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Uterine blood flow evaluation after artificial insemination on different uterine sites in mares

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Keywords: blood flow, mare, uterus.

The intensity of inflammatory response depends on concentration and volume of inseminate. In addition, insemination with very small volumes may result in less effective mechanical drainage, while highly concentrate semen may be more irritating because of more contacts between spermatozoa and endometrium, resulting in intense inflammatory response (Kotilainen *et al.,* 1994, Theriogenology, 41, 629-36). Doppler mode ultrasonography, when association with B mode, brings information about vascular architecture in real time and hemodynamic aspects of vessels in different organs (Carvalho *et al.,* 2008, Ciência Rural, 38, 872-79). The aim of this study was to evaluate both uterine horns and uterine arteries in mares inseminated into the uterine body or into the tip of the uterine horn by Spectral Doppler mode ultrasonography. Sixteen mares were inseminated with cooled jack semen until six hours after ovulation, into the uterine body (volume of 20mL and 500 million spermatozoa) or into the tip of the uterine horn, ipsilateral to the dominant preovulatory follicle (4mL and 100 million spermatozoa), with eight mares in each group. RI (resistance index) was determined for a mesometrial artery in each uterine horn and for both uterine arteries, using the mean of three values for data analyses. Analyses of variance and Duncan test were used for analyses of data and means, respectively. There were no difference between the group inseminated into the uterine body or into the tip of the uterus when compared the values of mesometrial and uterine arteries RI ($P > 0.05$). The following data was obtained: group inseminated into the uterine body (right = 0,78 ± 0,08 and left = 0,77 ± 0,075 mesometrial RI; right = 0,88 ± 0,06 and left = 0,87 ± 0,09 uterine arteries); group inseminated into the tip of the right uterine horn (right = 0,76 ± 0,07 and left = 0,78 ± 0,08 mesometrial RI; right = 0,89 ± 0,05 and left = 0,88 ± 0,05 uterine arteries); and group inseminated into the tip of the left uterine horn (right = 0,80 ± 0,08 and left = 0,81 ± 0,07 mesometrial RI; right = 0,88 ± 0,03 and left = 0,89 ± 0,06 uterine arteries). In conclusion, uterine vascular perfusion variation, owing to inflammatory response after exposure to different volumes and concentrations of inseminate doses, was not detected using only RI evaluation of mesometrial and uterine arteries.

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Effects of glycerol and ethylene glycol association on ovine sperm cryopreservation

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Keywords: cryoprotectant, semen extender, spermatozoa.

Glycerol is frequently used as cryoprotectant to freeze ruminants' semen, however it has toxic effects to the sperm. Alternatively, ethylene glycol has been used due to its cryoprotectant characteristics, lower molecular weight and easiness of crossing the plasma membrane. This study aimed to compare the effects of the combination of ethylene glycol and glycerol as cryoprotectants for freezing ram semen through the post-thaw assessment of total (MT) and progressive motility (MP), vigour (V), and through the evaluation of the integrity of plasmatic, acrosomal and mitochondrial membranes using the fluorescent probes propidium iodide, FITC-PSA and JC1 respectively. Ejaculates from 17 animals collected with artificial vagina and with values equal to or greater than 70% MV and MP were used. Semen cooling was performed in two steps, into polystyrene boxes. Initially semen was diluted in fraction A (FA: TRIS-egg yolk), and incubated for 2 hours when temperature reached 5°C, followed by the addition of fraction B (FA + cryoprotectant) (1:1) containing 14% glycerol, 6% ethylene glycol (control groups) or glycerol 7% + ethylene glycol + 3% (experimental group), with additional incubation for 30 minutes at 5°C. The semen was packaged in 0.25 ml straws and placed 6 cm above the liquid nitrogen (N2L) level, for 15 minutes and then dipped in N2L. The straws were thawed in water at 37°C. The physical parameters were analyzed subjectively. The evaluations of membranes were made using fluorescent probes by counting 200 cells of each sample, with the aid of epifluorescence microscopy. Data were subjected to analysis of variance and means were compared by t test at 5% significance. The results do not show significant differences in physical parameters between samples frozen with glycerol alone (MT = 48.52 ± 18.27; MP = 34.11 ± 8.52 and V = 3.47 ± 0.87) and ethylene glycol (MT = 37.05 ± 18.54; MP = 27.94 ± 13.58 and V = 3.12 ± 0.78), or with the combination of glycerol + ethylene glycol (MT = 47.35 ± 15.52, MP = 36.17 ± 10.68 and V =3.76 ± 0.56). The evaluation of sperm membranes by fluorescent probes propidium iodide, FITC-PSA and JC1 showed no differences between samples frozen in the presence of glycerol (63.39% ± 46.46, 65.83 ± 35.00% ; 28.21 ± 49.17%), ethylene glycol (54 ± 21% 47.54,% 63.83 ± 44.23, 50.51 ± 39.59%) or combination of glycerol and ethylene glycol (57 , 09% ± 38.83, 33.14 ± 41.64%; 52, 38% ± 23.16) respectively. Since the different treatments showed similar results for all parameters evaluated, the combination of cryoprotectants glycerol and ethylene glycol can be used in the cryopreservation of ovine semen, but it is important to evaluate other semen parameters such as the rate of pregnancy to obtain information about the possible benefits of cryoprotectants association.

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Omega 6 and 3 polyunsaturated fatty acids association during in vitro production did not alter the cryotolerance and membrane lipids profile of bovine embryos

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Keywords: cryotolerance, embryo, mass spectrometry.

Studies report positive effects of polyunsaturated fatty acids (PUFAs) supplementation, such as conjugated linoleic acid (CLA, omega 6) and docosahexaenoic acid (DHA, omega 3), during *in vitro* production of bovine embryos, on its cryotolerance. However, the effect of the association of omega 6 (n-6) and 3 (n-3) PUFAs families during embryo IVP, has not been evaluated. This study was conducted in order to evaluate the effects of supplementation of CLA and DHA association, during IVM and IVC on cryotolerance and membrane lipid profile by matrix-assisted laser desorption/ionization - mass spectrometry (MALDI-MS), of IVP bovine embryos. COCs (n=491) were IVM for 22 h, and after IVF, zygotes were IVC in SOFaa medium (5 mg/mL BSA + 2.5% FCS, 5% CO_2 in air) for 7 days. The experimental groups were: Control (C) and 100 µM CLA + 100 µM DHA (CLA+DHA). Blastocysts were vitrified (Ingámed®, Maringá-PR, Brazil) and after 3 h of thawing, embryo survival rates were measured. Viable embryos were transferred to microtubes containing 200 µL of methanol HPLC 50% in aqueous solution, stored at -20 °C and immediately transported for MS analysis. Each embryo was deposited at the center of the spot's plate. Before MALDI-MS analysis, 1 µL of matrix (1.0 mol/12.5 dihydroxybenzoic acid (DHB) in methanol) was deposited on each spot and dried at room temperature until its complete crystallization. Spectra were acquired in the mass range of *m/z* 700-1200, in the positive ion and reflectron modes using an Autoflex III (Bruker Daltonics, USA) mass spectrometer. After excluding isotope peaks, the most intense ions of each spectrum were considered as starting point for determining the *m/z* ratios corresponding to membrane lipids. Only *m/z* clearly distinguished from noise were included in the partial least squares discriminant analysis (PLS-DA). The re-expansion rates were evaluated by qui-square test. These rates did not differ ($P > 0.05$) between groups were assessed and 63.5% (C) and 62.1% (CLA + DHA). The association of PUFA n-6 and n-3 did not influence embryonic cryotolerance success rates. In agreement with the cryopreservation results, no variations on membrane lipid profile were observed in CLA + DHA group, compared with C group embryos. In this case, there was no separation of the groups in PLS-DA, indicating that sphingomyelin (SM) and phosphatidylcholine (PC) profiles were not affected by treatment. In conclusion, the supplementation with the association of CLA + DHA during IVM and IVC had no effect on cryotolerance and membrane lipid profile of IVP bovine embryos.

