, flushing AI index, IVP, OPU.

To shorten the generation interval, and to increase the genetic progress, CRV started to perform Ovum Pick Up (OPU) at 9 months instead of 12 months of age (Reproduction Fertility and Development 12/2014; 27;:209). We demonstrated that animals that had their (first) estrus before the first OPU produced significantly more embryos than animals that did not show estrus before OPU. It is however not known however what the effect is of performing OPU on such young animals on their fertility (i.e. flushing results and AI index).The aim of this study is to check the fertility of animals that have been used for OPU at young age. To investigate this we compared the flushing results and AI index of these animals. Embryos were produced by OPU-IVP (once every week during a period of 4-9 weeks), followed by flushing (two times) and insemination (AI) to make the animals pregnant. We used 3 groups of animals, (1) 12 young animals (9-10 months) that had their first estrus before the OPU, (2) 24 young animals that did not had their first estrus before the OPU and a (3) control group of 16 older (12-14 month) animals. The flushing results from young animals that had their first estrus before OPU (Group 1) were comparable with those of the control group (both 6.5 embryos per flush). However, flushing results from young animals that did not had their first estrus before OPU (group 2) were clearly lower and had only 4.1 embryo per flush.Interestingly, the insemination results (AI index) showed the same tendency, e.g. animals that had their first estrus before needed 2.1 semen straws to get pregnant, while animals that did not had their estrus before OPU needed 2.6 straws. It is therefore concluded that in young animals that showed estrus before the first OPU no difference in flushing results and AI index fertility results later in life were observed as compared to older animals. However, when no estrus was observed before the fist OPU, fertility results were lower. It is not known if this is due to the OPU at young age or that these are less fertile animals having estrus at a later stage.

A191E OPU-IVP and ET

The use of neutral red as a viability indicator hampers *in vitro* development of semi-nude bovine oocytes to the blastocyst stage

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Keywords: Neutral Red, oocyte viability, semi-nude oocytes.

Women suffering from premature ovarian failure due to cancer treatment can appeal to oocyte vitrification to preserve their fertility. An important factor to increase the effectiveness of the procedure is viability assessment of the cryopreserved oocytes after warming. To date, survival is predominantly assessed on the basis of morphological criteria by conventional light microscopy, a subjective assessment method that depends largely on the expertise of the observer. Therefore, there is a great need for an objective method to assess viability in a fast and non-invasive way. Oocytes can be cryopreserved at the immature or mature stage. After choosing to use mature oocytes, based on literature, this experiment examined whether the relative non-toxic stain Neutral Red (NR) can be used as an oocyte viability marker without affecting subsequent development to blastocysts. NR is taken up by lysosomes of metabolically active cells. Briefly, immature cumulus-oocyte-complexes (COCs) were subjected to routine *in vitro* maturation (IVM) for 21 or 24 hours, whereupon the 270 mature COCs were divided into 3 groups (2 replicates). A control group with an intact cumulus oophorus (24h IVM; LAB CTRL) and 2 groups of COCs with only the corona radiata (21h IVM), the semi-nude (SN) and Neutral Red group (NR) respectively. In view of future vitrification and IVF, cumulus cells were partially removed (semi-nude) by pipetting to facilitate oocyte handling and future cryoprotectant penetration. Following 30 minutes incubation with 15µg NR/ml maturation medium and a subsequent 1h washout period (NR group), all 3 groups were subjected to routine IVP (cultured under oil for 8 days). Cleavage and blastocyst rate were observed at respectively 2 and 8 days post-insemination. Developmental competence data were analyzed using a binary logistic regression including treatment as fixed factor and replicate as random factor (IBM SPSS version 22). Although there is a significant difference in cleavage (75 vs 55.8%) and blastocyst (36 vs 20.9%) ratio between the LAB CTRL and SN group, our results demonstrate that semi-nude oocytes still have an acceptable fertilization rate that can definitely be improved. However, oocytes from the NR-group significantly failed to cleave (42.9%) and develop to the blastocyst stage (2.4%) as compared to the CTRL and SN group. In conclusion, Neutral Red clearly affects cleavage and blastocyst formation of semi-nude oocytes in the above used conditions and therefore is not suitable for semi-nude oocyte viability assessment.

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A192E OPU-IVP and ET

Influence of caffeine supplementation prior to *in vitro* maturation on bovine oocyte developmental capacity

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Keywords: caffeine supplementation, cattle, *in vitro* maturation, oocyte.

Although, *in vitro* oocyte maturation (IVM) is common practice in the cattle industry, it is known that the mechanisms involved in meiotic resumption begin in a non-physiological way. The cyclic AMP pathway plays an important role in resumption of meiosis. When cumulus-oocyte-complexes (COC) are mechanically released from the follicle to perform IVM, cAMP levels in immature COC rapidly decrease, which in turn triggers meiosis continuation. It has been proposed that modulation of cyclic AMP prior to IVM can increase bovine blastocyst rates *in vitro*. Caffeine is a non-specific competitive phosphodiesterases (PDE) inhibitor and can inhibit meiotic resumption of oocytes due to maintenance of cAMP levels. It has been reported that gamete treatment with caffeine can increase developmental potential. The present study evaluated the influence of pre-IVM culture in the presence of different concentrations of caffeine on meiotic progress, developmental rates and blastocyst cell numbers. Bovine ovaries were collected from a local abattoir. A total of 4378 cumulus-oocyte-complexes were obtained by slicing. Four different concentrations of caffeine (Merk, Darmstadt, Germany) were used during slicing, searching and 2h pre-IVM culture: 1, 5, 10, 20 mM. A control group, using 2h pre-IVM without caffeine (0mM) and a standard control were also included. After pre-IVM, oocytes were washed and cultured for 24h *in vitro* without caffeine. Following maturation, oocytes were fertilized *in vitro* for 19h and zygotes were cultured *in vitro* for eight days to assess embryo development. Some oocytes were fixed in 2% glutaraldehyde at 9, 20 and 24 h after IVM. Hoechst staining was performed to evaluate nuclear status. Cleavage and blastocyst formation rates were evaluated. Expanded blastocysts from all treatments were submitted to differential staining. One-way ANOVA from R software was implemented to evaluate differences in progression through meiosis, cleavage and blastocysts rates and blastocyst cell numbers. Caffeine maintained the meiotic arrest after 9h IVM in a concentration dependent manner (GV: 100 ± 0.0%, 61.3 ± 21.3%, 40.7 ± 5.4 %, 36.2 ± 11.4% 11.9 ± 6.3%, 28.5 ± 10.0% for 20, 10, 5, 1, 0 mM and standard, $p<0.05$, mean ± SEM). Cleavage (57.7 ± 4.9%, 56.5 ± 3.8%, 62.7 ± 3.2%, 52.5 ± 5.1%, 54.4 ± 6.0%, 60.3 ± 2.3% for 0, 1, 5, 10, 20 mM and standard, $p>0.05$, mean±SEM) and blastocyst rates (26.2 ± 3.0%, 14.9 ± 2.8%, 22.4 ± 3.8%, 23.7 ± 2.1%, 21.4 ± 4.1%, 26.6 ± 2.4% for 0, 1, 5, 10, 20 mM and standard, $p>0.05$, mean ± SEM) and number of cells (ICM: 46.0 ± 4.1, 43,2 ± 3.7, 61.4 ± 7.8, 53.0 ± 6.5, 49.4 ± 5.6, 50.0 ± 4.4; TE:111.6 ± 13.6, 115.4 ± 7.8, 106.4 ± 3.5, 102.6 ± 8.3, 118.4 ± 14.6, 119.6 ± 11.7 for 0, 1, 5, 10, 20 mM and standard, $p>0.05$, mean±SEM) did not differ significantly among *in vitro* treatments. Although caffeine supplementation prior to IVM delayed resumption of meiosis, it did not affect subsequent embryo development and quality.

