



090 Assisted Reproductive Technologies

Prostaglandin F_{2α} as an inducer of ovulation in Fixed Timed Artificial Insemination in Zebu cows

F.B. Almeida^{*1,2}, O.A.C. Faria¹, B.D.M. Silva²

¹Universidade de Brasília, Brasília, Brazil; ²Embrapa Recursos Genéticos e Biotecnologia, Asa Norte, Brasília, Brazil.

Prostaglandin (PG) is a potent biological substance with varied applications in bovine reproductive control, and PGF_{2α} main effect is to induce luteolysis. Prostaglandin PGF_{2α} has been reported as an ovulatory stimulus in prepubertal heifers. Thus, the objective of this study was to evaluate the use of PGF_{2α} in fixed timed artificial insemination (FTAI) protocol as an inducer of ovulation in cows and characterize the blood perfusion of the pre-ovulatory follicles. The experiment was carried out at Embrapa Genetic Resources and Biotechnology. Twenty-four zebu cows were assigned to a 3 x 3 Latin square design with 30 days between treatment periods. Cows received at random stages of the estrous cycle an intravaginal device containing 1g of progesterone (P4) concurrent with an i.m. injection of 2.0 mg of estradiol benzoate (EB). The day of EB injection was considered study day 0 (D0). The intravaginal device was removed on D8. Cows were then randomly assigned to three treatments as estrous synchronization protocols. In the PGF_{2α}-D7 treatment, cows received 500 µg of PGF_{2α} i.m. (cloprostenol) in two applications on D7 and D8. In PGF_{2α}-D9 treatment, injections of 500 µg of PGF_{2α} i.m. were on D8 and D9. Control (CT) cows received 500 µg of PGF_{2α} i.m. on D8 and in D9 and 1 mg of EB on D9. Cows were evaluated with a color Doppler ultrasound (MyLab™30Gold VET, Italy) every 12 hours from D8 (0 hrs) to D10 (60 hrs), after which cows were evaluated every 6 hours until ovulation occurred or a maximum of 120 hours. Seven days after ovulation, cows were examined by ultrasound with color Doppler mode to evaluate the presence of luteal blood flow to assess functionality of the corpus luteum (CL). For follicular and luteal vascularization, a subjective classification of grades 1 to 5 was adopted, which considered the percentage of follicle wall or CL with blood circulation. Statistical comparison was performed for time of ovulation, and size of preovulatory follicle using ANOVA with the Tukey adjustment for treatment comparisons. Degree of vascularization of follicle wall and CL was analyzed using Friedman's test. Time of ovulation differed ($P < 0.05$) among treatments and CT cows ovulated earlier (72.4 ± 3.7 h) compared with the other treatments (PGF_{2α}-D7 96.0 ± 12.8 and PGF_{2α}-D9 90.0 ± 18.2 h). The mean preovulatory follicle size did not differ among treatments (PGF_{2α}-D7 13.1 ± 1.6 ; PGF_{2α}-D9 13.4 ± 1.8 ; CT 11.9 ± 1.5 mm). Similarly, the degree of irrigation of follicles did not differ among treatments (PGF_{2α}-D7 3.7 ± 1.0 ; PGF_{2α}-D9 3.5 ± 1.0 ; CT 2.3 ± 1.8). Ovulation rate in PGF_{2α}-D7 and PGF_{2α}-D9 averaged 62.5% (15/24) and it did not differ from CT (58.4%; 14/24). Diameter of the CL (PGF_{2α}-D7 15.5 ± 3.4 ; PGF_{2α}-D9 14.4 ± 3.2 ; CT 13.4 ± 2.7 mm) and its vascularization (PGF_{2α}-D7 4.7 ± 0.6 ; PGF_{2α}-D9 4.7 ± 0.6 ; CT 4.7 ± 0.4) did not differ among treatments. Administration of EB, but not PGF_{2α}, reduced interval to ovulation, although ovulation rate did not differ among treatments. EMBRAPA, UNB.



091 Assisted Reproductive Technologies

Cryopreservation of prepubertal bovine testicular tissue

P.M. Aponte^{*1,2}, F. Canet¹, S. Erazo¹, F. Salas-Diehl²

¹Colegio de Ciencias Biológicas y Ambientales (COCIBA), Universidad San Francisco de Quito (USFQ), Quito, Pichincha, Ecuador; ²Colegio de Ciencias de la Salud, Escuela de Medicina Veterinaria, Universidad San Francisco de Quito (USFQ), Quito, Pichincha, Ecuador.

Spermatogenesis is the complex process responsible for sperm generation in the testis. Spermatogonial stem cells (SSCs) are at the basis of the seminiferous epithelium constantly generating cohorts of daughter cells committed to differentiate. In the bull, type A spermatogonia population includes both SSCs and their differentiating daughter cells (de Rooij and Griswold, 2012). Germ stem cells can be cryopreserved and used for germoplasm preservation (Onofre *et al.*, 2016) and particularly posthumous preservation of elite bulls. During the early prepubertal stage, before the first wave of spermatogenesis, gonocytes, the precursors of A spermatogonia, are the only germ cells in the testis (Aponte *et al.*, 2005). We aimed at cryopreserving whole bovine testicular tissue as a fast procedure to save particular bovine male germ-lines. For this purpose, we took testicular tissue from 2-3 mo old Holstein calves (n = 4), isolated germ and somatic cells through enzymatic digestions, differential plating and recorded initial (day 0) cell composition and survival. Approximately 1 cm³ testicular pieces from the same testes were slowly infiltrated with either MEM + DMSO + FCS (Sol. A) or MEM + DMSO + BSA (Sol. B) in 3 fractions, dropwise, over 2 h, 4°C. Tissue pieces were then placed at -80°C for 3 d, and stored in liquid N₂ for 2 wk. Then, they were thawed in water at 39°C. Immediately, the tissues were subject to cell isolation. Cell composition and survival were compared pre and post-freezing through One Way ANOVA (SPSS, Windows, V. 17.0.). Results are expressed in mean ± SEM. Testicular morphology post-thawing was similar to that of fresh pre-freezing tissue. In both cases testes showed seminiferous tubules with Sertoli cells and gonocytes located near the basal membrane. The percentage of viable gonocytes in the resulting cell suspension was significantly lower after thawing, independently of the cryopreservation solution used (pre-freezing: 40.6 ± 14.7%; post-freezing: Sol A, 6.3 ± 3.3%; Sol B, 3.4 ± 0.4%; P < 0.05). Viability within the gonocyte population was similar among the treatments (pre-freezing: 93.1 ± 4.7%; post-freezing: Sol A, 81.0 ± 10.9%; Sol B, 81.5 ± 1.4%; P > 0.05). These results suggest that gonocytes are able to survive cryopreservation but absolute numbers obtained are considerably lower after thawing. This is perhaps caused by higher amounts of free DNA shed from dying cells during cryopreservation, making the surviving cells “sticky” and lost during cell isolation steps. Gonocyte numbers can probably be brought up by adding higher concentrations of DNase in the media and by using further purification steps. Post-freezing, apparently intact germ cells can be the basis for further reproductive biotechnologies aimed to bovine species such as spermatogenesis *in vitro* and transgenesis.



092 Assisted Reproductive Technologies

Effect of heat shock on developmental competence in bovine oocytes during *in vitro* maturation

F. Báez^{*1}, A. Camargo¹, C. Deragón¹, C. Viñoles²

¹University Center of Tacuarembó, UDELAR, Tacuarembó, Uruguay; ²Ruminants Reproductive Health Center in Agroforestry Systems / Agroforestry Pole, Bernardo Rosengurtt Research Station, UDELAR, Cerro Largo, Uruguay.

The aim of this study was to determine the effect of heat shock (HS) and its time of exposure during *in vitro* maturation (IVM) of bovine oocytes on meiotic progression and blastocyst yield. Intact immature cumulus-oocyte complexes (COCs) obtained from a local abattoir were IVM at 38.5°C for 24 h (control group, CG), or incubated for 6 h (group G-6h), 12 h (group G-12h), 18 h (group G-18h), or 22 h (group G-22h) at 41°C, and then matured at 38.5°C to complete 24 h. After maturation, oocytes from each group were fertilized *in vitro*, and the presumptive zygotes were cultured until reaching the blastocyst stage. The rate of meiotic progression of all oocytes was recorded at 24 h (n=60-68 oocytes/group) and analyzed by Chi-square test. Data on the percentage of cleavage (n=127-200 presumptive zygotes/group) on day 3, blastocyst rate, and total blastomeres in expanded blastocysts on day 9, were analyzed by one-way ANOVA using SAS PROC GLM. Exposure of bovine oocytes to 41°C for 12 h reduced (P<0.05) the percentage that reached the metaphase II (MII) stage. The rates of blastocyst development in CG and G-6h groups were greater than in G-12h, G-18h, and G-22 h (29.8±4.8 and 25.5±5.0 vs. 10.7±2.8; 18.9±3.9 and 7.5±5.3%, respectively; P>0.05). Moreover, the total number of blastomeres in CG (127.9±3) was greater (P<0.05) than all other groups, being similar among G-12h, G-18h, and G-22h groups (88.1±4; 86±6 and 90±5.7, respectively). In conclusion, exposure of bovine oocytes for 12 h to HS during IVM did not block development but reduced blastocyst rates. Therefore, exposure to HS during *in vitro* maturation reduces developmental competence of bovine oocytes.



093 Assisted Reproductive Technologies

Use of interspecific fertilization as a tool to evaluate fertilizing ability of buffalo (*Bubalus bubalis*) bulls in an *in vitro* fertilization program

J.A. Berdugo*¹, S. Palomres², L. Herrera²

¹BIOGEM, Universidad Nacional de Colombia-Sede Medellin Antioquia Colombia; ²Embriones del Sinú, Montería, Córdoba, Colombia.

In vitro production (IVP) of embryos is a wide spread biotechnology in cattle but it is not well developed in buffaloes, in which the maternal model for reproduction is highly applied. Buffalo and cattle are bovidae species and share significant physiological similarities, suggesting that no highly effective species-specific fertilization barrier exists. The limited availability of oocytes to evaluate the performance of candidate bulls for IVP programs is part of the problem. The objective of this work was to develop a strategy designed to evaluate the fertilizing capacity of buffalo males to be used for *in vitro* fertilization. This work was performed in Embriones del Sinú Laboratory, located in Montería North Coast Colombia, during 2015. Frozen semen from buffalo bulls was evaluated using matured cattle (*Bos indicus*) oocytes after OPU. Oocytes were obtained from 3 to 8 mm follicles, and matured for 24 h in SOF-supplemented medium. After thawing, sperm were prepared using a swim-up method and groups of 20 Grade I oocytes were inseminated with 2 million/mL motile sperm in 50 µl drops. Presumptive zygotes were transferred to culture medium for development until day 7, and cleavage and blastocyst rates (BR) were recorded. Of 272 oocytes used, 166 (61%) cleaved and 88 (32%) reached the blastocyst stage. Cleavage rate ranged from 50 to 74% and BR from 22 to 40%, in all the cases interspecific BR was higher than monospecific production (18%). These results are similar to those obtained by Kochhar et al. and Xiang Li et al., 86.3% and 71.3% cleavage and 25.9% and 33.7% BR, respectively. The feasibility of using cattle oocytes to evaluate the fertilizing capacity and the potential performance of buffalo bulls to be used for IVP has been shown, and as expected there was high variation in BR among the buffaloes evaluated. It is very surprising the low BR of embryos when cattle oocytes are fertilized by buffalo sperm. Others have reported fertilization of cattle oocytes with African buffalo epididymal sperm *in vitro*. Interspecies hybrids offer clues in the search for answers to several questions of genetics like interbreeding depression including over-dominance, hybrid vigor, and segregation distortion. Our next research will be conducted to evaluate the usefulness of the model to discriminate potentially fertile and infertile males. Embriones del Sinú - Asociación Colombiana de Criadores de Búfalos.



094 Assisted Reproductive Technologies

Comparison between ovine refrigerated spermatozoa from ejaculate and epididymal cauda

J.V.V. Brighenti*¹, T.G. Bergstein-Galan², R.R. Weiss², L.E. Kozicki¹

¹Postgraduate studies in Animal Science, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil;

²Postgraduate studies in Bioprocess Engineering and Biotechnology, Human and Animal Health, Federal University of Paraná, Curitiba, Paraná, Brazil.