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Biomodulation of MAPK expression by low level laser on oocyte in vitro maturation in cattle

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Keywords: Low-level laser, in vitro maturation, cattle.

Low-level laser irradiation (LLLI) is an alternative for the biomodulation of oocytes in order to improve blastocyst formation after fertilization. Two isoforms of MAPK, known as ERK1 / 2, are activated near the VG breakdown in bovine oocytes been essential for the transmission of maturation and cumulus cells expansion signals, both *in vitro* and *in vivo*. The objective of this study was to evaluate the effect of LLLI (λ 633nm HeNe - fluency 1J/cm^2 – 16:30 min) at the beginning of oocyte IVM in the MAPK (ERK1 / 2) expression when compared to non-irradiated oocytes (control). Control and irradiated samples (at least 3 replicates per group) were evaluated by the amount of Total MAPK and phosphorylated MAPK (MAPKP) after 30 min, 8 hours, 16 hours and 24 hours of IVM. The quantification of MAPK and MAPKP was performed by Western blot from 10 oocytes using a digestion buffer (1% NP40, 135 mM NaCl, 20 mM Tris pH 8, 10% glycerol). Protein integrity was analyzed by the Bradford method. Protein bands were quantified by densitometry and their densities calculated in arbitrary units. This value was submitted to 2WAY ANOVA with Bonferroni post test (Prism 5 GraphPad Inc). At the end of IVM oocytes were also evaluated for extrusion of the first polar body by optical microscopy and subjected to in vitro fertilization. There was an interaction between time and treatment for total MAPK ($p < 0.001$). There was no difference between irradiated and control group for total MAPK, except for 8 hours group in which the irradiated oocytes presented ten times higher amount of protein than the control. Despite the increase in total MAPK, there was no difference between the groups regarding MAPKP. This corroborates with the data related to the MAPK total / MAPKP ratio, which resulted in higher values 8 hours after the beginning of maturation. Despite this increase, the number of oocytes with nuclear maturation (extrusion of the first polar body) remained the same between groups, as cleavage and blastocyst rates after IVF. In fact it is expected an increase in levels of MAPK between 8 and 14 hours. However, the increase of MAPKP in 8 hours did not follow the pattern total MAPK. We concluded that the irradiation LLLT was able to induce increased expression of the total MAPK in in vitro matured bovine oocytes. More studies are needed to induce physiological changes induced by LLLT that can be used as an alternative aimed at better support for future events of fertilization.

Acknowledgments: FAPESP and UFABC.

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Synchronization of the follicular wave and blood flow of the dominant follicle in crossbred heifers treated with estradiol benzoate and cypionate

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Keywords: colour Doppler, follicular dynamics, FTET.

The study was designed to compare the synchronization of the follicular wave and the blood flow of dominant follicle after estradiol cypionate (EC) or benzoate (EB) treatment. Seventy-five crossbred heifers (HxZ) were distributed in 2 experiments (Exp.) and the same hormone protocol was given: Day zero (D0), insertion of the P_4 implant (1,0g Primer®, Tecnopec); D8, removal of the implant and injection of the sodium Cloprostenol (0.5 mg, Sincrosin®, Vallé). For Exp.1, 45 heifers were distributed in three groups (G), according to estradiol treatment on D0: G1a (n=15) 1ml of IM salina; G2a (n=15) 0.5 mg of IM EC (Von Franken®); and G3 (n=15) 2.0 mg of IM EB (Ric-BE®, Tecnopec). The diameter of the largest follicle – present at the ovary before treatment - was tracked on during days: 0, 2, 3, 4 and 5. The emergence of the new follicular wave – first observation of the dominant follicle at ≥4 mm – was also recorded. For Exp.2, the follicular wave was synchronized with 2.0 mg of EB (D0, n=30) and the ovulation was induced on D9 with one of the treatments: Gb1, 1 ml of IM saline (n=10); G2b, 0.5 mg of IM EC (n=10); and G3b, 1.0 mg of IM EB (n=10). From D8 to D10, the diameter and blood flow of the dominant follicle was monitored with an ultrasound device, equipped with color Doppler (7.5 MHz, M5, DPS-Equipamentos Médicos, São Paulo). In the cross-section of the follicle with the highest blood flow, score of vascularization was designated, according to percentage of the follicular wall occupied by color signals: 1 (>75%), 2 (>50 and ≤75%), 3 (>25 and ≤50%) and 4 (≤25%). Data for follicle diameter were checked for normality and analyzed with ANOVA, for the main effects of the group, day and interaction. The averages of the groups were compared by Tukey test to 5% (5-10% approach). Data for blood flow scores were analyzed in Kruskal-Wallis procedures. In Exp.1, the regression of the largest follicle was more effective ($P<0.07$) between 0 and 2 (-1.0±0.4 vs -0.2±0.2mm) in EB group. The effect of day was not significant ($P>0.05$) in control group (saline) group. The emergence of the new follicular wave was observed 3,5 days after the beginning of the protocol in CE and about 1 day later in the EB group (4.3d, $P<0.0013$). In Exp.2, the diameter of the dominant follicle did not differ ($P>0.1$) among the groups. The blood flow of the dominant follicle increased ($P<0.0006$) 24h (D9 to D10) after treatment in EB, which has not been observed in EC and saline groups. The ovulation rate did not differ ($P>0.05$) between groups (80, 70 and 60%, respectively for BE, CE and saline, respectively). It is concluded that the two estradiol treatments were efficient to synchronize a new follicular wave, however, the dose of BE may have contributed to the delay in follicular emergence. The increase in blood flow of the dominant follicle, 24h after EB treatment, might indicate close relation of the LH surge, as blood flow increases with the preovulatory peak of the LH surge (Acosta et al., 2003, Reproduction, 125, 759-67).

Acknowledgments: Biotran e FAPEMIG (financial support, project number APQ-1454-12).

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Ultrasound features of the corpus luteum 21 days after estrous and pregnancy diagnosis of bovine recipients: preliminary results

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Keywords: colour doppler, early diagnosis, FTET.