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A193E OPU-IVP and ET

Temporal pattern of steroid hormone concentrations during *in vitro* maturation of bovine oocytes

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Keywords: cattle, *in vitro* maturation, steroid hormones.

Present in vitro maturation (IVM) systems do not completely mimic the in vivo situation resulting in oocytes of reduced quality. Steroid hormones are regulators in the fine-tuned mechanism of follicular and oocyte maturation and development. During final maturation a switch from estradiol dominance to progesterone dominance within the follicle is well-described. This change is accompanied by the resumption of meiosis and results in the maturation of the oocyte. It also suggests the important role of these hormones in this process. Aim of the study was to determine the temporal pattern of steroid hormone concentrations in the IVM medium of bovine cumulus-oocyte-complexes (COC) supplemented with different gonadotropin concentrations. COC were obtained from abattoir-derived ovaries and were matured in medium TCM 199 (Tissue Culture Medium 199) supplemented with three different compounds of gonadotropins employing a standard protocol. The three combinations of gonadotropins were: 1. equine (eCG) and human chorionic gonadotropin (hCG), 2./3. follicle-stimulating hormone (FSH) and luteinizing hormone (LH), each in two different concentrations 0.05 IU or 0.01 IU, and 4. without any supplementation of gonadotropins. Groups of 30 COC were matured for 24 hours at 39°C and 5% CO^2 without oil overlay. 17β-estradiol (E2) und progesterone (P4) were measured in maturation medium before use (0h, control) and after specific time points of IVM via radioimmunoassay (RIA). So far, the following results could be obtained. *Treatment 1: TCM with eCG and hCG:* P4 and E2 could not be detected in the control medium (0h). During IVM, P4 concentrations increased in the medium (4h: 3.3 ± 1.0 ng/ml; 8h: 6.2 ± 3.3 ng/ml; 12h: 6.5 ± 2.0 ng/ml; 16h: 6.8 ± 1.1 ng/ml; 20h: 7.3 ± 1.8 ng/ml; 24h: 10.4 ± 1.6 ng/ml), whereas the E2 concentrations stayed similar (4h: 52.8 ± 12.1 pg/ml; 8h: 54.6 ± 7.9 pg/ml; 12h: 63.8 ± 15.2 pg/ml; 16h: 54.2 ± 16.3 pg/ml; 20h: 77.1 ± 40.1 pg/ml; 24h: 74.7 ± 32.4 pg/ml). Treatment 2/3: *TCM with FSH and LH:* Supplementation of 0.05 IU each, E2 concentrations stayed at the same level as with eCG and hCG (E2 0h: 6.2 ± 5.7 pg/ml, washing medium: 26.0 ± 10.8 pg/ml, after 24h: 59.7 ± 20.1 pg/ml). With the supplementation of 0.01 IU each, P4 and E2 concentration also stayed at the same level as with eCG and hCG (E2 0h: 3.3 ± 3.2 pg/ml, washing medium: 19.6 ± 4.2 pg/ml, after 24h: 58.4 ± 24.1 pg/ml, P4 0h: ≤0.25 ng/ml, washing medium: 0.3 ± 0.1 ng/ml, after 24h: 17.3 ± 3.5 ng/ml). *Treatment 4:* After 24 h of IVM *without gonadotropins* the following hormone concentration could be detected: E2: 129.4 ± 88.8 pg/ml and P4: 6.7 ± 0.8 ng/ml. During IVM, the temporal pattern of E2 and P4 did not correspond with the pattern during final maturation in vivo. This underlines that present conditions of IVM do not reflect the in vivo situation and require further optimisation.

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A194E OPU-IVP and ET

Time-lapse analysis of early cleavage in bovine embryos produced in serum-free medium

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Keywords: cattle, embryo cleavage, IVP, time-lapse cinematography, viability.

Two decades ago, early cleaving embryos were considered as developmentally more competent than slow cleaving embryos. But this theory has been challenged, since moderately developing embryos have decreased chromosomal abnormalities, normal *H19* and *Snrpn* imprint maintenance and potentially higher pregnancy rates. We want to analyze the incidence of chromosomal instability (CIN) in bovine cleavage stage embryos and relate this with developmental kinetics. Hence, we need a culture system allowing individual identification and selection of cleavage stage embryos for single cell analysis (SCA). In this preliminary study, we used time-lapse cinematography (TLC) as a non-invasive tool to describe kinetics and to use timing of early cleavages as a parameter predictive of blastocyst development. Bovine embryos were produced from immature oocytes derived from slaughtered cattle. Oocytes were matured in 500 µL TCM199 supplemented with 20 ng/mL epidermal growth factor (EGF). After in vitro fertilization with frozen-thawed bull semen, 9 presumed zygotes (7 replicates) were cultured in a WOW dish in 30 µL Synthetic Oviduct Fluid (SOF) supplemented with 0.4% BSA, 5 µg/mL insulin, 5 µg/mL transferrin and 5 ng/mL selenium (ITS), covered with mineral oil. In total, 63 zygotes were observed with TLC (Primo Vision®, VitroLife, Göteborg, Sweden), and images were taken every 15 min for up to 90 hours post insemination (hpi). At 192 hpi, blastocyst formation was set as endpoint. Timing of the first (t_1; cleavage into 2-cell stage) and second mitosis (t_2; cleavage into 4-cell stage) and the interval time between those two parameters were analyzed ($t_{\Delta 1\text{-}2}$). The median observation of each parameter was set as a threshold value (t_1 29.00h; t_2 38.83h; $t_{\Delta 12}$ 10.87h). All data were analyzed using a binary logistic regression model. Significantly more embryos reached the blastocyst stage when they cleaved before 29.00h into 2-cell stage or before 38.83h into 4-cell stage (48.3% and 51.2%, respectively), compared to embryos with a later first or second mitosis (16.1% and 18.9%, respectively) ($P<0.05$). Furthermore, when the interval between the first and second mitosis ($t_{\Delta 12}$) was shorter than 10.87h more embryos reached the blastocyst stage (42.3%), compared to a longer interval $t_{\Delta 12}$ (21.1%) ($P<0.01$). This indicates that timing of early cleavage is predictive for further developmental potential, which is confirming earlier studies (Van Soom *et al*., Theriogenology, 38:905-919, 1992; Grisart *et al*., J Reprod Fertil, 101:257-264, 1994). It is however the first time embryos have been cultured in WOW-dishes in serum-free medium and monitored using TLC. WOW dishes offer the advantage of small group culture with individual embryo follow-up, which allows specific embryo selection at any time of the development. Next, we want to identify CIN in embryos with particular cleavage patterns using TLC with SCA and eventually transfer embryos with high and low predicted viability.

A195E OPU-IVP and ET

3D visualization of bovine oocyte *in vitro* maturation by confocal laser scanning microscopy

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Keywords: bovine, fluorescence microscopy, oocyte maturation.