Recuperation and preservation of viable spermatozoa after the sudden death of an important breeding animal can be vital to manage breeding programs. The aim of this study was to compare sperm viability on refrigeration at 5°C when spermatozoa were collected in an artificial vagina (AV) or recovered from epididymal cauda (EPD). Semen was collected in an AV and 1 week after collection the same rams were slaughtered. Epididymal spermatozoa were recovered by slicing method immediately after slaughter of four randomly selected epididymis. After collection in AV or EPD the samples were diluted in medium containing glycine, 5% egg yolk and milk in final concentration of 400×10^6 spermatozoa/mL and refrigerated at 5°C. The parameters of total motility (TM), progressive motility (PM) and plasma membrane integrity (PMI) in hypoosmotic swelling test were evaluated 0 (R0), 24 (R24), 48 (R48) and 72 (R72) hours after the onset of cooling. The analyzes were performed in duplicate. ANOVA test was used to identify differences between groups (AV and EPD) and Repeated measures ANOVA test was used to analyze differences between moments (time after the onset of cooling). Tuckey test was applied on previous tests when $P < 0.05$. There was no difference ($P > 0.05$) in TM, PM and PMI between groups at any cooling moment. The initial TM was $86.4 \pm 1.0\%$ and $79.0 \pm 4.3\%$ in AV and EPD groups, respectively. TM decayed ($P < 0.05$) from 24 hours of refrigeration in both groups (AV: $68.6 \pm 6.3^{b\%}$ and EPD: $60.0 \pm 2.5^{b\%}$). However, PM differed ($P < 0.05$) from the initial moment more rapidly in AV group (R0: $73.2 \pm 1.4^{a\%}$; R48: $20.4 \pm 3.3^{bc\%}$; R72: $6.3 \pm 2.1^{c\%}$) when compared to EPD (R0: $62.0 \pm 4.4^{a\%}$; R48: $24.0 \pm 4.3^{ab\%}$; R72: $5.0 \pm 1.7^{b\%}$). PMI decreased ($P < 0.05$) from 12 hours of cooling in the AV group (R0: $90.4 \pm 1.3^{a\%}$ and R12: $65.4 \pm 2.9^{b\%}$). In EPD group, PMI remained the same throughout the refrigeration period (R0: $82.0 \pm 0.6\%$; R72: $66.5 \pm 3.9\%$). No difference in TM, PM and PMI was identified between AV and EPD groups during the preservation of spermatozoa in liquid stage at 5°C. Similar results were found when the spermatozoa were frozen (Bergstein-Galan *et al.*, 2017. Quality and fertility of frozen ovine spermatozoa from epididymides stored at room temperature (18-25°C) for up to 48 hours post mortem. *Theriogenology*, 96: 69-75). However, undesired changes, as the decrease in PM and PMI, occurred later in the EPD group when compared to AV. These findings are probably the result of greater resistance of epididymal spermatozoa to cold shock. In conclusion, there are no differences between ejaculated and epididymal spermatozoa preserved in liquid stage and cooled to 5°C.



095 Assisted Reproductive Technologies

Impact of GnRH administration at the time of AI on pregnancy and ovulation rates and its interaction with estrous expression

T.A. Burnett*, A.M.L. Madureira, J.W. Bauer, R.L.A. Cerri

University of British Columbia, Vancouver, BC, Canada.

Cows with reduced estrous expression have been found to have compromised fertility. The aim of this study was to determine if the administration of GnRH at the moment of AI could increase ovulation rates and fertility of animals expressing low estrous behavior. Cows were enrolled at the time of estrus from 3 commercial farms (n = 1629 AI events; Farm A: 757, Farm B: 305, Farm C: 567) and randomly assigned to receive GnRH at AI or not. At the same time, cows had their BCS and gait scored. On all herds, cows had their estrous expression monitored through leg-mounted activity monitors. Estrous expression was quantified as the maximum activity that occurred during the event; using the farm median, estrous expression was categorized as high or low. On Farm A, cows were assessed at alert and for ovulation at 24h (n = 160), 48h (n = 707) and 7d (total ovulation failure; n = 707) post-alert using transrectal ultrasonography; ovulation was determined by the disappearance of the dominant follicle. Pregnancy was confirmed at 31 ± 3d post-AI. Differences between treatments were tested using the GLIMMIX procedure of SAS where cow within farm was used as a random effect. Occurrence of ovulation at 24h was impacted by estrous expression, where animals with high expression had lower ovulation rates at 24h (23.3 ± 4.8 vs. 37.7 ± 5.5%; P = 0.05); no impact of GnRH was found. An interaction between GnRH and estrous expression was found for both the occurrence of ovulation at 48h and total ovulation failure. At 48h, ovulation rates were highest for cows with high expression that received GnRH, all other groups were the same (GnRH: High – 94.8 ± 2.5, Low – 85.9 ± 2.6; No GnRH: High – 88.5 ± 2.9, Low – 87.3 ± 2.6%; P = 0.05). Similarly, total ovulation failure was lowest for cows with high estrous expression that received GnRH (GnRH: High – 2.2 ± 1.9, Low – 6.2 ± 2.1; No GnRH: High – 8.2 ± 1.9, Low – 8.9 ± 2.0%; P = 0.05). Ovulation was not impacted by parity, gait or BCS. Fertility on all farms was impacted by parity, where primiparous cows had higher P/AI than multiparous (46.0 ± 3.1 vs. 35.6 ± 2.3 %; P < 0.01), but was not impacted by gait or BCS. Fertility was not impacted by treatment, but there was an interaction of treatment and estrous expression, where animals with low estrous expression receiving GnRH at AI had higher P/AI than those that did not receive GnRH; GnRH did not impact P/AI of high expression cows. In fact, cows with low estrous expression receiving GnRH had the same P/AI as those with high estrous expression with or without GnRH (GnRH: High – 43.3 ± 3.3, Low – 40.8 ± 3.6; No GnRH: High – 46.2 ± 3.5, Low – 33.0 ± 3.5%; P < 0.01). In conclusion, administration of GnRH at the time of AI resulted in an increased conception risk of animals with low estrous expression, however, this effect does not seem to be closely related to ovulation rates. Zoetis, Dairy Farmers of Canada and NSERC.



096 Assisted Reproductive Technologies

Factors affecting the ovum pick-up and *in vitro* embryo production in buffaloes

**N.A.T. Carvalho¹, J.G. Soares², O. Bernardes³, A.C. Basso⁴, B.M. Bayeux⁵, J.C.B. Silva⁵,
R.D. Mingoti⁵, P.S. Baruselli^{*5}**

¹Unidade de Pesquisa e Desenvolvimento de Registro/Centro de Zootecnia Diversificada/Instituto de Zootecnia, Registro, SP, Brazil; ²Universidade Federal de São Paulo, Escola Paulista de Medicina, Centro de Pesquisa em Urologia, São Paulo, SP, Brazil; ³Sítio Paineiras da Ingai, Alambari, SP, Brazil; ⁴In Vitro Brasil, Mogi Mirim, SP, Brazil; ⁵Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Reprodução Animal, São Paulo, SP, Brazil.

The objective of the present study was to evaluate the influence of variables related to donors and sires in the efficiency of OPU/IVEP in buffaloes. For this, data from 421 OPU/IVEP procedures (farm [A, B, C], parity [nulliparous-N, primiparous-P and multiparous-M], postpartum period [≤ 117 d, 117d to 217d and >217 d] reproductive status [pregnant-P or non-pregnant-NP at the OPU], body condition score [BCS; <3.0 , 3.0 to 4.0 and >4.0] and sire used for IVEP [A, B, C, D, E, F, G and H]) were evaluated. All buffaloes underwent a regular transvaginal ultrasound-guided ovum pick-up (OPU) for oocyte recovery at random stage of the estrous cycle. The HPMIXED procedure of SAS through the Best Linear Unbiased Prediction (BLUP) analysis was utilized to rank farms, parity, postpartum period, reproductive status, BCS and sires, in terms of oocytes per OPU, embryo produced per OPU and embryo rate. It was evidenced effect of farm ($A=9.6\pm 0.5^a$, $B=8.9\pm 0.3^{ab}$ and $C=6.9\pm 0.9^b$; $P=0.05$) and parity ($N=10.2\pm 0.7^a$, $P=11.1\pm 0.9^a$ and $M=8.34\pm 0.4^b$; $P=0.07$) on the number of retrieved oocytes per OPU. Nulliparous and primiparous produced higher number of retrieved oocytes per OPU than multiparous. Regarding the postpartum period and BCS, no differences were found for the number of retrieved oocytes ($P=0.92$ and $P=0.98$, respectively). Furthermore, according to the reproductive status, pregnant buffalo (30 to 120 days of gestation) produced lower number of retrieved oocytes per OPU than non-pregnant ($P=7.9\pm 0.6^b$ and $NP=10.0\pm 0.5^a$; $P=0.02$). However, only the sire variable had an effect on the number of embryo produced per OPU and embryo rate. There was a strong effect of the bull ($P<0.001$) on the efficiency of IVEP in buffaloes. The embryo rate (%) according to sires used ($n=8$) during IVEP was $A=37.7$; $B=29.7$; $C=25.2$; $D=22.0$; $E=20.4$; $F=17.5$; $G=6.6$ and $H=6.4$. It was concluded that semen used during IVEP procedures potentially influence IVEP results. Top ranking sires yielded outstanding embryo rates, while poor sires performers produced low embryo rates. Although we verified effect of farm, parity and reproductive status on the number of retrieved oocytes, no differences were observed in the number of embryos produced per OPU in the present study.



097 Assisted Reproductive Technologies

Modifications of a 5-d GnRH-based timed-AI protocol to optimize fertility in Holstein heifers inseminated with sex-selected semen

M. Colazo*¹, K. Macmillan¹, R. Mapletoft²

¹Alberta Agriculture and Forestry, Edmonton, AB, Canada; ²WCVM, University of Saskatchewan, Saskatoon, SK, Canada.

This study evaluated the usefulness of an estrus detection (ED) aid, timing of GnRH administration and AI on pregnancy per AI (P/AI) in Holstein heifers subjected to a 5-d GnRH-based protocol and inseminated with sex-selected semen. In Expt 1, Holstein heifers (n=591) received a progesterone releasing device (CIDR) on d 0, and on d 5 CIDR were removed, 500 µg of cloprostenol (PGF) was administered and ED patches (EstroTECT™) were applied. Patches were scored from 0 to 2, based on color change between initial application and 36 and 48 h after CIDR removal; 0 = unchanged, 1 = ≤ 50% color change, 2 = > 50% color change (defined as estrus). Heifers in the Control group (n=195) received 100 µg of GnRH and were inseminated 72 h after CIDR removal, regardless of estrus expression (TAI). Heifers in the treatment groups that were in estrus (n=110) were AI 56 h after CIDR removal and those not in estrus received 100 µg of GnRH at either 56 (GnRH56; n=142) or 72 h (GnRH72; n=144) after CIDR removal and were TAI at 72 h. In Expt 2, Holstein heifers (n=330) received CIDR and PGF treatments and ED patches as in Expt 1. Heifers in estrus at 36 or 48 h after CIDR removal were AI at 56 h as in Expt 1, but those not in estrus were TAI at either 72 (TAI72) or 80 h (TAI80) after CIDR removal, and those with an ED patch scored 0 or 1 at TAI received 100 µg of GnRH. All heifers in both experiments were inseminated by the same technician with frozen-thawed, sex-selected semen from sires available commercially. Pregnancy was diagnosed by ultrasound 27 d after AI. In Expt 1, the percentage of heifers detected in estrus was 31, 28 and 27% for Control, GnRH56 and GnRH72 groups. Overall, P/AI was greater (P=0.04) for GnRH72 (63%) than Control (55%), and intermediate for GnRH56 (59%) group. There was an interaction between estrus expression and treatment group; in heifers that expressed estrus, P/AI was greater (P=0.02) in GnRH56 (64%) and GnRH72 (65%) groups compared to Control group (43%). In Expt 2, 36% of heifers were AI at 56 h and P/AI was 63%. More heifers (P<0.01) in the TAI72 were given GnRH at TAI compared to TAI80 (40 vs. 22%), but P/AI did not differ between groups (68 vs. 66%). In summary, breeding heifers based on detected estrus increased P/AI with sex-selected semen. Administration of GnRH before TAI or delaying TAI did not increase P/AI, but delaying TAI reduced the number of heifers treated with GnRH. Estrus detection patches were considered useful to identify animals exhibiting estrus before TAI, increasing P/AI with sex-selected semen and reducing hormone usage. Authors thank Vetoquinol N.-A Inc. (Lavaltrie, QC, Canada) and Rockway Inc. (Spring Valley, WI, USA) for their in-kind support and Breevliet Ltd (Wetaskiwin, Alberta, Canada) for cooperation during the study.



098 Assisted Reproductive Technologies

Color Doppler ultrasonography for early pregnancy diagnosis in goats

I.O. Cosentino^{*1}, M.F.A. Balaro¹, F.S.C. Leal², A.L.C. Bade¹, L.R. Côrtes¹, E.K.N Arashiro¹, J.F. Fonseca³, F.Z. Brandão¹

¹Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil; ²Dairy goat farm Capril Vale das Amalthéias, Sapucaia, Rio de Janeiro, Brazil; ³Embrapa Caprinos e Ovinos, Coronel Pacheco, Minas Gerais, Brazil.