The study was designed to relate ultrasound (US) features – B mode and color Doppler (CD) mode - of the CL, 14 days after embryo transfer, and pregnancy diagnosis of the embryo recipients. Sixty-one (n = 61) recipients, synchronized (estrous = D0) and timed embryo transferred (TET, around D7) of frozen embryos (Ethylene glycol 1.5 mol) from South Africa (Embryo Plus, Brits). Fourteen days later (D21), the CL was classified according to its size and blood flow: score 1 (Large; High blood flow in most of the gland), 2 (Medium; Regular or Concentrated blood flow in certain areas of the gland) and 3 (Small; Poor or low blood flow). All procedures were performed by the same ultrasound operator. Approximately 400 frames in B mode and 100 in color-flow Doppler, containing entire cross-sections of the CL, were firstly stored in *avi* format on the ultrasound device (M5, DPS medical equipment, Sao Paulo), and further transferred to an external HD (Samsung 500 GB. The area of the CL at largest diameter and averaged area of the CL with colored pixels - indicative of blood flow – from three regions (central and opposite sides of the CL) were measured with ImageJ software (Image Processing and Analysis in Java). The predictive diagnosis (DG21) was based on CL at the same ovary of D7 and blood flow score of 1 or 2. The CL and blood flow areas were compared to US scores for size (B mode) and blood flow (DP). The PROC GLM procedure of SAS statistical software (version 9.0, SAS Institute Inc., Cary, NC, USA.) and the Duncan test were used to access the differences among means. Pregnancy diagnosis was performed on D35 (DG35) and the PROC FREQ procedure used to test the agreement of DG21 and DG35. The predicted pregnancy rate (DG21) was 57.4% (34/61) and did not differ (P> 0.05) from the ultimate diagnosis (DG35 = 50.8%, 31/61). Non-pregnant animals were accurately detected on DG21 (100% predictive value for negative) and its predictive value for positive was also high (88.6%). These results were similar to other early diagnosis of pregnancy based on functionality of the CL (Siqueira et al., In press). Thus, corpus luteum of pregnant animals had shown larger total (305.8±59.4 vs. 111.6±55.3 mm^2, P <0.0001) and blood flow area (64.5±22.3 vs. 5.2±7.2 mm^2, P <0.0001), when compared to non- pregnant animals. Noticing that the CL area was 3x larger and the difference in blood flow area was 12x higher in pregnant compared to non- pregnant recipients, it is possible to conclude that the diagnosis of pregnancy based on the size and blood flow of the CL is feasible as early as 14 days after embryo transfer.

Acknowledgments: Biotran e FAPEMIG (financial support, project number APQ-1454-12).

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Ultrasonography evaluation of fetal sexing in mares at 60 days of gestation after embryo transfer under farm condition

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Keywords: mare, sexing, ultrasound.

Embryo transfer technology in horses was developed to increase their genetic value. Recently, the improvement of ultrasound equipment has allowed more accurate identifications of fetal sex. The objective of this study was to evaluate in a program of embryo transfer in horses the efficiency of fetal diagnosis at 60 days of pregnancy. Twenty-eight mares from La Vanguardia haras, located in San Andrés de Giles-Argentina, were used. The embryos were produced by artificial insemination and collected seven days after ovulation. They were soon transferred to the twenty-eight recipients. Pregnancies were confirmed seven days post-transfer by ultrasound by visualization of the embryonic vesicle. Ultrasonographic evaluation was done using an ultrasound (Well D Medical Electronics ®, Shenzen, China) coupled to a transrectal probe from 5.5 to 7.5 MHz. The position of the genital tubercle was evaluated at 60 days of gestation. When the genital tubercle was located caudal to the umbilical cord the fetus was considered as male, and if it was near to the fetus tail it was considered as female. At 120 days of gestation fetal sex was confirmed according to Renaudin et al., 2000 (J Reprod Fertil Suppl., 56, 651). The accuracy of the gender diagnosis at 60 days was evaluated at 120 days, the correct sex of male and female were compared by Qui-square test. The accuracy of pregnancy diagnosis at day 60 after confirmation day 120 of gestation was 87.71% (24/28). The difference between male and female fetuses with incorrect diagnoses was significant (P<0,05), 20% (3/15) for female and 7.69% (1/13) for male. These results are similar to those found by Merkt et al., 1999 (Journal Medicine Vet Sci, 19, 90) as they reported that female fetuses are more difficult to diagnose than male fetuses. The accuracy of sexing at 60 days of embryo transfer has not reached 100%.

Acknowledgments: supported by funds from CAPES.

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Milk proteins associate to goat binder-of-sperm (BSP) homologs preventing the binding to epididymal sperm membrane in vitro

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Keywords: binder-of-sperm, goat, milk proteins.

Previous studies show that Binder-of-Sperm (BSP) proteins from bovine seminal plasma (SP) associate with milk proteins (Bergeron et al. 2006, 2007a, 2007b, Lusignan et al. 2011). Although studies confirm the importance of BSP proteins in the bovine, interactions of BSP homologs from other species and extender components have not been evaluated. Thus, the present study was conducted to evaluate if milk protein associate to goat BSP proteins *in vitro*, minimizing the binding to epididymal sperm membranes. Spermatozoa were recovered from the cauda epididymis from bucks by retrograde flushing and incubated with either skim milk (Pellicer-Rubio et al 1997) or citrate-glucose medium (2.37 g sodium citrate, 0.8 g glucose, 100 ml distilled water) at 37°C. Following incubation, an appropriate amount of crude goat SP was added. In parallel, ejaculated and epididymal sperm were subjected to the same approach as control. Sperm membrane protein extraction was performed according to the method described by van Tilburg *et al.* (2013). Protein samples from each preparation were separated by SDS-PAGE and the presence of goat BSP proteins was assessed by immunoblotting using a polyclonal antibodies raised directly against goat BSP protein homologs (GSP-14, GSP-15, GSP-20 and GSP-22). Immunoreactivity of goat BSP proteins was detected in the ejaculated sperm and citrate-glucose medium fractions, indicating that goat BSP proteins interact with sperm membrane. In contrast, when goat SP proteins were added to skim milk medium plus caudal epididymal sperm, the polyclonal antibodies could recognize weakly those goat BSP proteins, suggesting that these proteins associate to milk proteins, minimizing the binding to sperm membrane. The decrease of goat BSP proteins suggests that skim milk extender plays a significant role in protecting goat sperm during storage. Certainly, the protein–protein interaction prevents an extensive loss of lipids from sperm membranes which may be deleterious to sperm storage. In summary, our study showed that BSP proteins present in goat SP have affinity for the milk proteins. The results obtained in the present study confirm the hypothesis that skim milk components sequestrate BSP proteins, preventing the detrimental effects of these proteins on the sperm membrane. These findings are of considerable interest in view of the mechanisms of sperm protection by extender constituents.

Supported by CAPES foundation, Brazil, FUNCAP, Brazil and NSERC of Canada.