Regular nuclear and cytoplasmic oocyte maturation is a prerequisite for normal fertilization and embryo development. Improvement of *in vitro* maturation systems is a central issue in veterinary and human reproductive medicine. Thereby, better microscopic visualization of cellular processes and structures is essential for further extending our rudimentary knowledge and understanding of mammalian oocyte maturation. We used three-dimensional multicolor fluorescence microscopy to investigate critical steps of meiotic maturation *in vitro*. The primary aims of this study were to simultaneously gain information on the meiotic spindle apparatus, on the kinetics of meiotic progression, on the dynamic changes of the cytoskeleton and on the meiotic failures and aberrations. In cattle, the cumulus oophorus is considered to play an essential role for normal oocyte maturation. This makes direct microscopic live cell imaging of the oocyte rather difficult. Thus, cumulus-enclosed grade I and II oocytes were collected from slaughterhouse ovaries and allowed to mature for variable times from 0 to 28 hours *in vitro*. The oocytes were denuded and then fixed with formaldehyde in a microtubule-stabilizing buffer in such a way that the three-dimensional cell architecture was maintained, and were stained for DNA, microtubules and f-actin microfilaments. In addition, serine 10-phosphorylated histone H3 was used as a marker for chromosome condensation and the spindle midbody. For three-dimensional imaging of the oocytes *in toto*, confocal serial sections were captured at 1 µm distance using a 40x objective (NA = 1.3). For imaging details we used a high spatial sampling density (pixel size 50 x 50 nm, z-step size 200 nm) and image restoration by maximum likelihood estimation (MLE) deconvolution. A collection of more than 500 confocal image stacks gives a clearer and more detailed view of completion of meiosis I and progression to metaphase II. Qualitative and quantitative data analyses provide a basis for studies on molecular mechanisms e.g. on the dynamic localization and function of potential key proteins. Important is the detection of anomalies of meiosis I that result in irregular genomic configurations in the zygote: Main findings were (i) the failure of first polar body extrusion as a consequence of incorrect positioning or orientation of the meiotic spindle and (ii) lagging chromosomes, chromatin bridges and incomplete polar body cytokinesis due to irregular spindle formation, chromosome congression and segregation. 3D fluorescence microscopy allows to exactly determine the stage of oocyte meiosis and to diagnose fatal aberrations of meiotic maturation. Thus, high speed imaging systems could be used to test and to improve oocyte isolation methods and in vitro maturation systems.

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A196E OPU-IVP and ET

Concentration of procaine and exposure time influence *in vitro* fertilization rate in the equine

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Keywords: horse embryo, *in vitro* fertilization, procain exposure.

Most wild equids are currently endangered or threatened, as mentioned in the Red List of the International Union for the Conservation of Nature and several domestic horse breeds are at risk of extinction. Genome resource banking requires cryoconservation of semen, oocytes and/or embryos. Embryo production in equids is limited *in vivo*, since routine induction of multiple ovulation is still ineffective. Embryo production *in vitro* allows the production of several embryos per cycle that could easily be frozen owing to their small size. Intracytoplasmic Sperm Injection (ICSI) has been widely adopted to generate horse embryos *in vitro*, however ICSI is time-consuming and requires expensive equipment and expertise in micromanipulation. We have established an efficient *in vitro* fertilization (IVF) technique in the equine (Ambruosi et al., 2013 Reproduction, 146: 119-133) but IVF zygotes have a low developmental competence. Incubation of gametes with procaine, necessary for induction of sperm hyperactivation, may have a deleterious effect on embryos quality. Our objective was to increase the developmental competence of the IVF zygotes by decreasing procaine concentration or exposure time. Immature cumulus-oocyte complexes were collected from slaughtered mares in a local slaughterhouse, cultured for 26 hours in an *in vitro* maturation medium and pre-incubated for 30 minutes in oviductal fluid collected from slaughtered females. Fresh sperm was collected, diluted to $10x10^6$ spermatozoa/ml, incubated for 5 hours in a capacitating medium and diluted to $1x10^6$ spermatozoa/ml. Spermatozoa were then added procaine (1mM or 5mM) and co-incubated with oocytes for 2, 4 or 18 hours. Zygotes were cultured in DMEM-F12 for 48 hours post-IVF, fixed and analyzed. In experiment 1, spermatozoa were added 5mM procaine and co-incubated with oocytes for 2 hours *vs* 18 hours. The percentage of zygotes 48 hours post IVF was higher for 18 hours co-incubation (62%, 13/21) than for 2 hours (0%, 0/22) (Chi2 test $p<0.05$). In experiment 2, spermatozoa were added 5mM procaine and co-incubated with oocytes for 4 hours *vs* 18 hours. The percentage of zygotes 48 hours post IVF was similar for 18 hours (44%, 7/16) and 4 hours co-incubation (32%, 6/19) (Chi2 test $p>0.05$). In experiment 3, spermatozoa were added 5mM *vs* 1mM procaine and co-incubated with oocytes for 18 hours. The percentage of zygotes 48 hours post IVF was higher for 5mM procaine (48%, 13/27) than for 1mM (19%, 5/26) (Chi2 test $p<0.05$). In the 3 experiments, zygotes contained at least 2 highly decondensed pronuclei, pronuclei decondensation being the first step of embryo development. We also observed 2 cleaved embryonic structures in the group 5mM during 18 hours, but the quality of these embryos was poor. In conclusion, decreasing procaine concentration or exposure time influence IVF rate and doesn't improve equine embryo quality.

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A197E OPU-IVP and ET

Reproductive success in interbred ewes after fresh embryo transfer

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Keywords: MOET, pregnancy, Romanov sheep, Turkish native sheep.

Estrous response and ovulation rate of Turkish Native Sheep (N; n=27) and the prolific Romanov crossbred breed (NR; n=22) for the use as recipients in embryo transfer programs were evaluated in the anoestrus season (April). Estrus of recipient ewes was synchronized with vaginal sponges containing 30 mg FGA for 12 days and ewes received an i.m. injection of 400 I.U. PMSG at sponge removal. Estrus and ovulation rates, time to onset of estrus and site of the ovulation were determined. A total number of 3 Romanov breed donors ewes were superovulated using FSH-p with 200 mg NIH-FSH-P1 (total of 20 ml) (Folltropin-V; Vetrepharm, Canada) applied in 8 decreasing doses of 1.5, 1.5, 1.5, 1.25, 1.25, 1, 1, 1 ml i.m. at 12 h intervals, starting 60 h before sponge withdrawal. Donors received 1ml Estrumate and 100 I.U. PSMG 36h prior to sponge removal and finally an additional 200 I.U. PMSG was injected at sponge removal. Donors were mated with their own breed of rams. Ewes were observed for estrus (d 0) and were surgically flushed by laparotomy 6 or 7 d later to recover embryos. The number of ovulations and transferable embryos were 18.7 and 14.3, respectively. Embryos with Grade I, II and III with the stage of morula to expanded blastocysts were used in twin fresh transfer. The success rate of the synthetic progestagen treatment to establish an estrus out of season (April) was found to be 59.3% and 52.4% for N and NR, respectively. Time between the removal of the sponges and the onset of estrus was similar between the two breeds of recipient ewes (N: 53.06±0.95h and NR: 52.27±1.07h). For the recipient ewes at ET the ovulation rates were found significantly higher ($P<0.05$) in N ewes (1.0±0.00) than NR ewes (0.72±0.14); the ovulation site was mainly located on the right ovary in NR ewes (87.5%) compared to N ewes (42.9%). We transferred embryos as pairs to save number of recipients as advised by Gimenez-Diaz (2012) who indicated that pregnancy success for number of embryos transferred (single versus twin) was similar. Recipient ewes with fertile estrus (estrus accompanied with ovulation) received similar stages of embryos fallowing the laparoscopic measurements (location, number and quality score) of CL in recipients. Pregnancy and embryo survival rates were similar in N (64.3% and 77.8%) and NR (75.0% and 75.0%) ewes. The sex ratio of twin transferred embryos was higher in N (75% male) than those observed in NR (22.7% male) ewes. These preliminary results show that, Romanov crossbreed recipients with a lower ovulation rate and ovulation occurred mostly in right ovary had more overall MOET success (66% vs 57%; no of lambs born/no of embryo transferred) and were found more favourable with more female lambs from fresh embryo transfer compared to Turkish Native Sheep.

A198E OPU-IVP and ET

Estradiol route and non-surgical embryo recovery in synchronized Santa Inês ewes

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Keywords: embryo recovery, estradiol benzoate, Santa Inês ewes.