Subjective luteal blood flow analysis by color Doppler ultrasonography (US) was previously demonstrated in cows and sheep as an early and accurate method for pregnancy diagnosis. This study aimed to establish the best day for such diagnosis in dairy goats. 131 Saanen does 2.0±0.5 years old were used. In the first study, after a hormonal protocol for induction of synchronous estrus and AI, 60 goats were evaluated from Day 15 to Day 23 of the estrous cycle (Day 1 or D1 = ovulation day), by a subjective Color Doppler US assessment (score 1-4, where score 1 means no pregnancy and, score 2-4 means positive pregnancy) using portable equipment (Sonoscape S6, Shenzhen, China) with a 7.5 MHz linear rectal transducer. In the second study, 71 does received the same protocol and had the ultrasound exam performed at Day 21 (the best day detected in the first study) for luteal blood flow assessment. In both studies, B-Mode US at Day 30 confirmed pregnancy diagnosis (gold standard). The performance of the subjective luteal blood flow analysis and its agreement with the gold standard outcome in both studies was classified calculating Sensitivity (SEN), Specificity (SPEC), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Kappa index (K). In study 1, pregnancy diagnosis by subjective luteal assessment by Color Doppler US was not feasible at D15 and D16 (SEN 100%; SPEC 0%; PPV 49%; NPV not calculable, and K = 0) as all CL were considered viable (vascularization score was = 2) and consequently all animals were diagnosed as pregnant. From D17 to D21, the overall performance of the technique progressively increased (D17: SEN 96%; SPEC 4%; PPV 49%; NPV 50%, and K = 0.01; D18: SEN 100%; SPEC 12%; PPV 52%; NPV 100%, and K = 0.11; D19: SEN 100%; SPEC 42%; PPV 62%; NPV 100%, and K = 0.42; D20: SEN 100%; SPEC 73%; PPV 78%; NPV 100%, and K = 0.73). Results did not change from D21 to D23 (SEN 100%; SPEC 92%; PPV 93%; NPV 100%, and K = 0.92). Two animals diagnosed as non-pregnant on Day 30 had a well vascularized CL until Day 23. On D17, a doe diagnosed as pregnant on Day 30 had the CL scored as 1, even though it was evaluated as score = 2 on the following days. In study 2, the assessment presented a similar pattern of sensibility and specificity observed in study 1 (SEN 100%; SPEC 93%; PPV 91%; NPV 100%, and K = 0.92). The results showed that subjective luteal vascularization assessment by color Doppler US was a reliable tool for early pregnancy diagnosis in goats and can be efficiently used as early as 21 days post-breeding. Universidade Federal Fluminense, Infra-LabPesq/PROPPI, FAPERJ and the dairy goat farm Capril Vale das Amalthéias.



099 Assisted Reproductive Technologies

Effect of a single bST administration on follicular dynamics and ovulation during an interovulatory cycle in sheep

J.F. Cox*¹, F. Navarrete¹, A. Carrasco¹, J. Dorado², F. Saravia¹

¹Universidad de Concepción, Chillán, Chile; ²Universidad de Córdoba, Córdoba, España.

Insulin and IGF1 are major peripheral signals from the metabolic axis, and their plasma levels are seen as important for sound follicular function and fertility due to their ability to synergize with gonadotropins during terminal follicular development. Bovine somatotropin (bST) increases plasma IGF1 and insulin in sheep (Carrera-Chávez *et al.*, 2014. Anim Reprod Sci. doi.org/10.1016/j.anireprosci.2014.10.009) and was used in this study to assess its influence on follicular dynamics and ovulation as markers of ovarian function. The study used Highlander ewes housed in collective pens linked to a paddock. In Exp 1, 15 ewes were estrous-synchronized (P₄-6 days + PGF2 α d -6) and then divided into bST-treated (50 and 100 mg, Lactotropin[®]; n=5 ewes each) and untreated groups to assess the activity of bST through plasma IGF1 (RIA). In Exp 2, 12 ewes were synchronized and at d 6, they were grouped into a bST-treated (100 mg) and an untreated control (n=6 each) group. Starting at d 6 and up to 22 d after ovulation, each ewe was subjected to daily US (10mHz probe) to assess follicular and luteal (CL) dynamics and ovulation. The ultrasound included general ovarian features (interovulatory interval, ovulatory follicle and CL diameters and ovulation rate) and specific follicular wave features (number and duration of waves, growth rate, persistency and large follicle diameter). In Exp 3, the effect of bST was assessed under the restriction of anestrus, with estrus-synchronized ewes allocated to a bST group (100 mg at the start of the treatment), an eCG group (400 IU at the end of synchronization, d0) and a control group (n=15 each), the last two exposed to a male effect. At 36 h after d 0, all ewes were induced to ovulate with GnRH (4.2 μ g busserelin). Plasma estradiol was measured at d 0 and at GnRH treatment from 6 ewes in each group (RIA). Results showed that bST increased plasma IGF1 compared to controls by day 3 (P<0.01) and kept levels for at least 7 days before recovering pre-treatment levels. The IGF1 increase after bST doses was similar in terms of a day-to-day and AUC comparisons (P>0.10). In Exp 2, results showed that bST-treated ewes preserve all general and specific markers considered to monitor ovarian function in the study (P>0.10). However, in Exp 3, results showed that bST preserved the number, diameter and ovulatory potential of large follicles after synchronization, but reduced the estradiol production in term of mean plasma profiles (P=0.03) and in terms of ewes producing >10 pg/mL at GnRH administration (P=0.04), and also reduced the CL diameter at day 10 compared to eCG-treated ewes (P=0.003). Collective results in the study suggest that ewes respond to bST administration, and at high dosage it has no influence on follicular dynamics and ovulation during the reproductive season, but due to its ability to reduce E₂ production, bST may compromise ovarian function during the anestrus season.



100 Assisted Reproductive Technologies

Progesterone priming during follicular growth of Wave 1 improves pregnancy rate after FTAI in sheep

F. Cuadro^{*1}, R. Casali², J.M. Guillen-Muñoz^{3,1}, P.C. dos Santos Neto¹, N. Secco¹, A. Pinczak¹, A. Menchaca¹

¹Instituto de Reproducción Animal Uruguay, Fundación IRAUy, Montevideo, Uruguay; ²Universidade do Estado de Santa Catarina - UDESC, Centro de Ciências Agroveterinárias, Lages, Santa Catarina, Brasil;

³Universidad Autónoma Agraria Antonio Narro-Unidad Laguna, Torreón, Coahuila, Mexico.

The objective was to evaluate the effect of a short progesterone (P4) supplementation during preovulatory follicular growth of Wave 1 on pregnancy rate after fixed-time artificial insemination (FTAI) in ewes. The experiment was conducted during breeding season (33° S, Uruguay) on 804 multiparous cycling ewes that received three doses of prostaglandin (PG) F2 α analogue (125 μ g of cloprostenol, Ciclase DL, Zoetis, Argentina) im 7 d apart. This treatment (so-called Synchrovine protocol; Menchaca and Rubianes. 2004. New treatments associated with Timed Artificial Insemination in small ruminants. *Reprod Fertil Dev*, 16:403-414) is effective to synchronize the ovulation on average 60 h after the second PG treatment (ovulation occurs in a narrow window of 24 h). The Day 0 was defined at the time of the second PG dose, and 72 h later (i.e., after expected ovulation). The ewes were allocated into two experimental groups to receive (n=409) or not (n=395) a P4 treatment from Day 3 to Day 7 (i.e., in the early luteal phase during follicular growth of Wave 1). Progesterone was administered by using an intravaginal P4 device (0.3 g, DICO, Syntex, Argentina). This experimental model was recently validated in our laboratory to compare females with high vs. low serum P4 concentrations (~5.0 vs. 1.5 ng/mL, respectively; P<0.05; Cuadro et al., 2018. Serum progesterone concentrations during FSH superstimulation of the first follicular wave affect embryo development in sheep. *Theriogenology*; submitted). In both groups, all females received a third PG treatment on Day 7 to synchronize ovulation. Half of the flock with or without P4 treatment received cervical (n=387) or intrauterine (n=417) FTAI in a 2x2 factorial design, performed 48 h or 54 h after the last PG respectively, with 150 or 70 millions of spermatozoa, respectively. Pregnancy diagnosis was determined by transrectal ultrasonography 35 d after insemination. Statistical analysis was performed by GLMM. Pregnancy rate was improved by P4 supplementation (45.5%, 186/409 vs. 37.0%, 146/395; P4 vs. no P4 treated ewes, respectively; P<0.05) and by intrauterine insemination (45.1%, 188/417 vs. 37.2%, 144/387; intrauterine vs. cervical FTAI, respectively; P<0.05), without interaction between treatment and insemination method (P=NS). The number of fetuses/pregnant ewes was not affected by the P4 treatment and insemination method (P=NS). In conclusion, a short priming with P4 during the preovulatory follicular growth of Wave 1 increases pregnancy rate in sheep.



101 Assisted Reproductive Technologies

Effect of pre-maturation culture using EGFR kinase inhibitor on embryo development, lipid metabolism and gene expression profile

P.C. Dall'Acqua^{*1,2}, G.B. Nunes^{1,2}, C.R. Silva¹, P.K. Fontes³, M.F.G. Nogueira⁴, G.Z. Mingoti^{1,2}

¹São Paulo State University (Unesp), School of Veterinary Medicine, Laboratory of Reproductive Physiology, Araçatuba, SP, Brazil; ²São Paulo State University (Unesp), Graduate Program in Veterinary Medicine, School of Agrarian and Veterinary Sciences, Jaboticabal, SP, Brazil; ³São Paulo State University (Unesp), Institute of Biosciences, Botucatu, SP, Brazil; ⁴São Paulo State University (Unesp), School of Sciences, Humanities and Languages, Assis, SP, Brazil.

The epidermal growth factor receptor (EGFR) pathway is directly involved in oocyte meiotic resumption induced by a gonadotropic stimulus. Here, we used an EGFR kinase inhibitor (AG1478) to inhibit meiosis resumption in bovine oocytes and assessed the competence of such oocytes for embryonic development, lipid metabolism and gene expression. COCs (n=926) were pre-matured (PM) during 8h in TCM-199 with 1 μ M AG1478 (AG group). Next, COCs were washed for meiotic inhibitor removal and cultured for 22h in IVM medium (TCM-199 with bicarbonate, 0.5 mg/mL FSH, 100 IU/mL hCG, and 10% FCS). The control group (C group) was only cultured for 22h in IVM medium. After that, the matured oocytes were fertilized and cultured until day 7 or 9 to evaluate blastocyst and hatching rate, respectively. On day 7 of culture, expanded blastocysts (n=32) were collected and the abundance of 86 transcripts was assessed by RT-qPCR using a microfluidic platform (BioMark HD SystemTM, Fluidigm[®]). Relative mRNA abundance was calculated by Δ Ct (target genes were normalized by two reference genes: *ACTB* and *HPRT1*). Total cell number and neutral lipid content was evaluated on day 7 expanded blastocysts (n=31) and on day 9 hatched blastocysts (n=63), by Nile red and DAPI stain, respectively. Data with normal distribution were analyzed by t test, and non-parametric data were analyzed by Mann Whitney's test ($P < 0.05$). Blastocysts rate on day 7 (40.8%, averaged) and hatching rate on day 9 (77.4%, averaged) were unaffected by treatment ($P > 0.05$). Similarly, treatment did not affect ($P > 0.05$) the total cell number on day 7 (C 121.5 \pm 7.1 and AG 116.6 \pm 6.3) and on day 9 (C 198.9 \pm 9.04 and AG 180.1 \pm 6.9). Abundance of several transcripts was up-regulated ($P < 0.05$) in AG group, including genes related to embryo development and quality (*NANOG* and *RPLP0*), epigenetic regulation (*H2AFZ*), metabolism (*HMGCS1*), lipid metabolism (*ACSL1*, *GPAT3*, *FADS2*, *FASN* and *FDX1*), apoptosis (*BID*) and stress response (*GPX4* and *HIF1A*). Arbitrary lipid content in pixels did not differ between treatments ($P > 0.05$) on day 7- (C 1.1 \pm 0.1 and AG 0.9 \pm 0.1) and on day 9-embryos (C 0.3 \pm 0.0 and AG 0.4 \pm 0.0). Our results indicate that PM culture with an EGFR kinase inhibitor prior to IVM does not affect the subsequent embryonic development until blastocyst stage and hatching rate besides the increase of mRNAs related to embryo development and quality. The up-regulation of transcripts related to lipid metabolism did not reflect in an increase on embryonic lipid content. Also, the gene expression pattern suggests better embryonic stress resistance, but we still need to investigate the impact of these changes on conception rate and pregnancy development to term. In conclusion, the PM culture with AG1478 was not detrimental to embryo development and lipid metabolism. Financial support: FAPESP (#2015/06733-5 and #2012/50533-2) and CNPq (#307416/2015-1).



Autologous nuclear transfer in cattle does not increase cloning efficiency

T. Delaney-Cooper, J. Oliver, B. Oback, D. Wells*

AgResearch, Hamilton, New Zealand.