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Software based on artificial neural networks for embryo morphologic evaluation

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Keywords: classification, embryology, software.

The embryonary morphologic classification – of great importance for various researches, from basic to applied on assisted reproduction – is still based on subjectivity and have no precise, consistent and trustful method (Farin et al., 1995, Theriogenology, 44, 339-49). The main goal of this work was to develop a protocol for information extraction (based on digital bidimensional images) and a software, for the morphological classification of mammals embryos in a similar way that an embryologist would do, having Artificial Neural Networks (ANN) and Digital Image Processing as base. An image database was developed with 98 samples of mice blastocysts (between initial and expanded stages) obtained from superovulation of Swiss Webster mice. Using these images, a protocol of information extraction was developed, in a way that 12 variables were obtained. Among these variables, for example, there was the development stage, days-post- mating, embryo area and its circularity. The variables worked as an input for the ANN classification in four different degrees of quality (excellent, good, fair or bad). As base of training for the ANN, the same images were classified in a conventional way (visual evaluation from an experienced embryologist). From all images on the database, 80% were used for the learning process and 20% for the proof of efficiency (test data) of the ANN. The comparison of results (between the output of the ANN and the embryologist analysis) verified that 75% of the classifications were successful. To verify the consistency (repeatability) of the embryologist analysis was settled a blind test, on which the test images were categorized by the embryologist again (without the knowledge of the original classification). Between the first and second evaluation, there was variation of classification on 7 images. When both classifications made by the embryologist are considered as correct, that is, as possible variations of morphological quality, the success rate of the software increases to 95% (only one misclassification). For the final version of the software was developed a graphical user interface, in a way that it could be used by technicians and students of the field, only demanding a minimum knowledge of embryology. The process presented a high potential of applicability, specially having in mind the possibility of expansion for other mammal species with more commercial interest (as human and bovine). The whole process, or more precisely, from the mining of variables from the embryo image to the utilization of the final software for analysis, was framed as patentable and its deposit made at the Brazilian National Institute of Industrial Property (INPI). In conclusion, the software prototype, obtained from mice embryo images, presented high analysis objectivity, consistency and a higher precision then the visual embryologist analysis.

Financial support from FAPESP (FDM).

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Preimplantion genetic diagnosis using whole genome amplification of equine embryo biopsy

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Keywords: biopsy, equine, sexing.

Although Brazil is the world's leader in equine embryo transfer, the techniques of micromanipulation and preimplantation genetic diagnosis are still underdeveloped in the country. Recent studies showed that embryo biopsy can be performed without affecting the conception rate and can improve the results on vitrification of expanded blastocyst. The aim of this work was to develop the techniques for micromanipulation and molecular biology to identify the sex of equine embryos. The biopsy was performed by microaspiration using two micropipettes (Holding and Biopsy) coupled to mechanical micromanipulators (Narishige), enabling the drilling of embryonic capsule without using Piezo Drill, acid or laser. The standardization of PCR was performed with genomic DNA (gDNA) samples extracted from horse and mare blood, quantified (NanoDrop), adjusted to the concentration of 50ng/μL and subjected to serial dilutions (5ng/μL, 0.5ng/uL, 50pg/μL and 5pg/μL), enabling the assessment of sensitivity in two primers: Y-specific (SRY) and endogenous control (S4B). The result was visualized by electrophoresis in Agarose gel stained with ethidium bromide. Although the primer S4B showed great sensitivity, the primer SRY showed no band in male samples with concentrations below 50pg/μL, revealing that it will not be possible to identify the sex from embryo biopsy (10-20 cells). Thus, we used the Whole Genome Amplification methodology in order to increase the gDNA amount from aliquots of 10-20 fibroblastic cells of horse and mare obtained from *in vitro* culture. Subsequently, four thawed equine embryos were submitted to repeated biopsies of ~20 cells, enabling the standardization of WGA and PCR methodology with primers S4B (145bp) and SRY (182bp), totaling approximately 12 hours, from biopsy to electrophoresis. The result with fibroblastic cells allowed the visualization of the two bands on male sample and only one (S4B) in female samples. The result of embryo sexing revealed two male e two females, suggesting that the methodology developed in this work can be used for equine embryo sexing. Two equine embryos were biopsied immediately after collection and kept in maintenance medium (Holding) at 37°C for 12 hours. Evaluation of embryonic morphology was carried out at 3, 6 and 12 hours after biopsy, allowing to observe the reorganization of embryonic structures as well as the blastocoel reconstitution, which indicates the embryos remained viable after biopsy. The next stage of the work will be to conduct field experiments that will assess the conception rate of biopsied equine embryos and fresh transferred, as well as biopsied and vitrified embryos.

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Preliminary investigation on effect of fetal bovine serum in the expression of genes involved in via cGMP during in vitro maturation bovine oocyte

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Keywords: cGMP, FCS, maturation.

The sensitivity of IVP embryos to cryopreservation is often associated with lipid accumulation in the cytoplasm. Among the possible causes of this event, the presence of fetal calf serum (FCS) in the culture medium (Barceló-Fimbres et al., 2007, Mol Reprod Dev, 74, 1395-405) or other disorders in energy metabolism have been identified (Sanchez et al., 2006, Reprod Fert Dev, 18, 585-96). In adipocytes of primates, including humans, lipolysis-related hormones, such as hormone-sensitive lipase (HSL), are activated when phosphorylated by both cAMP- (PKA) and cGMP-dependent protein kinase (Lafontan et al., 2008, Trends Endocrin Metab, 19, 130-7). Thus, intracellular levels of cAMP and cGMP are involved in the regulation of the rate of lipolysis in adipocytes; when they are high they cause lipolysis while when reduced promote lipogenesis (Hass et al. 2009, Science, 2, 78-89). Both nucleotides are also present in bovine oocytes, together with their synthesis (GC and AC) and degradation enzymes (PDE3 and PDE5, Schwarz, 2011, PhD Thesis, FZEA-USP, 106f). The aim of this study was to evaluate the influence of fetal calf serum (FCS) on the transcripts of some components cGMP pathway. For this, COCs were cultured for 24h in maturation medium supplemented with different proportions of FCS (2% to 10%). The control group was matured in medium only with 0.4% BSA. The mRNA of pools of 20 denuded oocytes and their respective cumulus cells from each group was extracted with Trizol and converted using the High Capacity cDNA Reverse Transcription Kit. The relative amount of genes linked to the control of cGMP levels (GUCY1B3 and PDE5A) or enzymes activated by it (PKG1 and PKG2) and the gene for beta oxidation of long-chain fatty acids (CPT1B) was determined by Real Time PCR using SYBR Green. The data relating to five replicates were analyzed with PCR program LinReg. Based on the analysis performed with the program Genorm, the geometric mean of the expression of genes GAPHD and PPIA (Lonergan et al., 2003, Reprod Biomed Online, 7, 657-63) were used as endogenous controls. Statistical analysis was performed by ANOVA followed by Tukey post-hoc at a significance level of 5%. All analyzed genes were expressed in both cellular compartments. GUCY1B3 reduced expression in oocytes, whereas in cumulus cells PDE5A increased the expression and CPT1B decreased ($P<0.05$) when cultivating COCs in the presence of FCS in comparison with the control group. In conclusion, FCS affected the relative expression of genes of the cGMP pathway that may be related to lipid metabolism in COCs. Studies are being conducted to confirm the effect of FCS on lipid accumulation and to determine the correlation with the accumulation of cGMP pathway in bovine COCs matured in vitro.