Animal surgery procedures are being progressively restricted worldwide in the context of embryo transfer. In small ruminants, the needs for development of alternative and efficient non-surgical techniques for embryo transfer have been emphasized. Non-surgical embryo recovery is well consolidated in Brazil in cattle and goats, while in sheep it remains a challenge. The aim of this study was to check the efficiency of different ways of estradiol benzoate administration on cervix dilation and embryo recovery in synchronized Santa Inês ewes. A total of 23 pluriparous ewes were subjected to two doses of 37.5 μg d-cloprostenol by intravulvo-submucosal way seven days apart. After the second cloprostenol administration, ewes were checked for estrus at 12 h interval and mated with fertile rams during estrus. After mating, ewes were allocated according to estrous response into two treatment groups for embryo recovery seven days after estrous onset. In T1 (n=11), ewes received 37.5 μg d-cloprostenol and 1 mg estradiol benzoate 16 h before embryo recovery, plus 50 IU oxytocin i.v. 20 min before embryo recovery. In T2 (n=10), ewes received the same protocol as T1, but the way of estradiol administration was intravaginal. All ewes received 2 ml of lidocaine 2% without vasoconstrictor for epidural and 2 ml of lidocaine for contact cervical anesthesia plus acepromazine 1% (1 ml/kg live weight) before cervical passage as previously described in goats (Fonseca et. al.; Small Rumin. Res., 111:96-99, 2013). Qualitative and quantitative data were analyzed by chi-square test and ANOVA respectively with 5% significance. Estrous response after the second cloprostenol administration was 91.3% (21/23). There were no differences (P>0.05) in any parameter evaluated for T1 and T2: successful uterine flushing (90.9% and 80.0%), duration time of embryo recovery (20.3 ± 8.0 and 26.2 ± 5.3min), flushing recovery rate (PBS injected/PBS recovered; 90.1 and 90.5%), average structures recovered (1.0 ± 0.4, 20% viable and 1.4 ± 0.6, 33% viable). Considering that Santa Inês sheep have up to 1.3 lambs we can conclude that it is possible to perform efficient non-surgical embryo recovery in non-superovulated synchronized Santa Inês ewes, regardless the way of estradiol administration.

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A199E OPU-IVP and ET

Effect of Thymosin on *in vitro* fertilization and developmental competence and quality of pig embryos

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Keywords: IVF, pig embryo quality, thymosin.

Thymosin (TH) is biological active polypeptide released by thym. It plays a vital role in the repair and regeneration of injured cells and tissue. It protects cells from damage and blocks apoptosis (Goldstein et al. 2012, Expert Opin Biol Ther, 12(1), 37-51). In our recent study we demonstrated that supplementation of maturation medium for pig oocytes with synthetic TH increased the number of matured oocytes with lower morphological quality. The aim of the present study was to investigate the effect of supplementation of maturation medium for oocytes with TH on *in vitro* fertilization and developmental competence and quality of pig embryos. Cumulus-oocyte complexes (COCs) were obtained by aspiration from antral follicles of ovaries collected from slaughtered gilts. COCs were selected based on their cytoplasm morphology and cumulus cells appearance and cultured in modified TCM-199 medium supplemented with 0.5 mg/ml of synthetic TH (LipoPharm.pl) (experimental group) or without TH (control group) for 42 h at 39^{o}C and in a humidified atmosphere containing 5% CO_2 in air. After maturation, oocytes were assessed and *in vitro* fertilized (IVF). Semen for IVF was incubated in modified capacitation medium-M-199 for 1 h. Sperm fraction was introduced to the droplets containing oocytes and next gametes were coincubated for 4 h in modified TCM-199 medium. Presumptive zygotes were cultured *in vitro* for 144 h in NCSU-23 medium under the conditions stated above. Embryo quality criteria were cleavage, morula and blastocyst rates, total cell number per blastocyst and degree of apoptosis assessed by TUNEL. The results were analyzed statistically with Chi-square test. Treatment of oocytes with TH during culture slightly increased the ratio of matured oocytes (95/103, 92.3%) compared to the control group (134/150, 89.3%; no significant differences) cultured without TH. After IVF cleavage, and development to the morula and blastocyst stage, based on number of cleaved embryos, were not different between experimental (29.5, 71.4 and 32.1%, respectively) and control (25.4, 50.0 and 29.4%, respectively) group. The mean number of cells per blastocyst in experimental and control group was comparable (40.4 and 39.9; respectively). The mean number of apoptotic nuclei and apoptotic index was 0.67 and 1.66 in the experimental group and was significantly lower ($P<0.05$) than in the control group i.e. 1.66 and 4.35. In conclusion, the culture oocytes in a medium with TH supplementation had a positive effect on quality pig IVF blastocyst since they had a significant lower incidence of apoptosis. However, further studies are required to determine the competence of porcine blastocyst recovered from oocytes matured with TH for *in vivo* development.

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A200E OPU-IVP and ET

A retrospective study of *in vitro* embryo production from high genetic merit cows using unsorted or X-sorted sperm in a commercial program

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Keywords: breeding program, cattle, IVP, semen sexing.

X-sorted sperm can be used in embryo transfer programs to produce female progeny (Kaimio et al., Theriogenology 80, 950-954, 2013). However, X-sorted sperm is generally used in heifers as cow insemination results in lower numbers of transferable embryos (Hayakawa et al., Theriogenology 71, 68-73, 2009). We hypothesized that breeding programs based on IVP with X-sorted sperm may be a promising alternative. The aim of this study was to compare *in vitro* embryo production in cows using X-sorted or unsorted semen under commercial conditions performed at the Biotechnology MIDATEST Station located in Denguin, South West, France. Three to fifteen years old Holstein cows (n=26) and 16-22 months old heifers (controls: n=17) were used in an OPU-IVP program. Donor cows were stimulated with decreasing pFSH doses (Stimufol; Reprobiol, Liège, Belgium) twice daily during 3 days, (total dose: 350 µg for cows and 250 µg for heifers). Cumulus oocyte complexes (COCs) were collected by OPU 12 to 24 h after the last FSH injection and *in vitro* matured using a standard IVM protocol. Oocytes were fertilized with frozen–thawed unsorted (cows and heifers) or X-sorted (cows) sperm in modified Tyrode's bicarbonate buffered solution medium (fert-TALP) using different non pre-tested bulls (n=55). Presumptive zygotes were cultured in SOF medium (Minitub, Tiefenbach, Germany) plus 1 % cow serum up to Day 7 at 38.5 °C in 5% CO2, 5% O2 and 90% N2 atmosphere with maximum humidity. OPU/IVP was repeated one to 13 times (2.5 ± 2.6) for each donor cow or heifer. Grade 1 blastocysts and expanded blastocysts according to IETS classification were recorded on days 6.5 and 7. Viable embryos were frozen or transferred fresh. Embryo production was analyzed with ANOVA and blastocyst yield by Chi-Square. From 18 OPU sessions in heifers, a total of 168 COCs (9.3 ± 4.7 per session) were processed for *in vitro* maturation, and 5.4 ± 3.9 Grade 1 (G1) embryos were produced per session. In cows 42 sessions were performed with unsorted semen and 44 with X-sorted semen, 13.1 ± 9.6 and 8.9 ± 4.9 oocytes ($p<0.05$) were collected; 7.7 ± 5.5 and 4.1 ± 2.9 G1 embryos were produced, respectively ($p<0.05$). The mean embryo development rate (total number of G1 embryos / number of oocytes entering maturation process) was 59.1% (unsorted semen) and 46.3% (X-sorted semen; $p<0.05$). Although the number of collected oocytes was different, there were no differences in presumptive female embryos produced per session assuming a sex ratio of 90% (3.7 embryos per session) with sorted semen and a sex ratio of 50% (3.9 embryos per session) when using unsorted semen. In conclusion, our work confirmed the efficacy of OPU-IVP techniques to produce grade 1 embryos using X-sorted in high genetic merit cows. Furthermore this technique allows to get female calves based upon a lower number of recipients.