Correct nuclear-cytoplasmic interactions are vital to generate viable offspring following somatic cell nuclear transfer (SCNT). In standard (allogenic) SCNT, donor nuclei are introduced into foreign oocyte cytoplasm harbouring mitochondrial (mt)DNA from genetically unrelated animals. The two sources of mtDNA can result in clones displaying heteroplasmy, which is contrary to the unimaternal inheritance of mtDNA during mammalian sexual reproduction. Oocytes from specific maternal lines (Brüggerhoff *et al.* 2002, *Biol. Reprod.* 66:367) or with the same mitochondrial haplotype as the donor cell (Yan *et al.* 2010, *BMC Dev. Biol.* 10:31) are reported to be beneficial for development of SCNT embryos. To avoid any mismatch between nuclear and mitochondrial genomes, we investigated whether fusing cytoplasts and somatic cells from the same cow (autologous SCNT) could improve development. Cumulus oocyte complexes (COCs) were aspirated from pairs of ovaries from 16 commercially slaughtered cows, over six replicates. COCs were matured *in vitro* for 18-20h, keeping each cow separate. After maturation, metaphase II oocytes from each cow were allocated equally to either autologous or allogenic SCNT treatments and reconstructed using a zona-free methodology. Bovine follicular cells, collected from each cow during oocyte aspiration, were cultured in medium with 0.5% fetal calf serum for 18h before autologous SCNT. For allogenic SCNT, a proven ovarian follicular donor cell line from an unrelated cow was used, also after 18h serum starvation. Reconstructed embryos were cultured in modified synthetic oviduct fluid media for seven days. Selected SCNT embryos, plus *in vitro* fertilised (IVF) controls, were transferred singularly to synchronized recipients and pregnancy monitored until fetal recovery on Day 117-118 of gestation. Values are presented as mean \pm SEM. Statistical significance was determined using Fisher's exact test for both *in vitro* embryo development (expressed as a percentage of embryos cultured) and *in vivo* survival. On average, 40 \pm 4 COCs were recovered per cow. There was no difference in the rate of donor cell fusion between the two treatments (92 \pm 2%). Rates of cleavage were also similar (92 \pm 3% *v.* 94 \pm 2% for autologous and allogenic SCNT, respectively). However, the rate of *in vitro* development to transferable quality (grade 1-2) late morulae to expanded blastocyst-stage embryos on Day 7 following autologous SCNT was lower than for allogenic SCNT (53/223 = 24 \pm 4% *v.* 74/211 = 33 \pm 4%; P=0.05). *In vivo* survival on Day 30 of gestation was similar between autologous and allogenic SCNT (3/8 = 38% *v.* 2/9 = 22%) and tended to be less than IVF (5/9 = 56%). Losses by Day 117-118 were greater in the autologous and allogenic SCNT groups compared to IVF, with embryo survival being 13%, 11% and 44% respectively. Average fetal weight for SCNT fetuses was similar to IVF (698 \pm 6g *v.* 768 \pm 39g). In conclusion, our finding that autologous SCNT results in lower development compared to standard allogenic SCNT is contrary to previous reports (Yang *et al.* 2006, *Reprod.* 132:733). Nonetheless, specific nuclear-mtDNA haplotypes might be beneficial for embryo development and subsequent health and phenotypic performance of clones. Supported by MBIE, NZ.



103 Assisted Reproductive Technologies

Flow cytometry analysis of the mitochondrial membrane potential using two incubation times of JC1 probe in fresh bovine semen

E.A. Diaz-Miranda*, P.P. Maitan, T.P. Machado, J.M. Penitente-Filho, D.M. Araújo Lima, B.S. Camilo, V.E. Gomez-León, J.D. Guimarães

Department of Veterinary, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

Sperm mitochondria is located in the midpiece and shows different characteristics from somatic mitochondria. In addition, it plays an important role in sperm function including ATP production, maturation, capacitation, and apoptosis. Furthermore, mitochondria are key structures in sperm function suffering major alterations during the cryopreservation process. Mitochondrial membrane potential (MMP) is a fertility marker that can be assessed by the use of the 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1), a lipophilic cationic probe that exhibits the MMP depending on its accumulation, showing a green fluorescence in the inactive or depolarized mitochondria ($\Delta\Psi_m < 80-100$ mV), and an orange fluorescence when mitochondria exhibit high membrane potential. Despite the fact that MMP has become a routine analysis, remarkable variability in the results have been reported when used different times of incubation. We hypothesized that MMP would be a better marker of fertility after a shorter incubation period. The aim of this study was to evaluate differences in MMP between a 15 or 30 min incubation protocol of JC1 using fresh bovine semen. The MMP was assessed by using JC-1 dye (T4069, Sigma-Aldrich, Saint Louis, MO, EUA; excitation, 488 nm; emission, monomers 525–530 nm and aggregates 590 nm). The semen was collected by electroejaculation from 3 bulls in 5 ejaculates each, the bulls were classified as satisfactory potential breeders according to the standards of the Brazilian College of Animal Reproduction (CBRA). After collection, 3 μ L of JC-1 (153 μ M) was added to 500 μ L of sperm diluted in PBS 0.1 M (5×10^6 spermatozoa/mL) and incubated for 15 or 30 min at 37°C. The samples were analyzed by flow cytometer (BD FACSVerserTM - Beckton-Dickinson[®], SunnyVale, CA, USA). Data were analyzed using linear mixed models with repeated measures in time, and bulls were considered as random effect. Correlations among variables were evaluated by Pearson's simple correlation coefficient. The MMP percentage was higher ($P < 0.05$) in 15 than 30 min of incubation time (49.1 ± 2.1 vs 40.3 ± 2.1 , respectively). The average sperm motility was $73.1 \pm 2.9\%$; with minimum and maximum values of 55% and 90%, respectively. The MMP evaluated in both times were highly correlated between them ($r = 0.90$). However, there was no correlation between sperm motility and the MMP. Thus, the decrease of MMP in the 30 min protocol could be explained by the longer incubation time. Therefore, 15 min may be a more suitable incubation period for a MMP analysis in bovine fresh semen. This study was funded by CNPq, CAPES, and FAPEMIG.



Follicular emergence in zebu cows actively immunized against GnRH

O. Faria*¹, J.H.M. Viana²

¹Universidade de Brasília, Brasília, DF, Brasil; ²Embrapa, Brasília, DF, Brasil.

Cystic ovarian disease (COD) is a frequent problem in cows intensively used as oocyte donors for in vitro embryo production (IVEP) (Faria *et al.* 2017 Anim Reprod 14:782). Many of these animals fail to respond to conventional treatments with LH or GnRH analogs, and oocyte yield is compromised. Previous studies demonstrated that active immunization against GnRH resulted in remission of COD, without affecting oocyte development potential in vitro (Faria *et al.* 2018 Reprod Fertil Develop 30:190). The aim of the present study was to characterize the follicular dynamics in cows immunized against GnRH. Nelore (*Bos indicus*) cows were assigned to control (n=5) or treatment (n=8) groups. Cows in the treatment group received two SC injections of 1.0 mL anti-GnRH vaccine (Bopriva, Zoetis, Brazil), 28 d apart (weeks 0 and 4). Effectiveness of immunization (E-IM) was confirmed when no follicles ≥ 5.0 mm were detected on the ovaries, only cows with E-IM were used (n=5). The control group underwent a conventional follicular wave synchronization protocol, based on the insertion of 1 g intravaginal progesterone device and injection of 2 mg estradiol benzoate. Both groups were submitted to transvaginal ultrasound-guided follicle aspiration (OPU), aiming to remove all follicles larger than 2 mm after E-IM (treatment group) or 5 d after follicular wave synchronization (control group). Transrectal ultrasonography was performed daily to evaluate the number and size of follicles emerging after OPU. SAS MIXED procedure with repeated measure statement was used to evaluate the effects of treatment, time, and interactions on ovarian endpoints; and SAS GLM procedure to analyze follicular dynamics data. Results are shown as mean \pm SEM. There was no difference in the follicle population 96 h after OPU between treatment and control groups (10.8 \pm 2.8 vs 13.8 \pm 1.5, respectively; $P>0.05$). However, only in the control group the number of follicles reached a plateau later during the evaluation period (3.3 \pm 1.0^a, 9.0 \pm 1.4^{ab}, 15.0 \pm 3.0^b, and 13.8 \pm 1.5^b follicles at 24, 48, 72 and 96 h, respectively; $P<0.01$). In the E-IM cows, no follicle grew over 4 mm, so the diameter of the largest follicle differed between groups from 48 h on (3.1 \pm 0.1 vs 5.0 \pm 0.4mm, 3.0 \pm 0.0 vs 6.8 \pm 0.8mm, and 3.0 \pm 0.0 vs 8.5 \pm 0.2 mm at 48, 72, and 96 h after OPU for treatment and control groups, respectively). There was no difference ($P>0.05$) in the size of the largest and second largest follicles in the treatment group throughout the evaluation period, while in the control group follicle deviation began at 72 h, when the further dominant and the second largest follicles were 6.8 \pm 0.8 and 5.5 \pm 0.4 mm in diameter, respectively. In summary, the present results suggest that the lack of physiological FSH stimulus induced by immunization against GnRH does not impair, but delay follicle emergence. The lack of follicular dominance and the slower emergence may partially overcome the expected negative effects of the lack of physiological FSH support in immunized cows, and explain the maintenance of oocyte development potential and the good results of IVEP observed in previously studies (Faria *et al.*, 2018). Zoetis, UNB, FAP-DF and CAPES.



105 Assisted Reproductive Technologies

Effects of sire breed and season on in vitro embryo production and subsequent pregnancy rates

L.F.R. Féres¹, L.L. Santos², L.S.A. Camargo³, J.H.M. Viana*⁴

¹Unifenas, Universidade José do Rosário Vellano, Alfenas, MG, Brazil; ²Centro Universitário Unifaminas, Muriaé, MG, Brazil; ³Embrapa Gado de Leite, Juiz de Fora, MG, Brazil, ⁴Embrapa Cenargen, Brasília, DF, Brazil.

Climatic factors are known to influence reproductive efficiency in cattle. Thus, seasonality shall be considered in the development of breeding strategies to increase the reproductive performance of herds. The aim of this study was to analyze the influence of the season and sire breed in oocyte cleavage rate, blastocyst production and pregnancy rates. These traits were retrospectively analyzed using data from in vitro embryo production (IVEP) performed after OPU (n=3,371) sessions in Girbreed (*Bos indicus*, n=805) donors. The cumulus-oocyte complexes were in vitro matured and then in vitro fertilized with semen of Gir (n=27) or Holstein (n=52) sires in order to produce purebred Gir or F1 (Gir x Holstein) embryos. Data was analyzed by Kruskal-Wallis test followed by Dunn test. The effects of season and sire's breed were considered in the analysis. The highest overall cleavage rate (P<0.05) was found during the Spring (60.45±0.76%) and the lowest during the Summer (55.89±0.65%). Higher blastocyst production (P<0.05) was found during Spring (25.4±0.7%) than Summer (22.0±0.56%) and Autumn (21.8±0.55%). Fertilization with Holstein semen increased the cleavage rate (58.7±0.47%) compared to Gir semen (56.4±0.55%) but no difference (P>0.05) was found for blastocysts production. There was a season x sire breed interaction. Cleavage rate was higher in Winter for F1 embryos than for purebred embryos (61.53±1.08% vs 55.23±1.3%; P<0.05). No difference (P>0.05) between sire's breed was found for the other seasons. Cleavage rate was lower (P<0.05) during Summer and Autumn than during Winter (57.2±1.05% and 57.42±0.81% vs 61.53±1.08%, respectively; P<0.05) when fertilization was performed with Holstein semen. No difference (P>0.05) among seasons was found when fertilization was performed with Gir semen. There was no difference (P<0.05) between sire's breed on blastocyst production within seasons; however, lower (P<0.05) blastocyst rate was found during Summer with Gir semen (22.11±0.075%) and during Autumn with Holstein semen (21.23±0.71%) than during Spring (26.45±1.3% and 24.9±0.85% for Gir and Holstein semen, respectively). The pregnancy rate of embryos fertilized with Holstein semen (48.56±1.87%) was higher (P<0.05) than those fertilized with Gir semen (38.8±2.01%) during the Winter but no difference between sire's breed (P>0.05) was found in the other seasons. Pregnancy rate with Gir semen was lower during Winter than during Summer and Autumn (41.79±1.74% vs 44.4±2.13%, respectively; P<0.05), whereas no difference (P>0.05) between seasons was found with Holstein semen. In conclusion, the effect of season on oocyte competence and further in vitro embryo development and pregnancy rate can be influenced by sire's breed when oocytes are derived from Gir donors. Summer and Autumn are the worst season to produce blastocyst with Gir and Holstein semen, respectively. Interestingly, low pregnancy rate is achieved during Winter with embryos fertilized with Gir semen, even lower than embryos with Holstein semen. The understanding of such differences can be useful to reduce the effect of seasons on in vitro embryo production. Fazendas do Basa, Embrapa, FAPEMIG.



106 Assisted Reproductive Technologies

Ultrasonographic cervical evaluation in Lacaune ewes subjected to transcervical embryo collection

L. Figueira^{*1,2}, N. Alves¹, J. Souza-Fabjan², R. Batista^{2,3}, L. Souza⁴, Y. Diógenes⁵, V. Brair^{2,6}, G. Souza², J.F. Fonseca⁷

¹Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; ²Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil; ³UFVJM, Diamantina, Minas Gerais, Brazil; ⁴Cabanha Val di Fiemme, Soledade de Minas, Minas Gerais, Brazil; ⁵Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil; ⁶UNIGRANRIO, Rio de Janeiro, Rio de Janeiro, Brazil; ⁷Embrapa Caprinos e Ovinos, Coronel Pacheco, Minas Gerais, Brazil.