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RNA sequencing (RNA-Seq) of in vitro and in vivo bovine embryos

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Keywords: bovine, embryo, RNA-Seq.

Several technologies have been explored to highlight differences among embryos towards identification of those most likely to establish successful pregnancy. In this sense, transcriptome sequencing (RNA-Seq) is a powerful tool that can provide valuable information about the global gene expression of embryos. This information may help in understanding important metabolic pathways and identify patterns of in vivo development necessary for further improvement of IVP. The aim of this study was to evaluate the transcriptomic profile of bovine embryos produced in vitro from "fast" (4 cells at 40 hpi and blastocysts derived from this group) and "slow" (2 cells at 40hpi and blastocysts derived from this group) developmental groups as well as in vivo embryos. For this purpose, in vitro embryos were produced by conventional methods and cultured individually until the moment of analysis (in triplicate with 10 embryos per group). Blastocysts were produced in vivo and collected by conventional methods (in duplicate with 10 embryos per group). Total RNA was extracted, quantified and analyzed for integrity with an Agilent 2100 Bioanalyzer. The recovery of total RNA ranged from 131 to 521 pg per cleaved embryo and from 439 to 1319 pg per blastocyst. All RNA obtained was used for cDNA synthesis and subsequent amplification using the Nugen Ovation V2 kit. After amplification, the product obtained was quantified and evaluated. Between 2.23 to 5.2 µg of amplified cDNA was obtained from samples of cleaved embryos and between 2.38 and 4.76 µg from blastocysts. Samples with cDNA fragments between 600 and 3000 bp were considered suitable for the construction of sequencing libraries. The samples were then sonicated to fragments of approximately 300 bp. From these samples, 1 µg was used for construction of the libraries with the Illumina TruSeq Library system. Sequencing libraries were measured and checked for fragment size and concentration. Libraries were considered appropriate if fragments were sized between 350 and 500 bp (resulting from the previous cDNA fragment having adapters added). The samples were sequenced with Illumina HiSeq 2000 equipment and analyzed using CLC Genomics Workbench software. Sequence reads were checked for quality and alignment to known transcripts. At least 16000 transcripts were identified in all groups. Quantitative and qualitative information of the transcripts are still being processed. Based on the methodology described in which a very small number of embryos is necessary for quantifying thousands of transcripts, we conclude that RNA-Seq can be an important tool for studies in several areas of assisted reproduction such as embryo development, health, and genetic markers among others.

Acknowledgments: FAPESP and Gertec Embriões.

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Ultrasound features of the corpus luteum on the day of transfer and pregnancy diagnosis of bovine recipients: preliminary results

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Keywords: cattle, colour Doppler, FTET.

The goal was to evaluate ultrasound (US) characteristics – B and color Doppler mode (CD) - of the CL of embryo recipients on day 7 of the estrous cycle, relate the results with pregnancy diagnosis at day 35 (PD35) and compare the size of CL observed in B mode with the subjective dimensions of the CL by rectal palpation. Sixty-one crossbreed recipients (*Bos taurus* x *Bos indicus*) received frozen embryos (Ethylene glycol 1.5 mol) – produced in South Africa (Embryo Plus, Brits) - after hormone protocol for timed embryo transfer (TET) – estrous synchronization (D0) and embryo transfer (D7). On D7, the CL was classified according to its size and blood flow: score 1 (Large; High blood flow in most of the gland), 2 (Medium; Regular or Concentrated blood flow in certain areas of the gland) and 3 (Small; Poor or low blood flow), considering the size difference between the ovaries. The CL was also evaluated by rectal palpation (PR) in large, medium and small. All procedures were performed by the same US operator. Approximately 400 frames in B mode and 100 in color-flow Doppler, containing entire cross-sections of the CL, were stored in avi format. The area of the CL at largest diameter and averaged area of the CL with colored pixels - indicative of blood flow – from three regions (central and opposite sides of the CL) were measured with ImageJ software (Image Processing and Analysis in Java). The CL and blood flow areas were compared to scores for size (PR and B mode) and blood flow (CD). The PROC GLM procedure of SAS statistical software (version 9.0, SAS Institute Inc., Cary, NC, USA.) and the Duncan test were used to access the differences among means. Pregnancy diagnosis was performed on D35 and PROC FREQ was used to separate pregnant (G) of non-pregnant (NG) animals within the CL scores of D7. The pregnancy rate at 35 days was 50.8% (31/61), and it was similar to previous described for frozen embryos from *in vivo* production (Fernandes, 1999, ABMVZ, 51, 263-66). The subjective scores for size (PR and B mode) and blood flow of the CL, given on D7, were not efficient ($P < 0.5$, < 1.0 and < 0.4, respectively) to predict G and NG animals. Luteal and blood flow areas on D7 did not differ ($P < 1.0$, < 0.6, respectively) between animals later (DG35) diagnosed as G (320.4±64.6 and 55.9±19.0, respectively) and NG (321.3±71.2 mm^2 and 53.9±21.5 mm^2, respectively). The measured CL area was similar ($P > 0.05$) among RP scores for CL size. However, the B mode scores were more efficient ($P < 0.0001$) to separate CLs of different sizes (356.4±68.2a, 316.3±50.7a e 235.6±33.5b mm2, respectively for scores 1, 2 and 3). The real time observation CL in CD mode was sensitive ($P < 0.0001$) to detect blood flow changes of the CL tissue (66.8±18.4a, 45.2±15.7b e 38.4±16.1b mm^2, respectively for CD scores 1, 2 and 3). The interpretation is that the B mode was more efficient than the PR to differentiate corpus luteum of different sizes. The combined results of B and CD modes of the ultrasound give valuate information about size and blood flow of the CL, but were not efficient to predict the chances of the D7 recipient to become pregnant.

Acknowledgments: Biotran and FAPEMIG (financial support, project number APQ-1454-12).

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Cryopreservation of somatic cells of *Alouatta fusca*, *Cervus elaphus* and *Leopardus pardalis* using DMSO and PG as cryoprotectants

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Keywords: cell culture, cryopreservation, wild animals.