A201E OPU-IVP and ET

Presence of L-carnitine during maturation improves efficiency of fertilization in porcine oocytes

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Keywords: *in vitro* fertilization, *in vitro* maturation, L-carnitine, pig embryo.

The lipid-rich pig oocytes might be an excellent model to understand the role of fatty acid metabolism during oocyte maturation, their subsequent monospermic fertilization and preimplantation embryo development. Recently, it was described that L-carnitine stimulates mitochondrial oxidation of fatty acids and increases energy supply of mammalian oocytes. The aim of the study was to characterize the effect of L-carnitine during maturation on the efficiency of fertilization of porcine oocytes with different meiotic competence. Cyclic sows, checked for the ovarian cycle status, were used as oocyte donors. Meiotically more competent (MMC) and meiotically less competent (MLC) oocytes were isolated either from medium (6–9 mm) or small follicles (<5 mm). They were matured separately in IVM medium supplemented with 0, 4 and 10 mM L-carnitine (Sigma-Aldrich Co., Prague, Czech Republic) and fertilized by frozen-thawed spermatozoa of a boar proven in the IVF system using standard protocols (Hulinska et al. 2011, Anim Reprod Sci, 124: 112–117). The presumptive zygotes were incubated in PZM-3 medium (Yoshioka et al. 2002, Biol Reprod, 66: 112–211) for 15 h, fixed in 2.5% aqueous glutaraldehyde solution (v/v), stained with bisbenzimide-33258 Hoechst (Sigma-Aldrich Co., Prague, Czech Republic) and examined by epifluorescence at a magnification of 400 ×. The proportion of penetrated oocytes from the inseminated ones and proportions of monospermic and polyspermic oocytes from the penetrated ones were assessed. Total efficiency of fertilization (%) of oocytes was calculated according to the formula (ratio of monospermic oocytes (n) to inseminated oocytes (n) × 100). The results were statistically analysed by the ANOVA procedure using the Chi-square test. In MMC-oocytes total efficiency of fertilization increased (51.1, 54.3 and 57.6%) when the oocytes were matured with 0, 4 and 10 mM L-carnitine. Similarly in MLC-oocytes, total efficiency of fertilization increased (42.1 vs 48.8%) in oocytes matured with 4 mM L-carnitine compared to those matured without L-carnitine. On the other hand, total efficiency of fertilization decreased when MLC-oocytes were matured with 10 mM L-carnitine (37.9%). It can be concluded that supplementation of medium with L-carnitine during maturation positively influenced fertilization efficiency of porcine oocytes independently of their meiotic competence. However meiotically more competent oocytes were more capable of utilizing L-carnitine in comparison with meiotically less competent porcine oocytes in which the abundance of L-carnitine had a negative effect on fertilization.

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A202E OPU-IVP and ET

Effects of resveratrol supplementation during *in vitro* maturation and *in vitro* fertilization on developmental competence of bovine oocytes

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Keywords: bovine oocytes, *in vitro* fertilization, *in vitro* maturation, resveratrol.

Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin - isolated from various plant species, particularly grapevine peel. Recently, resveratrol gained scientific interest because of its strong antioxidant effects it may have health benefits, including protection against cardiovascular diseases. In addition, it has been shown to increase lifespan in several species and activates the SIRT1 gene. The aim of this study was to investigate its effects in bovine early embryo development. We employed three different resveratrol concentrations during *in vitro* maturation (IVM) and *in vitro* fertilization (IVF). Bovine oocytes (n=1648) were collected from slaughterhouse ovaries and subjected to IVM medium supplemented with 0.2µM, 1µM, and 20µM Resveratrol® (Sigma-Aldrich, Buchs, Switzerland) for 24 h followed by IVF with the same concentrations of resveratrol for 19 h. IVM and IVF medium without resveratrol (control) and DMSO supplementation as vehicle control were included in this experiment. Presumptive zygotes were cultured *in vitro* until day 8 to assess embryo development. Maturation rates, cleavage and blastocyst formation were determined. Maturation rates did not differ significantly (0.2µM: 64.2 ± 7%; 1µM: 82.3 ± 4%; 20µM: 68.8 ± 2%; control: 74.6 ± 5% and vehicle control: 70.2 ± 6%, respectively, $p \leq 0.05$) did not differ dramatically. Oocytes cultured in 1µM resveratrol supplemented maturation medium showed distinct detachment of cumulus cells. Cleavage was reduced in the 0.2µM and 20µM group (0.2µM: 44.21 ± 2%; 1µM: 58.4 ± 3%; 20µM: 40.9 ± 5%; control: 56.6 ± 2% and vehicle control: 55.2 ± 6%, respectively, $p \leq 0.05$). Blastocyst development was impaired in the low and high resveratrol concentration group compared to the other groups (0.2µM:11.3±1%;1µM: 28.4 ± 6%; 20µM: 8.2±4%; control: 22.7 ± 4% and vehicle control: 20.8 ± 2%, respectively, $p \leq 0.05$). These preliminary results indicate that very low and high concentrations of resveratrol impair the development to the blastocyst stage. In conclusion, a 1µM resveratrol supplementation during IVM and IVF seems to improve the developmental competence of oocytes, which is reflected not only in elevated blastocyst rates but also in the higher degree of expansion of cumulus cells after IVM and the maturation rates.

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A203E OPU-IVP and ET

Interpretation of equine *in vitro* produced embryo morphology

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Keywords: embryo, Hoechst, horse, ICSI.

To better understand the correlation between equine *in vitro* produced embryo morphology and nuclear status. Oocytes were recovered from abattoir-derived ovaries, matured *in vitro,* subjected to conventional ICSI and cultured *in vitro*. Assessment of nuclear status by staining with Hoechst 33258 and using fluorescent microscopy was performed at the following times after ICSI: Group A) 20 hours to evaluate pronuclear (PN) status; Group B) Day 2, 3, 4, or 7 to determine nucleus number and correlation of morphological cleavage; and Group C) Day 7 to 11 to determine blastocyst development. Only normal nuclei were included in the number of nuclei recorded; nuclei with signs of degeneration (vacuolization, condensation or fragmentation) were disregarded. Confirmed blastocysts contained > 64 normal nuclei and showed arrangement of an outer rim of nuclei in a presumptive trophoblast layer. Two Day-9 presumptive blastocysts were transferred to the uterus of a recipient mare to evaluate viability. A total of 109 oocytes were subjected to ICSI in groups A and B. In Group A, the rate of PN formation was 43%. In Group B, 64% demonstrated apparent morphological cleavage but only 17% had ≥ 2 normal nuclei on staining and only 6.5% had a number of nuclei that matched the number of visible blastomeres and were appropriate for age. The other stained embryos that appeared cleaved morphologically possessed only degenerated nuclei or were completely anuclear. In Group C (n = 138 injected oocytes), 17 embryos were presumed to have developed to the blastocyst stage based on morphological criteria. Of these, 7 were confirmed blastocysts by staining and 8 were degenerating embryos. One embryo, presumed to be degenerated, was also revealed to be a blastocyst. Notably, as uncleaved oocytes were placed in a separate droplet at Day 4 but were kept in culture, we could evaluate changes in these oocytes over time. Several known uncleaved oocytes increased in diameter on Day 9, which on simple morphological evaluation, could have led to mistaken classification as blastocysts. Transcervical transfer of two Day-9 presumptive blastocysts to the uterus of a recipient resulted in 2 embryonic vesicles detected on Day 14 after ICSI. The smaller vesicle was manually reduced and the remaining vesicle developed normally and is > 250 days gestation. Overall in Group C, including the two transferred embryos, the rate of confirmed blastocyst development per injected oocyte was 7.2%. To the best of our knowledge, this is the first report documenting the morphology and DNA staining of equine *in vitro*-produced blastocysts vs. blastocyst-like structures. Our findings reinforced the importance of removing uncleaved oocytes to limit uncertainty in later assessments of blastocyst development, and of staining embryos for DNA to definitively establish blastocyst development.