The degree of completeness and interdigitation of the cervical rings affects cervical passage, which is a necessary step for transcervical embryo collection. This study assessed the use of cervical ultrasonography (US) to select the most suitable animals for transcervical embryo collections. Lacaune ewes (n=24) were synchronous estrus-induced with medroxyprogesterone acetate sponges (60 mg, Progespon[®], Syntex, Buenos Aires, Argentina) for nine (n=12) or six days (n=12), 37.5 µg d-cloprostenol i.m. (Prolise[®], Tecnopec, São Paulo, Brazil) and 400 IU eCG i.m. (Novormon 5000[®], Syntex) 24 h before device removal. After sponge removal, ewes were checked twice daily for estrus detection. At 12 h after the onset of estrus, the cervix was longitudinally evaluated by transrectal US (8.0 MHz, Mindray M5VET[®], Shenzhen, China), to count the number and disposition of rings to classify the degree of misalignment in three scores: grade I – lined up, grade II – intermediate, and grade III – misaligned (Fonseca, CT 45, Embrapa Caprinos e Ovinos, 2017). Cervical passage was performed at day 7 of estrus cycle by transcervical technique (Fonseca *et al.*, Theriogenology, 86:144-151, 2016). Cervical transposition rate was tested by Fisher's Exact test, and the number of rings counted by US or during cervical passage were evaluated by ANOVA and paired t-test at 5% significance, using SPSS Statistics (IBM[®] Inc., Chicago, USA). Association of variables was evaluated by Pearson correlation. The percentage of ewes for each degree of cervical score on US during estrus was: grade I 20.8% (5/24), grade II 20.8% (5/24) and grade III 54.5% (14/24). Cervical surpass was possible in 100%(5/5) of grade I, 80%(4/5) of grade II and 79.5% (11/14) of grade III (P<0.05). The number of rings counted during procedure (6.5±0.2) or by US (6.1±0.1) did not differ (P>0.05). However, the association of these variables was poorly correlated (r=0.20, P>0.05); in 20% the count in US was overestimated and 35% underestimated related to count in procedure. In 45% of ewes, the number of rings was the same in both by US and the procedure, and in 85%, the difference in the number did not exceed one ring. In conclusion, US cervical evaluation allows estimating the number of rings, and the ranking of animals by US cervical misalignment, making possible to select animals suitable for transcervical embryo collection. Financial Support: EMBRAPA (Project SUPEROV / 02.13.06.026.00.04). CAPES and Vicente M. Munhoz (Cabanha Val di Fiemme).



Efficient transcervical embryo collection in synchronous estrus-induced Lacaune ewes

L. Figueira^{1,2}, J. Souza-Fabjan², R. Batista^{2,3}, L. Souza⁴, Y. Diógenes⁵, M. Filgueiras⁶, G. Souza², N. Alves², J.F. Fonseca^{*7}

¹Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil; ²Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil; ³UFVJM, Diamantina, Minas Gerais, Brazil; ⁴Cabanha Val di Fiemme, Soledade de Minas, Minas Gerais, Brazil; ⁵Universidade Estadual do Ceará, Fortaleza, Ceará, Brazil; ⁶UNIGRANRIO, Rio de Janeiro, Rio de Janeiro, Brazil; ⁷Embrapa Caprinos e Ovinos, Coronel Pacheco, Minas Gerais, Brazil.

Induction of cervical dilation allows efficient non-surgical embryo recovery, depending on the sheep breed, and thus its usefulness needs to be assessed in each breed. This study checked the efficiency of two estrus induction treatments and the feasibility of transcervical embryo recovery in Lacaune ewes. Ewes (n=28) received medroxyprogesterone acetate sponges (60 mg, Progespon[®], Syntex, Buenos Aires, Argentina) for nine (G9, n=14) or six (G6, n=14) days. Both groups received 37.5 µg d-cloprostenol i.m. (Prolise[®], Tecnopec, São Paulo, Brazil) and 400 IU eCG i.m. (Novormon 5000[®], Syntex) 24 h before sponge removal. Ewes were checked for estrus twice daily and were naturally mated by fertile rams (6:1 ratio) while in estrus. Ultrasonography monitoring (Mindray M5VET[®], Shenzhen, China - 8.0 MHz) was performed twice daily from sponge removal to ovulation detection and 5 days after to count the number of corpora lutea (CL). Ewes received 37.5 µg d-cloprostenol and 1 mg estradiol benzoate i.m. (Sincrodiol[®], OuroFino, Cravinhos, Brazil) 16h before plus 50 IU oxytocin (Ocitocina forte UCB[®], São Paulo, Brasil) i.v. 20 min before uterine flushing. Embryo collection was performed at day 6 after ovulation by transcervical technique (Fonseca et al., *Theriogenology*, 86:144-151, 2016). Qualitative data were tested by chi-square test, and quantitative data were evaluated by ANOVA and t-test at 5% significance, using SPSS Statistics (IBM[®] Inc., Chicago, USA). Estrous behavior rate was 85.7% for both treatments. There was no difference (P>0.05) for interval to estrus and estrus duration, respectively for G9 (37.0 ± 2.3 and 22.0 ± 2.9 h) vs G6 (42.0 ± 7.0 and 25 ± 2.3 h). Ovulation rate was 100% (G9) and 92.9% (G6) (P>0.05). The number of CL was higher (P<0.05) in G9 (2.9 ± 0.3) than G6 (1.9 ± 0.3). Overall, cervical transposition was possible in 85.7% (24/28) of ewes and 78.6% (22/28) were successfully collected for embryos. The time to cervical passage was lower (P<0.05) in G9 (3.6 ± 0.5) than G6 (6.4 ± 1.2 min). The total procedure time in G9 (23.8 ± 1.8) and G6 (26.5 ± 2.5 min) did not differ (P>0.05). In G9 ewes, the number of recovered structures was higher (1.6 ± 0.4 vs 0.6 ± 0.2; P<0.05) and there was a tendency (P<0.10) to recover more viable embryos (1.2 ± 0.4 vs 0.4 ± 0.2). Possibly, eCG dosage promoted multiple ovulations (> 2) in 50% of the ewes that ovulated, and two ewes had five ovulations. This is the first report in the literature of transcervical embryo collection in the Lacaune breed. In conclusion, both estrous induction treatments showed a high rate of estrus and ovulation, but the dosage of 400 IU of eCG seems excessive. The protocol for cervical relaxation was efficient to allow the transcervical embryo recovery of Lacaune ewes. CAPES and Vicente M. Munhoz (Cabanha Val di Fiemme). Financial support: EMBRAPA (Project 02.08.02.005.00.04) and Fapemig (Project CVZ-PPM 00201-17).



Equine chorionic gonadotropin (eCG) impacts the transcriptional profile of genes involved with competence of *cumulus*-oocyte complexes and embryo quality in superstimulated Nelore cows

F.F. Franchi*¹, P.H. Santos¹, P.K. Fontes¹, E.M. Razza¹, E. Mareco², M.F.G. Nogueira³, A.C.S. Castilho²

¹Universidade Estadual Paulista, Botucatu, SP, Brasil; ²Universidade do Oeste Paulista, Presidente Prudente, SP, Brasil; ³Universidade Estadual Paulista, Assis, SP, Brasil.

The impact of superstimulatory protocols using gonadotropins such as FSH or eCG on competence of *cumulus*-oocyte complexes (COCs) and embryo quality remains unknown. Thus, this study aimed to quantify the mRNA abundance of genes related to quality of COCs and embryos from Nelore cows (*Bos taurus indicus*) submitted to ovarian superstimulation using FSH (FSH group, n=10) or replacement of the last two doses of FSH by eCG (FSH/eCG group, n=10). All animals were slaughtered 12 h after removal of the intrauterine progesterone device. Ovarian antral follicles from superstimulated groups (>9 mm) were aspirated for COC gene expression and *in vitro*-embryo production. Total RNA was extracted and reverse transcribed from each sample (n=3 samples/type); sample were pools of 20 oocytes, their respective *cumulus* cells and a pool of 5 blastocysts (BL). The mRNA abundance of 96 genes related to COC development and 96 genes related to embryo quality were measured by RT-qPCR using the microfluidic platform BioMark™ HD system (Fluidigm®) and the expression of target genes was normalized by geometric mean of three reference genes (*ACTB*, *GAPDH*, *PPIA*). Bioinformatic analysis was performed using R and Bioconductor software and the gene expression heatmaps were generated using the average of calibration samples to normalize each group. Genes and groups were sorted by hierarchical clustering using the “heatplot” function from the “made4” package. Heatmap analysis successfully clustered similarities and differences between groups and showed a pattern of expression among genes. Means were compared with Student’s t-test and differences were considered significant when P=0.05. In the oocytes from the FSH/eCG group, 36 genes involved with lipid metabolism (*ACACA*, *ACSL3*, *ACSL6*, *CPT1B*, *DGAT1*, *ELOVL5*, *SCD*), oxidative stress (*CAT*, *SOD1*, *TXNRD1*), transcriptional control (*DNMT1*, *DNMT3A*, *DNMT3B*, *HPI1*) and cellular development (*BMP15*, *GDF9*, *HIF1O*, *IMPDH1*, *IFG1R*, *COX2*) were up regulated. In *cumulus* cells, *CPT1B* and *VCAN* mRNA abundance was higher in the FSH/eCG group whereas *SREBF1-2*, *ADCY3*, *NDUFA1*, *GPX4* and *NPR3* demonstrated a lower abundance. In BLs, *CPT1B*, *SLC2A3* and *BID* was higher in the FSH/eCG group and *SREBF1-2* and *DDIT3* showed a lower abundance. In summary, the present findings suggest that use of FSH combined with eCG could increase the quality and competence of blastocysts and COCs by regulation of genes related to oxidative stress protection, oocyte competence, and especially by modulation of genes involved with lipid metabolism in COCs and bovine embryos. Supported by São Paulo Research Foundation (FAPESP), grants #2011/50593-2, 2012/50533-2, and 2013/11480-3.



109 Assisted Reproductive Technologies

Evidence that pregnancy-associated plasma protein A (PAPP-A) plays a role in bovine *in vitro*-embryo production

**A.B. Giroto*¹, J.V. Amoris¹, F.F. Franchi², P.K. Fontes², P.H. Santos², M.A. Maioli³,
G.P. Nogueira³, G.B. Nunes³, G.Z. Mingoti³, M.F.G. Nogueira⁴, A.C.S. Castilho¹**

¹Universidade do Oeste Paulista, Presidente Prudente, São Paulo, Brazil; ²Universidade Estadual Paulista, Botucatu, São Paulo, Brazil; ³Universidade Estadual Paulista, Araçatuba, São Paulo, Brazil; ⁴Universidade Estadual Paulista, Assis, São Paulo, Brazil.

The pregnancy-associated plasma protein A (PAPP-A) is able to disrupt IGF's association with IGF binding proteins (IGFBPs) to allow the increase of free IGF. The aim of the present work was to access the effects of PAPP-A during oocyte *in vitro* maturation (IVM) on IGF1 bioavailability, embryo yield, post vitrification survival and transcriptional profile of blastocysts (BLs). The cumulus-oocyte complexes (COCs) from a local abattoir were submitted to IVM for 24 h with TCM199, serum free medium supplemented with PAPP-A (100 ng/mL; P100 group) or not (P0 group). The maturation medium was collected for quantification of free IGF1 by ELISA. The matured COCs were submitted to *in vitro* fertilization followed by *in vitro* embryo culture for 7 d. On day 3 and 7, the cleavage and BL rates were evaluated. On day 7, BLs (3 pools/group; n=5 BLs/pool) were used to analyze the transcriptional pattern of 96 genes and to verify the post vitrification survival. The free IGF1 concentration was transformed to fold change and cleavage and BL rates were calculated as percentage and transformed to arcsine. The mRNA abundance of target genes was normalized by geometric mean of reference genes and data were transformed to fold change. Bioinformatic analysis was performed using R and Bioconductor softwares. Gene expression heatmaps were generated using the average of P0 group levels to normalize each group. Genes and groups were sorted by hierarchical clustering using the “heatplot” function from the “made4” package. The means were compared with t-test using JMP software (SAS Institute Cary, NC). Differences were considered significant when P=0.05. The PAPP-A addition increased by 1.27 fold the free IGF1 concentration in IVM medium but did not impact the cleavage rate or BL yield. Moreover, the rate of post vitrification survival did not differ (P>0.05) after 24 h and was 54% for the P0 group and 43% for the P100 group. The heatmap analysis was able to cluster similarities and differences between groups and also to show the pattern of expression between genes. In BLs, the mRNA abundance of *DMNT3A*, *CASP9*, *CPT2*, *TFAM* and *KRT8* were up regulated in the P100 group. On the other hand, *ATF4*, *IFITM3* and *CASP3* were down regulated in the P100 group. In conclusion, the addition of PAPP-A during IVM provided an increase of free IGF1, and although it did not influenced the BL rate or *in vitro* post vitrification survival, the greater amount of free IGF1 could impact positively the embryo competence by modulation of genes involved with apoptosis, mitochondrial biogenesis, DNA methylation, specially, embryonic lipid metabolism.



110 Assisted Reproductive Technologies

***In vitro* embryo production in Angus breed with semen sorted for male: effect of semen preparation method**

J.G.V. Grázia¹, A.C.N. Gois², C.A.G. Pellegrino³, A.P. Marques Júnior¹, L.G.B Siqueira⁴, J.H.M.Viana^{*5}

¹Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; ²Apoyar Biotech, Alta Floresta, Mato Grosso, Brazil; ³Prole Ltda., Belo Horizonte, Minas Gerais, Brazil; ⁴Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil; ⁵Embrapa Genetic Resources and Biotechnology, Brasília, Distrito Federal, Brazil.