The cell cryopreservation is an important tool for genetic material preservation from endangered animals, for their long-term use. The formation of genetic resources includes gametes, embryos and cells (Holt and Pickard 1999 Reviews of Reproduction 4, 143-50) facilitating the creation of a genetic pool of endangered species. The aim of this study was compare survival, viability and injury in DNA, in wild animals cells cryopreserved with DMSO or propylene glycol (PG). Muscle tissue samples of *Alouatta fusca* (baboon), *Cervus elaphus* (deer) and *Leopardus pardalis* (ocelots) received in Veterinary Hospital - CAV/UDESC, obtained post mortem, were collected aseptically and transported to the laboratory. Cells were obtained by explant technique (Fresnhey 2005, Culture of Cells 175-197) and maintained in DMEM + 10% FCS at 37 °C with 5% CO_2. The cells were cryopreserved in cryovials at a concentration of $1x10^6$ cells/mL using a solution with 10% PG or DMSO in culture medium. After wadding, the cryovials were placed in Mr. Frosty® apparatus (Nalgene Nunc Cooler, USA) which was kept at 4 °C for 15 min and for 12 h at -80 °C. Then the cryovials were transferred to N_2L. For thawing, cryovials were kept in the water bath at 37 °C and the contents transferred to a tube containing 2 mL of culture medium, centrifuged and resuspended in 500 µL of medium. One sample was used for determination of survival by trypan blue staining. In the remainder, 10.000 cells were cultured in well with 1.9 cm^2 in triplicate and them viability was assessed by counting the number of living cells after 24 h of cultivation. Another part of the samples were used for the comet assay (duplicate) (Collins 2004, Molecular Biotechnology 26, 249-261). In all tests cells not cryopreserved were used as control. Statistical analyzes were performed using SAS (SAS Institute, 2000), PROC GLM and subsequent comparison of means by Tukey test (5%). Rates of cell survival for DMSO and PG, immediately after thawing, were respectively, 81.2% and 71.8% (baboon), 68.8% and 68.8% (deer), 68.7% and 69.1% (ocelot) no significant difference in the same animal. The viability after 24 h of culture cells from the same animal, frozen with different cryoprotectants showed no significant difference ($P<0.05$), and during this time, the increase of the cell number for DMSO and PG were respectively, 43.3% and 46.7% (baboon), 50.0% and 53.3% (deer), 22.5% and 22.5% (ocelot). The DNA integrity assessed by comet assay showed no difference between the categories of damage when compared to cells frozen with DMSO or PG, with respectively 74.5% and 77.5% (baboon), 68.5% and 75.5% (deer), 64.5% and 67.5% (ocelot) showed no injury, as well as controls baboon (85%), deer (82%) and ocelot (80%). Therefore, the use of DMSO or PG in cryopreservation of cells these animals did not affect the cell viability by parameters analyzed.

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Identification of CAE virus by qPCR in embryos recovered from seropositive dairy goats

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Keywords: caprine, CEA, real time PCR.

The aim of the present study was to identify by qPCR the presence of the CAEV (caprine arthritis-encephalitis virus) in goat embryos produced in vivo following embryo washes recommended by the IETS, 1998 (Stringfellow, DA, Seidel, D.A., Manual International Embryo Transfer Society). Embryos were flushed according to Fonseca, J.F. et al., 2012 (In press) from ten (n=10) dairy goats aged 4 and 5 years naturally infected and positive for CAE virus by AGID test. Subsequently embryos with intact ZP (morule and initial blastocysts) were divided into two groups; the washed embryos (n = 44) and unwashed embryos (n = 44), totaling 44 pools. The RNA of two embryos pooled from the same flusing was extracted using the MinElute Virus Spin Kit® (Qiagen, Düsseldorf, USA). After that the material was subjected to Real Time PCR, the positive control was goat synovial membrane. All samples were negative confirming the results reported by Ahmad et al., 2008 (Theriogenology, 69, 408-415) where the presence of CAE virus in goat embryos produced in vivo was not detected by conventional PCR. The present study shows that embryos derived from CAE-positive animals are free of the pathogen independently of washing. However, considering embryo commercialization rules the recommended washes must be maintained. Further studies are being carried out to determine the risk of transmission of pathogens in naturally infected animals, in order to achieve the international trade of embryos from CAE-positive goats.

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Adaptation and evaluation of negative pressure effects on pre-freezing of ram semen

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Keywords: Controlled stress, cryopreservation, heterologous IVF.

The induction of controlled stress by positive pressure increases the cryotolerance of mammal gametes and embryos. However, the device to apply positive pressure is difficult to obtain and to transport. Our group showed that the negative pressure obtained in a low cost and easy to handle equipment (Nitrocooler) has similar effect to positive pressure. The aim of this study was to evaluate the effect of 5 min negative pressure treatment of ram semen, before freezing. Semen was pooled from ejaculates of 2 rams collected with the aid of an artificial vagina, and diluted 1 + 1.5 in Tris-yolk medium, fractionated into 4 aliquots and submitted to: control; pressure of 200 mBar (P200); pressure 500 mBar (P500) and pressure of 800 mBar (P800). Just after, an identical fraction containing 10% glycerol was added to the each aliquot, and loaded in 0.25 mL straws, cooled and frozen in a TK3000 Compact machine (5 replications). Post-thaw evaluations performed were: progressive motility (PM) just after thaw and during the Thermo-resistance-test - TRT (1, 2, and 3 h) evaluated by optical microscopy; acrosomal integrity (AI) by FITC staining; membrane integrity (MI) through the hiposmotic test, PM after selection by percoll (PMPP), acrosome integrity post percoll (AIPP), membrane integrity after percoll (MIPP) and cleavage rate after heterologous IVF with bovine oocytes. Data were analyzed as a randomized block design, with no effect of repetition, by Fischer LSD tests with 5% significance. Just after thaw the PM of Control group (49±7.4) was higher than P200 (40.9±9.7), P500 (38.9±7.4) and P800 (38.9±7.4) that did not differ among them. There were no differences in PM between Control, and P200, P500 and P800 groups during the TTR 1 h (43.4±9.7; 37.9±7.5; 36.9±7, and 37.9±7, respectively), 2 h (40.8±8.6; 31.8±6; 34.8±7.7 and 36.8±8.7, respectively) and 3 hours (34.8±10; 28.7±8.2; 33.8±9.7 and 29.8±16 respectively), and yet in AI (55.4±13.2; 52.4±5.8; 49.5±7 and 49.8±11.4 respectively), PMPP (66±4.1; 59.2±10.2; 51.7±19.2 and 63.2±9.1, respectively), AIPP (46, 4±4.6; 45.7±4.1; 46.2±5.7; 49.3±8.9, respectively) and MIPP (45.4±10.2; 39.9±4.9; 47.3±9.4 and 41.1±8.9, respectively). For MI evaluation the Control group (36.6±7.7) was higher than P200 (30.2±5.8) and P800 (30.4±6.6) but did not differ from P500 group (34.3±7). Regarding cleavage, the P800 group (34.5) was lower than all other groups (control 44.3, P200 51.2 and P500 50.9) that did not differ among them. Data show that even with an initial reduction in PM, as the time passed the negative effects of P200 and P500 pressures disappeared. However, it was still observed a negative effect of P800 pressure. We concluded that the P200 and P500 applied to the ram semen do not reduce its viability after freezing, being necessary to evaluate a possible effect in pregnancy rates after the use of negative pressure treated semen.