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A204E OPU-IVP and ET

Transfer of cattle embryos produced with sex-sorted semen results in impaired pregnancy rate

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Keywords: cattle, embryo transfer, pregnancy, sex-sorted semen.

This study compared the pregnancy rates after transfer of day-7 *in vivo* embryos produced either with conventional or sex-sorted semen from numerous bulls commercially available and extensively used. In addition, mortality of calves born from sexed embryos and conventionally produced embryos was studied. The data consisted of 12,438 embryo transfers, of which 10,697 embryos were produced using conventional semen (CONV embryos) and 1,741 using sex-sorted semen from 97 bulls (SEX embryos), predominantly of Ayrshire and Holstein breeds. Quality codes of embryos were similar in both groups. Of the CONV embryos, 27.4% were transferred fresh, the proportion being 55.7% for SEX embryos. Recipient properties (breed, parity, number of previous breeding attempts and interval from calving to transfer) were similar for both embryo types, heifers representing 57.8% of recipients in the CONV group and 54.8% in the SEX group. Recipients that were not inseminated or did not have a new embryo transferred after the initial one, and had a registered calving in fewer than 290 days after the transfer, were considered pregnant. Data were analyzed with IBM SPSS Statistics, Version 21.The effects of sexing protocol, embryo type (fresh vs. frozen), developmental stage, quality and breed of embryo as well as parity (heifer vs. cow) and breed of a recipient on conception were analyzed using binary logistic regression. Pregnancy rate for recipients receiving CONV embryos was 44.1% and for those receiving SEX embryos 38.8%. The odds ratio for pregnancy in recipients receiving CONV embryos was 1.34 compared with SEX embryos ($P<0.001$). Other factors affecting the pregnancy rate were embryo quality ($P<0.001$), being highest for grade 1 (CONV 45.2%, SEX 42.8%) and lowest for grade 3 (CONV 29.2%, SEX 22.2%) embryos, and developmental stage of an embryo ($P=0.038$). Transfer of earlier developmental stages, i.e. compact morulas, resulted in lower pregnancy rates than transfer of later stages. Also recipient parity affected pregnancy rate ($P<0.001$), the odds ratio for pregnancy for heifers was 1.18 compared with that for cows. There was no effect of the breed on pregnancy rate, neither of an embryo nor of the recipient. The proportion of female calves was 49.6% and 92.3% in CONV and SEX groups, respectively. Calf mortality was 9.0% and 8.9% in CONV and SEX groups, respectively. Mortality of female calves was similar in CONV and SEX groups, 6.6% and 7.7%, respectively. For male calves, mortality was 9.2% in the CONV group but significantly higher, 16.0% ($P<0.05$), in the SEX group. This study showed that transfer of embryos produced with sex-sorted semen decreased the pregnancy rate by about 12% compared with embryos produced using conventional semen. Mortality of male calves born from SEX embryos was higher than for those born from CONV embryos.

A205E OPU-IVP and ET

Supplementation of culture medium with foetal calf serum or insulin – transferrin – selenium affects the integrity of equine oviduct explants

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Keywords: foetal calf serum, horse, insulin, IVP, oviduct explants, selenium, transferring.

Equine oviduct explants provide an excellent tool to unravel embryo-maternal interactions. They can be cultured *in vitro* for several days in DMEM F12 and serum whilst remaining functionally intact and highly differentiated. However, dark cell degeneration (DCD) has been observed inside the explants (Nelis et al. 2014 RFD 26 954-966). Since serum has been reported to negatively affect cell and embryo culture (Fernandez-Gonzalez 2004 PNAS 101 5880-5885), we aimed to assess the effect of serum and the serum replacer insulin-transferrin-selenium on the prevalence of DCD, ciliary activity, membrane integrity and ultrastructure of equine oviduct explants. Oviducts ipsilateral to the ovulation side were gathered from mares in the early postovulatory stage. Oviduct explants were harvested by scraping and cultured for 6d in 50 µl drops under oil in 5% CO_2 in air in DMEM/F12 (control; Invitrogen, Merelbeke, Belgium), in DMEM/F12 with 10% foetal calf serum (FCS; Greiner Bio-one, Wemmel, Belgium) or in DMEM/F12 supplemented with 5 µg/ml insulin, 5 µg/ml transferring and 5 ng/ml selenium selenite (ITS; Sigma, Schnelldorf, Germany). Three replicates of 60 droplets per condition were performed. With an inverted microscope, every 24h, the percentage of explants with dark zones and the percentage of explants showing ciliary activity were determined. In addition, the percentage of membrane-damaged cells was determined using Trypan blue (Sigma-Aldrich, Diegem, Belgium). At d0, 3 and 6, ultrastructure was assessed by TEM. To compare DCD prevalence, ciliary activity and membrane integrity, binary logistic regression was implemented (SPSS 21 for windows; SPSS IBM, Brussels, Belgium). During the first two days, the prevalence of DCD was significantly lower in the FCS group (36%), when compared to ITS (68%, $P<0.0005$) and the control (67%, $P<0.0005$), indicating an initial protective effect of FCS. From d3 on, significantly more DCD was observed in the presence of ITS and FCS (87% resp. 92%, $P<0.0005$) compared to the control (81%). FCS and to a lesser extent ITS seem to sustain the percentage of explants showing ciliary activity (97%, $P<0.0005$ and 94%, $P<0.0005$) compared with the control (87%). In all groups, as shown by Trypan blue, the explants consisted of >98% membrane intact cells ($P=0.9$). No qualitative differences in the development of DCD was detected by TEM. The outer surface of explants in all groups was highly differentiated and intact. In conclusion, without affecting morphology, components of FCS, which may be depleted after 2 days of culture, turn out to partly protect while ITS enhances the development of DCD. Furthermore, FCS and ITS seem to preserve ciliary activity. Since the toxic margin of insulin and transferrin, but not of selenium, is far above the applied levels in our culture system, amongst others, selenium may play a role in the development of DCD. Further research is needed to unravel the exact cause in the development in DCD in oviduct explants.

A206E OPU-IVP and ET

Reproductive response of prolific breed and its crosses in intrauterine insemination program

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Keywords: conception, laparoscopic AI, Romanov sheep, synchronization, Turkish native sheep.