In Brazil, *in vitro* embryo production (IVEP) has become the technique of choice in cattle to increase the number of offspring from high-value and genetically superior donors. However, animal breeding programs are a limited market for embryo transfer (ET) services. In the past 10 years, ET numbers in Brazil have increased mainly due to the use of IVEP in association with X-sorted sperm to supply crossbred heifers for dairy production. There is also a great potential for large-scale production of F1 calves in beef, however, there are few reports of the use of Y-sorted sperm for IVEP. In this regard, we hypothesized that semen preparation is a critical step for *in vitro* fertilization, as observed with X-sorted sperm. The aim of the present study was to evaluate the effect of semen preparation procedure on IVEP, using Y-sorted sperm from Angus sires. Cumulus-oocyte complexes were obtained from ovaries of Nelore cows at the slaughterhouse and *in vitro* matured in TCM199, following standard procedures. Matured COC were randomly allocated to be *in vitro* fertilized with a commercially available Y-sorted sperm from an Angus sire prepared either by: a) centrifugation in a Percoll gradient (n = 921 oocytes) or washing with IVF medium (n = 3,112 oocytes). After IVF, the presumed zygotes were cultured in SOF medium at 95% humidity at 38.8°C. A mixed system was used for *in vitro* embryo culture (IVC), in which the first 72 h used 5.5% CO₂, while the last 96 h of IVC used a defined atmosphere of 5.5% CO₂ and 5% O₂. Cleavage rate, embryo rate at days 7 and 8, and percentage of grade I embryos were analyzed using the Chi-square test. There was no effect of semen preparation method on cleavage rate or on the blastocyst rate and percentage of grade I embryos at D7 (52.0% vs 49.5%, 22.3% vs 20.5%, and 76.8% vs 75.1% for washing with IVF medium and Percoll, respectively; P>0.05). However, washing with IVF medium increased the blastocyst rate at day 8 and the overall embryo rate compared to Percoll (8.5% vs 6.4% and 25.6% vs 21.8%, respectively; P<0.05). The results show that the preparation method used for Y-sorted Angus sperm has significant effects on IVEP in beef cattle. Thus, large-scale IVEP programs for F1 production in beef cattle should optimize procedures, considering the particularities of each breed and kind of semen used. National Council for Scientific and Technological Development (CNPq) and FAPEMIG.



111 Assisted Reproductive Technologies

The effect of prostaglandin F_{2α} (PGF_{2α}) on cumulus expansion and glucose metabolism of *in vitro* matured bovine cumulus-oocyte complexes

K. Grycmacher*, D. Boruszewska, E. Sinderewicz, I. Kowalczyk-Zieba, J. Staszkiwicz-Chodor, I. Woclawek-Potocka

Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Olsztyn, Poland.

Cumulus cells play a pivotal role during oocyte maturation and accomplishment of its developmental competence. During development of bovine ovarian follicles, cumulus cells undergo expansion, which is regulated by various intracellular signaling cascades. It also depends on the synthesis of the extracellular matrix, the main component of which - hyaluronic acid - is synthesized from various compounds including glucosamine and glucose. Moreover, glucose is utilized for substrates that oocytes can then use for energy production. It was also shown that PGF_{2α} takes part in many reproductive processes. Taking all of the above into consideration, the aim of present study was to examine the role of PGF_{2α} in cumulus expansion, glucose metabolism and expression of genes involved in these processes. Cumulus-oocyte complexes were aspirated from subordinate follicles and matured *in vitro* in the presence or absence of PGF_{2α} (700 pg/mL) for 24 h. Following maturation, cumulus expansion was visually assessed and cumulus cells were used for gene expression analysis. The mRNA expression of amphiregulin (AREG), epiregulin (EREG), betacellulin (BTC), ADAM metalloproteinase domains (ADAMs), epidermal growth factor-like family receptor (EGFR), tumor necrosis factor alpha-induced protein 6 (TNFAIP6), prostaglandin-endoperoxide synthase 2 (PTGS2), pentraxin 3 (PTX3), hyaluronan synthase 2 (HAS2), glutaminefructose-6-phosphate transaminases (GFPTs), glucose transporters (GLUTs), phosphofructokinase (PFK), and lactate dehydrogenase (LDH) was determined by real-time PCR. The data were analyzed using student's t-test (GraphPad PRISM 6.0. Software). Cumulus expansion was similar in the control and PGF_{2α}-treated group. PGF_{2α} stimulated mRNA expression of EREG, EGFR, TNFAIP6, PTX3, GLUT4 and PFK and reduced transcript abundance of GLUT1 and LDH. The results suggest that PGF_{2α} supplementation of the maturation medium influences the expression patterns of genes associated with oocyte maturation. Moreover, we suspect that PGF_{2α} might facilitate oocyte maturation in cows. Supported by Grants-in-Aid for Scientific Research from the Polish National Science Centre: 2015/17/B/NZ9/01688 and the grant of KNOW Consortium "Healthy Animal - Safe Food", MS&HE Decision No. 05-1/KNOW2/2015.



112 Assisted Reproductive Technologies

Influence of parity category on conception rate of Holstein females submitted to timed artificial insemination in semiarid conditions

S.I. Guido*¹, F.C.L. Guido², L.F. Alencar¹, P.R.L. Azevedo¹, J.C.V. Oliveira¹, A.S. Santos Filho¹, J. Evêncio Neto²,

¹Agronomic Institute of Pernambuco, IPA, São Bento do Una, Pernambuco, Brazil; ²Federal Rural University of Pernambuco, UFRPE, Recife, Pernambuco, Brazil.

This study was conducted at the Experimental Station of São Bento do Una (ESSBU/IPA) Pernambuco, Brazil, (Latitude 08 31' 35'' and Longitude 036 27' 34.8''), with the objective to evaluate the influence of category on conception rate of timed artificial insemination (TAI) in Holstein females in semiarid conditions, presenting in the period, mean temperature-humidity index (THI) of 63.5. For the study, 368 cyclic females were used, up to 60 days in milk (DIM), production with an average of 24.8 kg/milk/day, and age ranging from 28 to 108 months for primiparous and pluriparous. The nulliparous females were cyclic at 12 months of age. The animals were kept in semi-intensive system, receiving a diet composed of cactus pear (*Opuntia ficus-indica* Mill), sorghum silage (*Sorghum bicolor* (L) Moench) and protein concentrate with 18% of crude protein (CP) besides mineral supplement and water ad libitum. Females used were submitted to gynecological examination by rectal palpation, being randomly distributed into three groups G1 (nulliparous), G2 (primiparous) and G3 (pluriparous). All females of the three groups were treated with an intravaginal device containing 1.9g of progesterone on day 0 (D0) and 2mg of estradiol benzoate (EB). On day 7 (D7) females were treated with 0.530 mg of sodium cloprostenol and 300 IU eCG, and on day 8 (D8) intravaginal devices were removed. Twenty-four hours after device removal (D9), 1mg EB was given and 54 hours after device removal all cows were fixed-time AI at day 10 (D10) with semen from Holstein bulls of a reputable center. All females were subjected to ultrasound examination for pregnancy detection 30 days post-AI. The data were subjected to statistical analyzes carried out by means of computational SAS version 9.1.3. The conception variable was assumed to have a binomial distribution (P = pregnant; N = Not pregnant). Initially a descriptive analysis was performed to observe the percentages of successes and failures and the chi-square test was used to verify if there were significant differences through the FREQ procedure of SAS (2003). The conception rate was 50.2% in G1, 59.6% in G2 and 44.7% in G3, the result of G2 being superior (P<0.01) to the other groups. In conclusion, the primiparous presented better conception results with the TAI protocol used. CNPq/FINEP.



113 Assisted Reproductive Technologies

Effect of GnRH on day 5 or 7 on pregnancy rate in dairy cows after AI with sex-sorted sperm

C. Guzman², W. Huanca*¹, A. Pozo², W.F. Huanca¹

¹Laboratory of Animal Reproduction, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, San Borja, Lima, Peru; ²Veterinary Medicine School, Faculty of Agricultural Sciences, Ayacucho, Peru.

Reproductive performance in dairy cows after AI with sex-sorted sperm is poor. The aim of this study was to evaluate the effect of a single administration of buserelin acetate (AB), an analogue of the gonadotropin release hormone (GnRH), on pregnancy rate per AI with sex-sorted sperm in lactating dairy cows. A total of 162 cows were assigned to one of three treatments. T1 (n=54): Control; T2 (n=53): 0.05 mg AB on day 5 post AI; T3 (n=55): 0.05 mg AB on day 7 post AI. The same technician inseminated cows, 10 to 12 hours after estrus detection and with sex-sorted sperm (Select Sires) from three bulls using the rectal vaginal technique. The animals were observed twice per day for heat detection, and pregnancy diagnosis was done 30 days after AI with a portable ultrasound (ESAOTE, The Netherlands) and a 5.0 MHz transducer. Data were analyzed by Chi square. Pregnancy rate of 9.3%; 30.2% and 16.4% was observed in T1, T2 and T3, respectively, with a significant difference ($P < 0.05$) between T2 compared to T1 and T3 but there was no difference between T1 and T3. The results suggest that the use of GnRH on day 5 improved pregnancy per AI in dairy cows inseminated with sex-sorted sperm.



114 Assisted Reproductive Technologies

Impact of recombinant bovine somatotropin, progesterone, and estradiol benzoate on the ovarian follicular dynamics of *Bos taurus taurus* cows in protocols for synchronization of estrus and ovulation

A.P. Kaminski¹, M.L.A. Carvalho¹, M.S. Segui¹, L.E. Kozicki*¹, F.R. Gaievski¹, V.B. Pedrosa², R.R. Weiss³, T.G. Bergstein-Galan⁴

¹Pontificia Universidade Católica do Paraná, Curitiba, Paraná, Brazil; ²State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil; ³Federal University of Paraná, Curitiba, Paraná, Brazil; ⁴Alamos Genetics Flushing Station, São Luis do Purunã, Paraná, Brazil.

The objective of this study was to evaluate the use of recombinant bovine somatotrophin, progesterone (P4), and estradiol benzoate (EB) on ovarian follicular dynamics for the synchronization of estrus and ovulation protocols in crossbred *Bos taurus taurus* cows. Twenty-four non-lactating multiparous cows were randomly divided into two groups: recombinant bovine somatotrophin group (GbST; n = 11) or the control group (GC; n = 13). The GbST group on day zero (d0; start of the study) received an intravaginal device with P4 (1.9 g), EB (1.0 mg, IM), and bST (500 mg, SC); ovarian ultrasonography (US) to examine the follicles and corpus luteum (CL) and transrectal ultrasonography were also performed. On d8, D-cloprostenol (150 µg, IM) was injected, US was carried out, and P4 was removed. On d9, EB (1.0 mg, IM) was injected and on d10 and d15 US was performed. The same protocol was applied to the control group, except bST was not administered on d0. The follicles and CL were evaluated and analyzed by US on d0, d8, d10, and d15. The data were statistically analyzed using the Statistical Analysis System (SAS) with a significance level of 5%. The effects on the groups (GbST and GC) with respect to the follicle size, CL size (F test), and ovulation rate (Fisher test) were evaluated. The GbST group showed a significantly higher follicle diameter (14.5 vs 12.1 mm, $P < 0.03$) on d10, CL diameter (19.7 vs 16.9 mm, $P < 0.01$) on d15, and ovulation rate (90.9% vs 69.2%, $P = 0.09$) compared to the GC group. In conclusion, bST, when used in combination with P4 and EB in timed artificial insemination protocols, significantly increased preovulatory follicle size and the percentage of ovulated cows and promoted a higher follicular growth rate after P4 removal compared to that in the control group. We thank the Pontificia Universidade Católica do Paraná for the availableness of the structures to the execution of this study.



115 Assisted Reproductive Technologies

Effect of a one-time, strategic donor FSH-treatment on oocyte and embryo production in a commercial buffalo IVP program

**J.L. Konrad^{*1,2}, J.A. Berdugo³, R. Yuponi¹, N. Vallejos¹, M.A. Ledesma⁴, G. Crudeli¹,
M. Sansinena^{1,2}**

¹Facultad de Ciencias Veterinarias, UNNE, Corrientes, Corrientes, Argentina; ²Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina; ³BIOGEM, Universidad Nacional de Colombia, Sede Medellín; ⁴Fecunda -Valdez y Laurentis, Corrientes, Argentina.