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Uterine blood flow in bitches with cystic endometrial hyperplasia-pyometra

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Keywords: bitch, diagnosis, Doppler.

The Cystic Endometrial Hyperplasia (CEH)-Pyometra Complex comprises a group of uterine diseases named CEH-mucometra, endometritis and pyometra. The differential diagnosis of these diseases is accomplished through the combined results of two-dimensional ultrasound, clinical signs and uterine macroscopic and histological evaluation. The aim of this study was to characterize the uterine blood flow in bitches with distinct uterine pathological conditions in order to develop a noninvasive and early method of diagnosis. We allocated 28 bitches into 3 groups, according to clinical signs, ultrasound analysis and uterine histological examination (H/E stain): Control-Diestrus Group (n=6), CEH-Mucometra Group (n=10) and Endometritis-Pyometra Group (n=12). With the use of colour Doppler, the uterine vessels were identified for quantitative assessment of the overall uterine vascularization and the pulsed-wave Doppler was employed to classify the flow velocity waveforms. Parameters of blood flow velocity (PS, ED and TAMAX) and hemodynamic parameters (RI, PI and S/D) were calculated by the ultrasound software. A total of nine stable uterine artery waves was obtained to calculate the average for each variable. The degree of uterine vasculature was subjectively scored as 1 to 4, being 1 the minimum and 4, the maximum degree. Data were compared by ANOVA and LSD ($p \leq 0.05$). Control (PS: 47.3±2.6; ED: 1.3±0.5; TAMAX: 8.5±1.1) and CEH-Mucometra (PS: 60.8±4.5; ED: 9.8±0.8; TAMAX: 20.5±0.1) groups showed lower blood flow velocities when compared to Endometritis-Pyometra Group (PS: 86.7±6.3; ED: 33.3±3; TAMAX: 48.3±4.1). Conversely, Control (RI: 0.97±0.01; PI: 6.4±0.6; S/D: 78.8±9.8) and CEH-Mucometra (RI: 0.8±0; PI: 2.6±0.1; S/D: 6.7±0.4) groups presented higher hemodynamic indices in comparison to Endometritis-Pyometra Group (RI: 0.6±0.02; PI: 1.2±0.1; S/D: 2.9±0.2). In the latest Group, uterine perfusion was characterized as continuous blood flow. The quantitative uterine vascularization was: Control Group - minimum and maximum of score 1, CEH-Mucometra Group - minimum of score 1 and maximum of score 2; Endometritis-Pyometra Group - minimum of score 2 and maximum of score 4. Our study demonstrated that the severity of the uterine disease is associated with higher blood flow, fundamental in the compensatory mechanisms against the infectious agent. Doppler velocimetric evaluation may allow for the differential diagnosis between CEH-Mucometra and Endometritis-Pyometra, whenever clinical symptoms and ultrasonographic findings are inconclusive. Thus, when the two-dimensional ultrasound evaluation does not allow differential diagnosis, analysis of hemodynamic indices (RI, PI and S/D) of the uterine artery can be complementary. We conclude that Doppler ultrasound is a noninvasive diagnostic method, with high sensitivity and specificity for the differential diagnosis of HEC-mucometra-Endometritis and Pyometra in bitches.

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Effect of the addition of pentoxifylline to the semen extender on seminal characteristics of stallion thawed semen

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Keywords: CASA, membrane, spermatozoa.

Pentoxifylline, a methylxanthine derivate, is an inhibitor of the enzyme phosphodiesterase, that increase the intracellular cAMP concentration. The pentoxifylline stimulates flagellar motility therefore increasing sperm motility (STANIC et al., 2002, Intern J Androl, 25, 186-90). The purpose of this study was to examine the effects by adding pentoxifylline to seminal extender on cryopreserved equine semen after thawing. Eight batches from a unique stallion was cryopreserved ($100x10^6$ spermatozoa/0.5 mL straws). Two semen straws were thawed and divided in two aliquots. One of the aliquots was diluted in skim milk extender, the other aliquot was diluted in the same skim milk extender plus pentoxifylline (7.18 mM). Aliquots of each treatment were warmed in a water bath at 37°C and analyzed at different time after dilution: T0 (5 min), T30 (30min), T60 (60 min) e T120 (120 min). The sperm movement was evaluated using the Computer-Assisted Semen Analysis (CASA), integrity of plasma and acrosomal membranes and mitochondrial membrane potential, using the fluorescent probes association (PI, H342, FITC-PSA and JC-1) and chromatin denaturation accessed by Toluidine blue. To all variables was utilized the analysis of variance (ANOVA), and the range was compared by *LSD* test, SAS 9.3 version (2010). For statistical analyses, it was considered effect of treatment, time and treatment x time interaction. No improvement was found on total motility, progressive motility, beat cross frequency (BCF), and percentage of rapid cells in none of the analyzed periods. However, pentoxifylline increased ($P<0.05$) VAP (71.68±1.52 x 65.71±0.89 μm/s), progressive velocity (VSL, 63.63±1.29 x 59.20±0.80), curvilinear velocity (VCL, 131.24±2.51 x 118.50±1.57 μm/s) and displacement lateral of head (ALH; 5.17±0.09 x 4.69±0.07 μm) when compared to controls. Pentoxifylline decreased ($P<0.05$) straightness (STR; 88.61±0.40 x 89.67±0.27%) and linearity (LIN; 51.45±0.49 x 53.45±0.45%). Membrane integrity wasn't affected by pentoxifylline addition and it did not provide any protective behavior in the length of incubation. Chromatin integrity also was not affected by extender, neither the time of incubation. In conclusion, pentoxifylline increase some motility parameters but it does not affect cellular membranes or chromatin membrane.

Acknowledgments: FAPESP, process numbers 2012/14927-6 e 2012/15736-0.

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Bovine embryo genotyping on high-throughput SNP platform

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Keywords: biopsy, marker-assisted selection, WGA.