The success of laparoscopic AI (LAI) depends on events and factors that interrelate in a complex way. Once the selection and preparation of the ewe have been accomplished, one of the most important steps in the program is the successful synchronization of the ewe to deliver good quality ova to the site of fertilization at a specific time. However, a considerable variation in success rate exists when using this technology whereby conception rates range from 10 to 85%. A major and highly consistent finding from the studies reported showed to be a major difference among the different ewe breeds with respect to pregnancy rate after LAI regardless of the source of that semen. These results confirm the importance of the breed and therefore possible reasons for this effect needs to be elucidated. In this study, we aimed to investigate the reproductive performance of yearling prolific Romanov breed and its half and quarter crosses with Turkish native breeds in a LAI program conducted during the breeding season. In addition, to breed effect we also examined vaginal electrical resistance (VER) values which was reported by the previous researches (Bartlewski et al., 1999; Rezac, 2008) that ewes with lower VER, which means higher estrogen levels. A total number of 30 ewes, equally distributed for each genotype (Romanov: 10, F1 Romanov crosses (F1): 10 and quarter Romanov (Q breed): 10) were included in the experiments. All animals were treated with a vaginal sponge containing 30 mg fluorgestone acetate (FGA; Chrono-gest, Intervet, MSD, Turkey, for 12 d. Immediately following sponge removal, ewes received an injection of 500 IU, i.m. eCG. An experienced laparoscopic AI operator performed the inseminations using fresh diluted semen (100 x 10^6 motile spermatozoa/0,4ml) at 52-55h after sponge removal. The animals were screened for estrus beginning at 24 h after sponge removal and continuing up to 57 h. Animals that did not show any mating marks by 57 h were not inseminated. Electric resistances of vaginal secretions (VER) were measured with a vaginal probe (DRAMINSKI, Poland) that was gently inserted into the vagina prior to LAI. *Conception rate* was determined by *ultrasound* 40 days after AI. The Romanov breed showed the highest estrus response (83%; $P<0.05$)) and, the F1 (40%) and Quarter Romanov crosses (50%) were found similar estrus rates. Conception rates (CR) were 80%, 75% and 57% for Romanov, F1 and Quarter Romanov crosses, respectively ($P>0.05$). Correlation coefficient between vaginal mucous impedance and conception rates was computed as 0.025 and showed to be not significantly correlated with CR. However, compared to F1 and Q ewes Romanov ewes showed more tight VER values which is possibly related to the variation in the moment of estrus.

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A207E OPU-IVP and ET

The application of bovine *in vitro* embryo production technology to develop an in vitro test battery for the screening of estrogenic compounds

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Keywords: 17β-estradiol, bovine IVP, diethylstilbestrol, *in vitro* assays.

The objective of this study was to develop a battery of tests able to identify the two main mechanisms of action of estrogenic compounds: the receptor-mediated mechanism, naturally occurring in hormone-responsive tissues carrying specific receptors (Erα and Erβ) and the direct mechanism through which estrogen and estrogenic compounds bind spindle components and cause a depolymerizing effect on microtubules therefore inhibiting the correct formation of the meiotic spindle. For this purpose two well-known compounds, diethylstilbestrol (DES) and 17β-estradiol (EST), were tested on four different *in vitro* assays: bovine oocyte *in vitro* maturation (bIVM) assay, bovine embryo *in vitro* culture (bIVC) assay and MCF-7 (human breast adenocarcinoma) and BALB/3T3 cell lines (mouse fibroblasts) proliferation and cytotoxicity assays, respectively. For the bIVM assay immature oocytes were aspirated from abattoir ovaries, washed and transferred to oocyte maturation medium, which was supplemented with the test compounds. At the end of maturation the oocytes were denuded, fixed with acetic acid/ethanol (1:3) for 18-24h and stained with lacmoid solution. The completion of meiosis up to the metaphase II stage was considered as the toxicological endpoint. For the bIVC assay, bovine embryos were obtained by IVM and IVF, followed by *in vitro* culture. At day 7 after IVF, embryos were selected at the early blastocyst stage and exposed to test substances from this stage onwards. The toxicological endpoint was the development of embryos up to the expanded hatched blastocyst stage at day 11. For the other two assays MCF-7 cells were cultured in MEM without glutamine and phenol red supplemented with 10% Foetal Bovine Serum (FBS) charcoal stripped, 4 mM α-glutamine and 1 mM pyruvate and BALB/3T3 cells were cultured in DMEM:TCM199 (1:1) supplemented with 10% FBS charcoal stripped. Both cell lines were exposed to test compounds at increasing concentrations. The AlamarBlue® test was performed and data were analysed with a TECAN plate reader (Infinite F200 Pro). Results indicate that only the MCF-7 proliferation assay can detect the receptor-mediated mechanism in the picomolar range of test compounds whereas a cytotoxic effect appeared in both cell lines in the micromolar range of test compounds. Moreover, the bIVM assay can detect the direct mechanism inducing spindle depolymerisation and abnormal nuclear configuration in the range of 1-20 microM. Finally, the bIVC assay does not seem to be informative because only a cytotoxic effect is evident at the highest concentration tested, as for the BALB/3T3 assay. In conclusion, this battery of four tests can allow to discriminate between the two major mechanisms of action of estrogenic and estrogen-like compounds, the receptor-mediated pathway and the direct one.

A208E OPU-IVP and ET

Using progesterone assay before superovulatory treatment in bovine farms

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Keywords: bovine embryos, progesterone, superovulation.

A French ET team had systematically realized progesterone assay for 15 years to help in the decision to start or not the superovulatory treatment for an embryo flushing in farm.Since 2010, 2210 progesterone assays have been done from 1561 females (1 425 heifers; 1119 Holstein, 30 other dairy breeds, 266 dual-purpose breeds and 146 beef breeds) in 665 farms. The blood samples for progesterone assay were performed by the farmer from 4 to 13 days after a reference heat and sent to a hormonology lab (LNCR, Maisons-Alfort). The superovulation protocol (8 FSH injections) was: D-16 to D-8 = reference heat; D-10 to D-3 = blood sample for progesterone assay; D-2 = input of an implant of norgestomet (Crestar®); D0, 8:00 = first FSH (Stimufol®) injection; D4 = 2 AI depending on heat observation; D11 = embryo flushing. The ET team received the quantitative result from the lab 2 to 6 days after the blood sample. A qualitative result was determined: negative for quantitative result inferior to 1.2 ng/mL, positive for results superior to 1.8 ng/mL and dubious between 1.2 to 1.8 ng/mL. The interval between the reference heat and the first FSH (from 8 to 16 days) didn't influence the number of collected embryos. No clear effect of parity (0, 1, 2, 3, 4 or 5 and more) or kind of breed could be shown, due to the great predominance of Holstein heifers (1 125). Among the 2210 progesterone assays, 1961 (89 %) gave a positive result, 114 (5%) a dubious result and 135 (6%) a negative one. Among the planned embryo collections, 108 (5%) were not performed, 42 due to a negative progesterone result, 66 for other different reasons. The mean progesterone level increased significantly from 4 to 6 days after heat, but this increase was no more significant after 7 days. Actually, 70 % of the negative results were all the same followed by an embryo flushing (result received too late, recipient already prepared…). Of course negative progesterone levels, led to significantly ($p<0.0001$) less total and viable embryos collected than for positive ones: respectively 10.3 ± 8.6 and 5.7 ± 5.3 for positive versus 6.1 ± 5.2 and 3.0 ± 3.6 for negative. But, for the positive results, no effect of the level of progesteronemia on the number of collected embryos has been observed. Because of the very low ratio of embryo flushing finally cancelled due to negative result of the progesteronemia (2%), it has been decided to stop the use of systematic progesterone assay.

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A209E OPU-IVP and ET

Effect of low oxygen tension on mitochondrial activity in cultured pig embryos

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Keywords: IVF, mitochondrial activity, oxygen tension, pig embryo quality.