One of the main limitations for the establishment of commercial IVP programs in buffalo resides in the reduced number and competence of OPU-derived oocytes. The objective of this study was to evaluate the effect of a one-time, strategic FSH-treatment in the number of available follicles for aspiration, oocyte quality and subsequent in vitro embryo development. Eleven, mature and fertile Mediterranean and Murrah donors in excellent body condition were available for ovum pick-up; aspirations were conducted during the reproductive season in Argentina. Animals were subjected to two sequential weekly aspirations T0-DFR (initial, unsynchronized complete follicular ablation), T0-control (OPU after 7 days post DFR) and were then treated 180 mg FSH (Follitropin, Vetpharma) distributed in twice daily i.m. injections for 3 days. Subsequent aspirations were conducted 7 or 15 days post FSH treatment (T1-FSH7 and T1-FSH15) and the FSH-residual effect after initial aspiration was also analyzed (T2-Residual). Continuous data was analyzed with ANOVA and Tukey post-hoc comparisons and categorical data was analyzed using Fisher's exact test. All analyses were performed in GraphPad Prism v.7; statistical significance was established at $P < 0.05$. There were no significant differences in the average number of oocytes available for puncture between treatment groups (T0 = 8.7 ± 0.5 , T0-DFR = 4.7 ± 0.7 , T1-FSH7 = 7.6 ± 1.8 , T1-FSH15 = 8.0 ± 0.7 , T2-Residual = 6.0 ± 1.2) or total oocytes recovered (T0 = 4.9 ± 0.9 , T0-DFR = 2.0 ± 0.3 , T1-FSH7 = 4.2 ± 1.1 , T1-FSH15 = 3.5 ± 0.4 , T2-Residual = 1.4 ± 0.4). However, there was a significant improvement in the number of better quality oocytes (grades 1 and 2) in FSH-treated animals (T1-FSH7 = 3.0 ± 0.9 and T1-FSH15 = 1.5 ± 0.5 versus T0 = 0.5 ± 0.3 , T0-DFR = 0.9 ± 0.1 and T2-Residual = 0.4 ± 0.1 ; $P < 0.05$). In addition, cleavage (T1-FSH7 = 26%, T1-FSH15 = 71% versus T0 = 7%, T0-DFR = 0 and T2-Residual = 10%; $P < 0.05$) and blastocyst rates (T1-FSH7 = 21%, T1-FSH15 = 30% versus T0 = 5%, T0-DFR = 0 and T2-Residual = 0; $P < 0.05$) were significantly improved by FHS treatment. Our results indicate one single, strategic FSH treatment results in significant improvements in oocyte quality and embryonic development; these effects are observed when OPU is conducted 7 or 15 days post FSH treatment. Residual effects of FSH treatment do not result in sustained improvement or embryo production after this period. This strategy could have a positive impact on commercial buffalo IVF programs. Universidad Nacional del Nordeste, UNNE, Fecunda Biotecnología Paraguay y Valdez y Laurentis, Argentina. Universidad Nacional de Colombia, Sede Medellín.



Down-regulated genes in *in vitro* produced bovine day 8 blastocysts due to an insulin challenge during oocyte maturation

D. Laskowski^{*1}, G. Andersson², P. Humblot¹, M. Sirard³, Y. Sjunnesson¹, R. Båge¹

¹Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala Sweden; ²Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Uppsala Sweden; ³University Laval, Departement des Sciences Animales, Quebec Canada.

Insulin is a key metabolic hormone that has mitogen functions during early embryo development. Deviation of its physiological levels occurs in metabolic disorders due to overfeeding or negative energy balance and can be detrimental for oocyte quality. Reproductive disorders are often related to metabolic imbalance. The effect of insulin during oocyte maturation on gene expression of bovine day 8 blastocysts (BC8) by transcriptome analysis was investigated. Abattoir- derived oocytes (n=882) were divided into 3 groups and *in vitro* matured for 22 h by adding insulin (INS10=10µg/mL; INS0.1= 0.1 µg/mL and INS0= control). This was followed by standard *in vitro* production up to blastocyst stage. BC8 (n=120) were pooled in groups of 10 and total RNA was extracted (AllPrepDNA/RNA micro kit, Qiagen®) for transcriptomics. Amplified aRNA was hybridized on the Agilent EmbryoGENE-slides in a 2-color dye swap design. An empirical Bayes moderated t-test was applied to search for the differentially expressed transcripts (DET) between control and insulin groups, using the 'limma' package in R (www.r-project.org). DET were defined as having a 1.5 fold-change difference between treatment and control and P<0.05. In total, 202 DET were found in INS10 and 142 DET in INS0.1, whereof only 5 respective 4 DET were under-expressed compared to INS0. Thus, as a global pattern, insulin treatment induced an over-expression of most genes. However, some of the under-expressed genes have interesting functions that might be coupled to insulin influence in health and disease as hCTD1 (control of embryo development, involved in cell cycle regulation), UPK1A (mediates signal transduction events that play a role in the regulation of cell development, activation, growth and motility), ERAL1 (depletion of ERAL1 leads to apoptosis, cell death occurs prior to any appreciable loss of mitochondrial protein synthesis or reduction in the stability of mitochondrial mRNA) in INS 10; and ULBP1 (activates PI3K and CD38 immune system with functions in glucose metabolism) in INS0.1. To summarise, although only a few genes were underexpressed after insulin challenge, they demonstrate a potential difference in cellular functions and pathways coupled to mitogenic, mitochondrial or immunological functions of insulin. Thus, under- expression of these genes are potentially related to the pathomechanisms of hyperinsulinemia on the cell level and also involved in reproductive and metabolic disorders. Funded by FORMAS.



117 Assisted Reproductive Technologies

The effect of ceftiofur hydrochloride on pregnancy rates of beef cattle synchronized with intravaginal progesterone implants

L.M. Löff², A.N. Silva^{*2}, F.L. Facioli², G.C. Zanella², C. Bondan¹, A. Rodrigues³, E.L. Zanella¹, R. Zanella¹

¹Professor at Faculty of Agronomy and Veterinary Medicine, College of Veterinary Medicine, University of Passo Fundo, Passo Fundo, RS, Brazil; ²Undergraduate Student at Faculty of Agronomy and Veterinary Medicine, College of Veterinary Medicine, University of Passo Fundo, Passo Fundo, RS, Brazil; ³Human Doctor, Fazenda Amigos da Boa Vista, Paniel, SC, Brazil.

In the past years with the intensification in the livestock sector, attention was given to improve reproductive efficiency to maximize production. The use of estrous synchronization protocols for artificial insemination with fixed time (FTAI) were able to reduce the problems associated with heat detection, maximizing the reproductive efficiency. Studies from our group have identified changes in the vaginal microbiota of cows synchronized with intravaginal device with progesterone and also vaginitis. It is known that the use of antibiotics is a common procedure to treat and to control bacterial infection. Considering that changes in the uterine microbiota may compromise pregnancy, the objective of this work was to evaluate the effect of ceftiofur hydrochloride in beef cattle submitted to FTAI protocol in the pregnancy rates. For this study, 64 adult crossbred beef cows (Angus X Charolais X Hereford) were used. Animals were allocated randomly to two groups (G1 and G2) and further submitted to the same FTAI protocol with the CIDR® implant and 2.0 mL of Estradiol Benzoate (IM) on day 0, followed by the administration of 2.5 mL prostaglandin (IM) on day 7, on day 9, 0.3 mL of ECP® IM and the removal of the implant, on day 11 AI was conducted in all animals by the same veterinarian, using conventional frozen-thawed semen from 2 bulls (Hereford-HF or Maine Anjou-MA). In addition to that, G1 received 2.2 mg/kg of Ceftiofur - Excede® by IM route on day 9 the protocol, but G2 did not receive any additional treatment. Pregnancy detection was conducted in all the cows 60 days after AI. Data were analyzed using a X^2 test. No differences were observed in pregnancy rates among the bulls used. However, cows inseminated with MA Semen and treated with Ceftiofur presented 25.5% more pregnancies compared to the ones non-treated (58.82% MA-G1, 33.33% MA-G2; $P < 0.05$). This difference was not observed in the cows inseminated with HF semen. In general, the pregnancy rate of G1 was 57.57% and G2 was 41.93% ($P < 0.03$). Our results have shown that the use of antibiotic to modulate the bacterial proliferation in the vaginal microflora of beef cattle was able to reduce the losses of pregnancy by 15.64%. In conclusion, the use of the antibiotic ceftiofur hydrochloride was able to improve the reproductive efficiency of beef cattle submitted to a synchronization protocol.



Effects of using either estradiol benzoate or GnRH at the beginning of a 7-d P4-based FTAI protocol with or without GnRH at the time of AI in Nelore heifers

G. Madureira*¹, J.N. Drum¹, C.E.C. Consentini¹, J.C.L. Motta¹, L.O. Silva¹, A.B. Prata¹, M.C.V. Elias¹, R.L.O.R. Alves¹, J.R.S. Gonçalves², M.C. Wiltbank³, R. Sartori¹

¹“Luiz de Queiroz” College of Agriculture of University of São Paulo, Piracicaba, SP, Brazil; ²“Hildegard Georgina Von Pritzelwiltz” Experimental Station, Londrina, PR, Brazil; ³University of Wisconsin, Madison, WI, USA.

Evaluation of physiology and fertility variables of a 7-d FTAI protocol in *Bos indicus* heifers was the main objective of this study. Moreover, two treatments at the beginning of the protocol [estradiol benzoate (EB) vs buserelin acetate (GnRH)], including or not GnRH at the time of AI, were compared. A total of 958 heifers [26.4±2.0 mo old, BCS of 2.9±0.1 (1-5), and body weight of 307.6±22.5 kg; mean±SD] were used. At first, the presence of a corpus luteum (CL) was evaluated by ultrasound. Heifers with CL were assigned to the experimental treatments described below, and heifers without a CL were submitted to a protocol for induction of cyclicity and after 12 d (D0), all heifers, independent of CL presence, were randomly assigned to the treatments: B0 (EB at D0 + no GnRH at AI; n=239), BG (EB at D0 + GnRH at AI; n=246), G0 (GnRH at D0 + no GnRH at AI; n=239), or GG (GnRH at D0 and AI; n=234). On D0, all heifers received a P4 device (0.5 g) for 7 d and were treated with EB (1.5 mg; B) or GnRH (20 µg; G). On D7, concomitant with device withdrawal, all heifers received cloprostenol sodium (PGF; 0.530 mg), estradiol cypionate (EC; 0.5 mg) and had tail-chalk spread on the base of their tailhead. Heifers from the G group also received an extra PGF (24 h before the second PGF) and eCG (200 IU) treatment on D6, whereas B heifers received eCG on D7. At AI (48 h after D7), only BG and GG groups received GnRH (10 µg), and all heifers were checked for estrus. Hormones were donated by Globalgen Vet Science, Jaboicabal, BR. Ultrasound was used to determine presence of CL (D0, D7 and D16) and diameter of the dominant follicle (DF; D7) and pre-ovulatory follicle (OF; D9). Pregnancy per AI (P/AI) diagnosis was performed 40 d after AI. Statistical analyses were done by GLIMMIX and MIXED of SAS (LSM±SEM; P=0.05). Heifers with CL at first ultrasound represented 15.5% (147/951) of the total. In addition, 77.4% (628/804) ovulated after the induction protocol. After treatment on D0, G heifers ovulated more than B [62.5% (292/467) vs 14.2% (68/479)]. Further, more B heifers underwent luteolysis between D0 and D7 [39.4% (146/371)] than G [23.2% (88/379)]. On D7, DF (mm) was greater for G (10.4±0.19; n=54) than B (9.7±0.17; n=68), whereas at AI there was no difference between treatments for OF [mm; 11.8±0.2 (n=54) vs 11.6±0.18 (n=68)]. Also, more heifers in G than B were detected in estrus at AI [81.7% (384/470) vs 70.7% (343/485)]. Induced heifers detected in estrus at AI had greater P/AI [57.3% (423/726)] than heifers not in estrus [46.9% (111/228)]. There was no effect of CL on D0 (cyclicity) on P/AI, thus data were combined. There was no overall effect of treatments on P/AI [B0=57.3% (137/239), BG=53.7% (132/246), G0=54.2% (130/239), or GG=59.0% (138/234)]. There was also no association between estrus and GnRH at AI on P/AI. Ovulation rate after AI was not different among treatments [91.7% (n=139)], and only one heifer had double ovulation. In summary, there was no improvement in P/AI by treating with GnRH at the time of AI. Moreover, despite producing a larger DF on D7 and more estrus on D9, the GnRH-based protocol had similar P/AI compared to the EB-based protocol. FAPESP, CNPq, CAPES, Globalgen, GENEX and Figueira Farm.



Murrah buffalo antral follicle count in ovaries from a slaughterhouse

N.F.S. Marques*^{1,3}, D.C. Arantes^{1,3}, C.B. Silva², H. Atique Netto³, S.M. González^{3,2}

¹Universidades Estadual de São Paulo-UNESP-FMVZ, Botucatu, São Paulo, Brasil; ²Universidade Estadual de Londrina, Londrina, Paraná, Brasil; ³Centro Universitário de Rio Preto - UNIRP, São José do Rio Preto, São Paulo Brasil.