Despite the great development of animal reproduction biotechnology, such as embryo *in vitro* production (IVP), preimplantation genetic diagnosis (PGD) is still applied with restraint in animals embryo transfer. Recent advances in genomics have associated phenotypic characteristics with molecular information, allowing the development of marker-assisted selection. The aim of this study was to perform PGD in bovine embryos using high-throughput SNP platform (BeadChip/6,909 SNP). The small amount of genomic DNA (gDNA) obtained from embryo biopsy is the main limitation for the high-density SNP analysis. Thus, the Whole Genome Amplification (WGA) (Repli-g Mini Kit, Qiagen, Hilden, Germany) was used to increase the amount of gDNA from embryo biopsy and allow the analysis of thousands SNP simultaneously. Eighty-eight IVP bovine embryos were subjected to micromanipulation by microaspiration, allowing the formation of three groups with different numbers of biopsied cells: G1) 5-10 (n=28); G2) 10-20 (n=37); G3) > 100 - hatched blastocyst (n = 23). All samples were subjected to the same WGA protocol, and 4μL of each sample were used for genotyping on iScan/Illumina platform. The genotyping quality was assessed using the Call Rate (CR), GenCall Score (GC10), Alelle Drop In (ADI) and Alelle Drop Out (ADO). Kruskal-Wallis test was applied to investigate differences in the distribution of variables among groups. Spearman`s rank correlation coefficient revealed a significant correlation between all variables. The results showed a positive correlation between CR and GC10 (0.99/P <0.001), while ADI and ADO rates were negatively correlated with CR and CG10 (ADI/CR: -0.87; ADI/GC10: - 0.88; ADO/CR: -0.87; ADO/GC10: -0.86), P<0.001 for all variables. Kruskal Wallis pointed to significant differences in all variables (CR, GC10, ADO and ADI) among the 3 groups of biopsies (G1, G2 and G3). The CR average was 59.26%, 78.47% and 95.97% for G1, G2 and G3, respectively. It was developed a script (mendellFix) based on the "Law of Segregation", for inference of not determined genotypes based on the parents genotypes, thus increasing the CR of group 1, 2 and 3 to 79.69%, 88.20% and 97 28%, respectively. The results of this study show that it is possible to perform the genotyping of bovine embryos in high-throughput SNP platform with samples subjected to WGA/MDA protocol, but the number of cells obtained by embryo biopsy affects the quality of genotyping. The association of biotechnologies described in this work allows the application of marker-assisted selection in bovine embryo, contributing to further accelerate the process of animal breeding.

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Active immunization of cows in the pre-partum period and transfer of passive immunity to newborn calves for bovine viral diarrhea virus and bovine herpesvirus

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Keywords: BoHV-1, BVDV, vaccine.

The aim of this research was to evaluate the humoral immune response of the commercial vaccine Cattle Master Gold FP5 + L5 (Zoetis®, Madison, USA) for BVDV and BoHV-1 in the pre-partum of Holstein cows, as well as the transfer of specificantibodies (AB) from mothers to their calves for passive immunity. Cows screened (n=11) were negative in the serum neutralization test (SN) for BVDV and BoHV. Of these, 6 were immunized by subcutaneous injection of 5 mL of vaccine, which contains5960 strain of BVDV-I, 53637 of BVDV-II inactivated and RLB103 of BoHV-1 attenuated at 42 and 21 days (d) of the expected delivery date. Control unvaccinated cows was maintained (n=4). Calves were fed with six liters of colostrum from their mothers in the first 12 hours of life. Blood samples were collected from mothers in three moments: M0 - before the 1st dose, M1 - before the 2nd dose, M2 - 21 d after the 2nd dose. The samples of calves were obtained before (T0) and after feeding of colostrum at 48 hours of life (T1). The measurement of neutralizing ABs mothers and calves by SN for BVDV and BoHV followed similar protocols (OIE 2010, Terrestrial Manual, 1-17). The frequency of seropositivity for BVDV-1 and BoHV in the group of immunized mothers was 33.33% (2/6) and 100% (6/6), respectively. The mothers of the non-vaccinated group remained seronegative. All calves in this study showed no evidence of antibodies to BVDV and BoHV-1 at T0, however, it was possible to detect seroconversion of newborns from seropositive mothers after active immunization for BVDV and BoHV-1. Geometric mean titers (GMT) obtained in positive mothers in SN was zero in M0, 16 in M1 and 64 in M2 to BVDV, and M0=0, M1=13.45 and M2=71.52 for BoHV-1. The GMTs of calves in T1 was 32 to 77.71 for BVDV and BoHV-1. Thus, maternal vaccination during pre-partum period induced the arising of neutralizing ABs in mothers, especially to BoHV. Moreover, the transfer of maternal ABs to newborn was detected after colostrum feeding. It is worth noting that only inactivated BVDV vaccines can be commercialized in Brazil, however, these have lower biological immunogenicity when compared to live vaccines. Previous study (Silva, 2006, Federal University of Santa Maria, 35-55) evaluated six Brazilian commercial vaccine for BVDV and only 1/6 was able to induce neutralizing antibodies in vaccinated cattle. Thus, despite the lower response to BVDV comparing with BoHV in this study, the seroconversion rate obtained was better than those obtained in the aforementioned study. In addition, the immune response of the mothers and calves should not be evaluated only for the production of neutralizing ABs, since the immunogen must also confer cellular immunity. Thus, studies are being conducted to evaluate the lymphocyte subpopulations, as well as its activation, and this information is essential to complement the data obtained and preliminarily presented.

A267 Supporting Biotechnologies: Cryopreservation and Cryobiology, Image Analysis and Diagnosis, Molecular Biology and "Omics"

Pregnancy monitoring of vitrified in vitro produced embryos in buffaloes

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Keywords: birth, buffaloes, vitrification.

Vitrification has proven more appropriate technique of cryopreservation of mammalian *in vitro* embryos. However, even with several studies being conducted in the species, there are few trials evaluating pregnancy and calving rates after embryo transfer (ET) (Presicce, 2007, Reprod Domest Anim, 42, 24–32). For this reason, the aims of the present study were to monitor pregnancy after transfer of vitrified embryos and to collect data of calves and recipients after delivery. Expanded blastocysts grade I, produced *in vitro* (IVP) from ovun pick up (OPU) buffaloes donors were vitrified by the cryotop method (De Rosa et al., 2007, Ital J Anim Sci, 6, 747-50) on days 5, 6 and 7 of culture. At eight sessions, 70 vitrified embryos were transferred in the period of 01/02/11 to 02/04/12 to recipients previously synchronized at day 6 after ovulation, utilizing embryo transfer fixed time protocol (Baruselli et al., Rev Bras de Reprod Anim, 31, 285-92). All embryos were warmed in the lab (De Rosa et al., 2007, Ital J Anim Sci, 6, 747-50) and loaded into straws for ET and there weren't temporary culture in CO_2 incubators or selected. On day 30 after ET, positive pregnancy was diagnosed in 26 animals (37.1%). A total of 9 fetal mortalities (34.6%) were verified from 30 days until calving, in which 4 occurred from 30 to 60 days after ET (15.4%), and 5 occurred from 60 days until calving (19.2%). In this last period, 5 pregnancy losses were due to abortion. From these 17 pregnancies, a total of 10 female calves (58.8%) and 7 male calves (41.2%) were born. Pregnancies from female and male calves had a mean length of 313.2 and 316.4 days, respectively (range 302 to 332 days, and 311 to 323 days, respectively). Weight at calving was a mean of 33,6 and 34 kg for female and male calves, respectively (range 30 to 38 kg, and 32 to 35 kg, respectively). All calving occurred without intervention and dystocia was observed in only one case. No large offspring syndrome, hydramnios, hydroallantois, or umbilical cord anomalies were observed in calves. Delivery was normal and without induction in all recipients, and any puerperal infections, or retained placenta occurred. Suckling assistance was necessary in only one newborn. Results are unprecedented in science and opens possibilities for the commercial use of the techniques of OPU, IVP and ET of vitrified embryos in buffaloes.

Acknowledgments: FAPEMIG (APQ - 0591-5.04/08), FINEP, FIEMG.