Mitochondrial membrane potential (ΔΨm) is a key factor of the normal pre-implantation embryonic development due to high correlation with the cellular energy production. Consequently, alteration of ΔΨm may improve performance of pig embryo produced *in vitro*. Therefore the goal of this study was to check whether decreasing of oxygen tension may influence ΔΨm during *in vitro* development of pig embryos. Pig zygotes were collected surgically from superovulated gilts after flushing the oviducts. Zygotes were cultured in NCSU-23 (North Carolina State University-23) medium at 39°C in an atmosphere containing 5% CO_2 and: (A) 21% O_2, (B) 5% O_2, (C) 2% O_2. Embryos at 2- to 4-cell, 8- to 16 cell and morula stages were selected on days 2, 3 and 4 of culture, respectively. To estimate ΔΨm embryos were labeled with 0.5 μM MitoTracker Orange CMTMRos (Molecular Probes Inc.) for 30 min. at 39°C and subsequently analyzed in LSM 510 META confocal microscope (Carl Zeiss GmbH). The amount of fluorescence emitted from the mitochondria in arbitrary unit which proportional to the ΔΨm were measured. Data were analyzed using one-way analysis of variance and post-hoc Tukey test. For zygotes ΔΨm (mean±standard error of the mean) equals 8.07±1.28 (N=19). In group (A) ΔΨm was: 7.74±1.65 (N=17), 14.28±2.45 (N=16) and 15.1±2.44 (N=17) for 2- to 4 cell, 8- to 16 cell and morula stage respectively. In group (B) ΔΨm was: 9.73±0.96 (N=11, 2- to 4 cell), 24.52±2.37 (N=20, 8- to 16 cell) and 28.3±1.33 (N=18 morula). For group (C), ΔΨm was: 10.15±1.19 (N=21, 2- to 4 cell), 26,45±1.88 (N=13, 8- to 16 cell) and 32.57±1,04 (N=21, morula). In all analyzed groups, at the 2- to 4 cell stage ΔΨm was very low with no differences between groups, while significantly increased later, at 8- to 16 cell and morula stage ($p<0.01$). In conclusion, significant differences between embryos at the same developmental stages cultured in different oxygen tension were detected. Mitochondrial membrane potential for 8- to 16 cell and morula cultured at ambient oxygen tension was lower than that of stage matched embryos cultured in hypoxia conditions. Further investigations regarding the oxygen-sensitive hypoxia-inducible factors expression during *in vitro* cultured of pig embryos under different oxygen tensions are required.

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A210E OPU-IVP and ET

Serum-free *in vitro* culture of equine embryos

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Keywords: embryo development, horse, IVP, serum free in vitro culture.

While bovine embryos are routinely cultured in serum-free conditions since serum culture is associated with the occurrence of the large offspring syndrome, equine embryo culture is still conducted in the presence of fetal calf serum (FCS). In the horse, a negative effect of in vitro culture on the foals has not been observed, but early embryonic loss and development of trophoblast-only pregnancies have been associated with in vitro production of equine embryos (Hinrichs et al, Theriogenology 68:521-529, 2007). Therefore, the aim of this study was to evaluate equine blastocyst development and quality in serum-free culture medium. Equine embryos were produced as reported previously (Smits et al. Reproduction 143:173-181, 2012). Briefly, oocytes were aspirated from abattoir ovaries, matured in DMEM/F12 based medium in 5% CO_2 in air and fertilized by piezo-assisted ICSI. Presumptive zygotes were further cultured in DMEM/F12 supplemented with either 1) 10% FCS, 2) 10% serum replacement (SR, Life technologies, Gent, Belgium) and 5 ng/ml selenium, or 3) 0.4% BSA (Sigma-Aldrich, Diegem, Belgium), 5µg/ml insulin, 5µg/ml transferrin and 5 ng/ml selenium (BSA-ITS) at 38.2°C in 5% O_2, 5% CO_2 and 90% N_2. At day 2.5 cleavage was assessed and at day 9 blastocyst rate was evaluated. Subsequently blastocysts were fixed in 2% paraformaldehyde during 20 minutes and stored in PBS with 0.5% BSA at 4°C until staining. Differential apoptotic staining was performed as described previously (Wydooghe et al, Anal Biochem 416:228-230, 2011) to determine total cell number (TCN), inner cell massa/trophectoderm (ICM/TE) ratio and apoptotic cell ratio (ACR). Cleavage and blastocyst rates were compared using binary logistic regression. Data concerning blastocyst quality (i.e. TCN, ACR and ICM/TE ratio) were analyzed using a mixed-model analysis of variance (SPSS statistics 22). Cleavage rates were similar in FCS (22/29, 75.8%), SR (20/28, 71.4%) and BSA-ITS (22/28, 78.6%). No blastocysts developed in the BSA-ITS. Blastocyst rates were not significantly different between FCS (7/29, 24%) and SR (4/28, 14%) and TCN and ICM/TE were not affected either. However, ACR was significantly higher in SR (4.16 % ± 0.49), when compared to FCS (0.88% ± 0.20 , $p<0.001$). In conclusion, serum-free IVC of equine embryos in the presence of SR does not impair embryonic development, but ACR in the resulting blastocysts is significantly increased, when compared with ACR in blastocysts cultured in the presence of FCS.

A211E OPU-IVP and ET

The effect of dimethylsulphoxide on bovine embryonic development in vitro

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Keywords: cattle, dimethylsulphoxide, embryo development, IVP.

DMSO is routinely used as cryoprotectant or solvent for in vitro production (IVP) of embryos. Based on its high glassforming characteristics it is essential for vitrification but DMSO is also known for its toxicity at higher concentrations. Earlier studies deemed concentrations of up to 0.4% in in vitro maturation and 0.1% in in vitro culture (IVC) as safe with regards to morphological criteria. In the present study, bovine IVP embryos employing standard protocols were exposed to the following DMSO concentrations during IVC: 0% (control group), 0.05%, 0.1%, 0.15%, 0.2% and 0.25%. At day 8 cleavage and developmental rates were recorded. The morphological quality of expanded day 8 blastocyst was assessed with differential cell stainings; live-dead-staining (live-dead ratio) and TUNEL staining (apoptotic index). Fat accumulation was determined by red-oil staining. So far, the following results could be obtained: Cleavage and developmental rates did not differ ($p \geq 0.05$) between embryos of the various groups. Mean cleavage and development rates averaged at 58.3% ± 10.6% and 28.4% ± 9.2% (0 %), 59.5% ± 11.5% and 26.1% ± 7.4% (0,05%), 57.6% ± 6.6% and 21.7% ± 7.1% (0,1%), 58.1% ± 7.8% and 27.8% ± 5.6% (0,15%), 56.6% ± 7.3% and 24.5% ± 7.0% (0,2%), 56.3% ± 10.9% and 23.5% ± 9.9% (0,25%). The live/dead cell ratio was significantly higher ($p \leq 0.05$) in those embryos derived from the 0.1% group [40.1% ± 23.1%] than that from embryos of the other groups [22.6% ± 13.5% (0%), 23.4% ± 10.4% (0.05%), 24.2% ± 14.6% (0.15%), 22.7% ± 14.0% (0.2%), and 20.3% ± 9.9% (0.25%)]. Apoptotic cells in embryos exposed with 0.1 % and 0.2% DMSO were significantly lower than in those of other groups and with 0.05% DMSO the apoptotic cells in this group are also slightly lower compared to those of control group ($p = 0.08$). Apoptotic index was lower in embryos out of the groups supplemented with 0.1% and 0.2 % DMSO compared to those of the control group (0% DMSO: 3.8%±1.6%, 0.05% DMSO: 2.6%±1.6%, 0.1% DMSO: 2.3%±1.8%, 0.15% DMSO: 3.2%±1.5%, 0.2% DMSO: 2.2%±1.5%, 0.25% DMSO: 3.1%±1.7% [$p = 0.09$; $p = 0.06$]). Fat accumulation was significant higher [$p \leq 0.05$] in embryos stemming from the group supplemented with 0.15 % DMSO (0% DMSO: 6616.9 μm^2±2703 μm^2, 0.05% DMSO: 7346.3 μm^2±1981.3 μm^2, 0.1% DMSO: 6975.5 μm^2±1847.9 μm^2, 0.15% DMSO: 9301.1 μm^2±1703.3 μm^2, 0.2% DMSO: 8675.1 μm^2±2271.4 μm^2, 0.25% DMSO: 8300.7 μm^2±2711μm^2). These findings show that DMSO concentrations of 0.1% and 0.2 % used during in vitro culture influences the quality of embryos at the morphological level. However, further analyses to verify these results at the molecular level via RT-qPCR are still needed.

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