The study of antral follicle count (AFC) is currently widespread in bovine animals to obtain parameters for identifying the best females in terms of reproductive efficiency. The parameters related to AFC for buffaloes are still unknown. Therefore, the objective of the present study was to determine parameters of AFC for the ovaries of bubalina species from the slaughterhouse, in view of the significant increase in the production of these animals. According to the AFC of the female, it is ranked as the number of follicles present in a pair of ovaries, thus resulting in three groups: high, medium or low AFC. For the study, a pair of ovaries was obtained from buffaloes (n = 26) collected in the slaughterhouse of São José do Rio Preto and transported in a thermal container at 36°C with 0.9% saline solution. These were identified (left/right) and kept in a water bath until the AFC was determined by macroscopic visualization. We measured the diameter of the largest follicle antral found in ovary with a Trident® ruler. The antral follicles = 1 mm (visible to the naked eye) were counted to obtain the high (G-high), average (G-average) and low (G-low) AFC groups. Statistical analysis for comparison between the AFC of high, medium and low groups was performed using Fisher test, with 5% level of significance through the Statistical Programme Action 3.1 R version 3.0.2 (Campinas, SP, Brazil). For comparison between the AFC of the right and left ovaries we used t-test using the same statistical programme referred to above. The parameters obtained for AFC of G-high was = 19, for G-average = 18, and for G-low = 8 follows follicles, with the number of animals classified in the groups: n = 7; n = 11 and n = 8, respectively. On comparison between the buffaloes with high, medium and low AFC, there was a significant difference. Regarding the comparison between the AFC of the right and left ovaries between G-average and G- high, there was no significant difference. But we observed a significant difference when comparing right and left ovaries for animals of G-low (P < 0.05). Therefore, we find that with the few antral follicles Murrah buffaloes presented in the ovaries, resulting in smaller differences for high, medium and low AFC. In addition, the buffaloes with low AFC showed a difference in follicular number between the ovaries, suggesting that there is an ovary with greater follicular recruitment and possibly functionality. We thank the veterinary hospital Halim Atique for giving up the space to do the work.



120 Assisted Reproductive Technologies

Plasma and acrossomal integrity of bull spermatozoa cryopreserved with iodixanol

F. Marqui*¹, A. Martins Júnior², T. Cruz¹, T. Berton³, C. Freitas-Dell'Aqua¹, J.A. Dell'Aqua Jr¹, E. Oba¹

¹UNESP - São Paulo State University, Botucatu, São Paulo, Brazil; ²UNESP - São Paulo State University, Araçatuba, São Paulo, Brazil; ³Tairana Artificial Insemination Station, Presidente Prudente, São Paulo, Brazil.

The oxidative stress has been reported one of the most important factor contributing to sperm quality and fertility reduction. Recently, iodixanol showed to protect bull, ram, buffalo and equine sperm membranes when added to freezing extender, either by altering the formation of ice crystals, or by preventing reactive oxygen species generation. Therefore, this study was carried out to verify the effects of addition of iodixanol as a cryoprotectant for bovine semen freezing on post-thaw plasma and acrossomal membrane integrity (PAMI), translocation of phosphatidylserine (TPS) and plasma membrane destabilization (PMD). Thus, we compared a commercial freezing medium (CFM; BotuBOV[®], Botupharma, Botucatu, Brazil) with 7% glycerol (C; control), CFM with 3.5% glycerol plus 3.5% iodixanol (GI), and CFM with 7% iodixanol (I). After routine sperm assessment (motility, concentration and morphology), the ejaculates (n=18) from three Nellore bulls were extended according to the groups previously described. The samples were filled in 0.5 straws, cooled for 5h at 4°C, frozen in a programmable freezer machine (Digitcool, IMV, L'Aigle, France) and stored in liquid nitrogen until evaluation. Plasma and acrossomal membrane integrity were simultaneously assessed using Propidium Iodide and FITC-PSA probes, respectively, while TPS was identified by Annexin V and PMD by YO-PRO-1. All sperm samples were analyzed by flow cytometry (BD LSR; Fortessa, Becton Dickinson, Mountain View, USA). Sperm parameters were expressed percentage (%) for the total number of cells. ANOVA and Tukey's test were used for statistical analysis (six replicates), with P<0.05 taken as significant. Higher MPAI was observed for CO (56.7 ± 2.1) and GI (57.1 ± 1.3) than for I (33.5 ± 2.9). Furthermore, GI (53.3 ± 1.6) showed higher percentage of spermatozoa without TPS than CO (45.6 ± 2.4) and both than I (32.5 ± 2.9). For PMD, the I group (12.6 ± 1.7) exhibited lower rate of membrane without destabilization than GI (42 ± 2.5) and CO (62.4 ± 2.7), which were significantly different. In conclusion, freezing medium with iodixanol as cryoprotectant was not able to effectively conserve the membranes integrity of bull spermatozoa as well as tests using fluorescent probes (data not showed) observed that it was not able to control oxidative stress as expected. However, the association of iodixanol and glycerol is positive by reducing the concentration of glycerol which is toxic to spermatozoa. CAPES, Tairana AI Station and Botupharma, Brazil.



121 Assisted Reproductive Technologies

Effects of moxibustion during the mid-luteal phase on blood flow to the corpus luteum in cattle

M. Matsui^{*1}, M. Maruyama¹, K. Hazano^{1,2}, J. Tomiyasu^{1,2}, S. Haneda¹

¹Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan; ²Gifu University, Gifu, Gifu, Japan.

It is known that moxibustion improves the fertility of the dairy cattle. However, the mechanisms of the effects of moxibustion are not clear. To investigate the physiological effects of moxibustion on ovarian function of the cow, the effects of moxibustion on the function of corpus luteum (CL) was evaluated. In the present study, the changes in luteal blood flow (LBF) and plasma progesterone (P₄) concentration in the mid-luteal phase and PGF_{2α}-induced regression phase were examined after moxibustion treatment. Non-lactating Holstein-Friesian cows (n=3) were applied a series of control experiments (control group, without moxibustion treatment), and thereafter, in another estrous cycle, moxibustion treatment was conducted (moxa group). Soybean paste (miso, 30g each) were placed on nine specific acupoints on the back skin of the cow. Dried mugwort (moxa, 2.5g each) were put on miso and burnt for 20 min. Moxibustion treatment was performed on 5 days from day 9 to day 13 of the estrous cycle (ovulation = day 1). After moxa on day 13, PGF_{2α} was injected to induce luteal regression. To evaluate the size of the CL (CLS) and LBF, transrectal color Doppler ultrasonography examination was carried out before moxibustion treatment on day 9, on day 13 and just before PGF_{2α} injection (0 h), and at 1, 2, 4, 8 h after injection. Examination of CLS by transrectal ultrasonography was carried out continuously at 12, 24, 36, and 48 h after injection. To assess the change of LBF, the ratio of the colored area to the area of the maximum diameter of CL was used. Blood samples were collected at each time point, and plasma P₄ concentrations were measured by EIA. Data were analyzed using repeated measures ANOVA followed by Dunnett's multiple-comparison test. There was no significant effect of moxa during the mid-luteal phase on CLS, LBF, or plasma concentrations of P₄. A decrease in CLS was observed in both groups at 4 h after PGF_{2α} injection. At 1 h compared with 0 h, LBF significantly increased in both groups but no significant difference was found between moxa and control. A decreasing ratio of the changes in LBF from 1 h after PGF_{2α} injection was significantly larger at 4 h compared with 2 h in the moxa group, but not in the control group. A decrease in P₄ concentrations was observed in both groups at 8 h. There was no significant difference in P₄ concentrations between the two groups at all examination time points. An earlier decrease in LBF observed in the moxa group may indicate that moxibustion influenced vasoconstriction or vasodilation and changed the LBF after PGF_{2α} injection. In contrast, moxibustion may have no effects to decrease of plasma P₄ level after PGF_{2α} injection. These results suggest that the changes in responsiveness of LBF to PGF_{2α} by moxibustion may affect to the function of CL, and this effect may contribute to improving fertility in the cow.



122 Assisted Reproductive Technologies

Blastulation timing affects the blastocyst development and its secretion of extracellular vesicles

E. Mellisho, F.O. Castro, L. Rodriguez-Alvarez*

Laboratory of Animal Biotechnology, Department of Animal Science, Faculty of Veterinary Science, University of Concepcion, Chillan, Bio Bio, Chile.

Blastocyst formation is an essential event in preimplantation development. An early blastulation has been related to a better synchrony of embryo development and hence better embryo quality. The normal embryo development needs a constant crosstalk with the environment. This interaction is mediated by molecules and extracellular vesicles (EVs) secreted by the embryo. The nature of this molecules and vesicles may vary depending on embryo stage and quality. Thus, a better understanding of the embryo secretome during blastulation time can help in improving the selection accuracy of competent embryos. To examine the effect of blastulation time on blastocyst development and secretion of EVs, cattle ovaries were collected at an abattoir and transported within 2 h to the laboratory. Cumulus-oocyte-complexes (COCs) were recovered and matured in four-well dishes (25 to 30 COCs per 500 μ L well) for 22-23 h. Oocytes were fertilized using frozen-thawed semen that was used before in other in vitro fertilization procedures with good blastocyst production (control bull). Presumptive zygotes were in vitro cultured (IVC) in groups in four-well plates (25 to 30 zygotes per 500 μ L well) using SOFaa culture medium at 39°C under 5% CO₂, 5% O₂ and 90% N₂ until morula stage (day 5 post IVF). At day 5, morulae were selected and cultured individually in 96-well plates in SOFaa medium depleted of EVs (SOFdep) and monitored for blastulation at day 6.5 (early) and 7.5 (late) post fertilization. At day 7.5, blastocyst diameter was measured and culture media from individual blastocysts was collected and EVs were isolated and analyzed using a nanoparticle tracking analysis. Comparison analyses between early (n=189) and late (n=174) blastulation were performed using ANOVA, the non-parametric U Mann-Whitney test, and clustering analysis and principal component analysis to discriminate groups according to blastulation time. Statistical analysis was performed with SAS system V8 for windows (SAS Institute Inc). Early blastulation rate (day 6.5) was 16.2% (222/1371) while late blastulation rate (day 7.5) was 21.3% (292/1371), showing a significant difference (P = 0.0046). Embryos derived from early blastulation (day 6.5) were larger at day 7.5 (163.7 \pm 22.6 vs 154.0 \pm 18.6 μ m; P = 0.0001), secreted a greater number of vesicles (28.7 \pm 18.2 vs 23.4 \pm 9.0 x 10⁸ per mL; P = 0.0001) and these particles were larger (108.4 \pm 25.9 vs 99.3 \pm 19.0 nm; P = 0.0001), compared to those derived from Day 7.5 blastocysts. However, there was no correlation between blastocyst diameter at day 7.5 and characteristics of secreted EVs (size, r = 0.25, P = 0.0001; and concentration, r = 0.040 and P = 0.007), independently of blastulation time. Clustering analysis of populations of EVs did not allow discrimination between embryos of early and late blastulation. In conclusion, blastulation time affects the diameter of bovine embryos as well as the characteristics of populations of EVs secreted by the embryo during the period from morula to blastocyst stage. Work supported by Grants Fondecyt 1170310 and Corfo 17Cote-72437, Government of Chile.



123 Assisted Reproductive Technologies

Extracellular vesicle-depleted culture media improves quality of *in vitro* produced bovine embryos

B. Melo-Baez*, E. Mellisho, A.E. Velasquez, S. Saez, L. Rodriguez-Alvarez

Laboratory of Animal Biotechnology, Department of Animal Science, Faculty of Veterinary Science, University of Concepcion, Chillan, Bio Bio, Chile.

Extracellular vesicles (EVs) are currently considered a mechanism of cell communication. The EVs are secreted into the extracellular environment by different cell types including embryos, and can be identified *in vivo* in different biological fluids as well as *in vitro* in embryo culture medium. Usually, media used for *in vitro* culture of embryos are supplemented with serum or other protein sources that favor cell proliferation and development. These protein sources also may contain EVs, including microvesicles and exosomes that in principle can be internalized by embryonic cells. The aim of this study was to determine whether the absence of EVs in culture media could affect *in vitro* bovine embryo development. Several variables were evaluated, including embryo developmental rate up to blastocyst stage, blastocyst quality (based on morphological criteria of the IETS) and gene expression. Bovine embryos were produced by *in vitro* fertilization (IVF). Cumulus-oocyte-complexes (COC's) were recovered from slaughterhouse ovaries and *in vitro* matured for 22-23 h in four-well dishes (25 to 30 COCs per 500 μ L well), and fertilized with commercial frozen-thawed semen previously proved for IVF. Embryos were *in vitro* cultured in groups (25 embryos/well in 4-well plates) in global® total® medium (GTc) or global® total® EV-depleted medium (GTd) at 39°C under 5% CO₂, 5% O₂, and 90% N₂. Presumptive zygotes were randomly assigned to experimental (GTd) or control (GTc) groups. Medium was depleted by ultrafiltration (centrifugal filter devices 100 kDa, Amicon) for 15 min at 3000 RPM. EV populations from GTd and GTc were analyzed using a nanoparticle tracking analysis (NTA). Blastocyst rate and quality was determined at day 7 and 8 after IVF. At day 8, blastocysts total cell number was analyzed. Also, expression analysis of 9 genes (OCT4, SOX2, NANOG, BCL2, CDX2, GATA6, TP1, BAX and CASP3) was performed by RT-PCR; ACTB was used as the housekeeping gene. Data were analyzed with Wilcoxon non-parametric test using InfoStat program. The ultrafiltration protocol eliminated 98% of culture media EVs (original culture media 2.54×10^{10} particles per mL versus depleted culture media 4.4×10^8 particles per mL). Blastocyst rate at day 7 was 18.7% (37/198) and 13.5% (27/201) in GTc and GTd, respectively, whereas at day 8 it was 22.7% (45/198) and 19.4% (39/201) in GTc and GTd respectively; no statistical differences were observed for blastocyst rate at day 7 or at day 8 ($P > 0.05$). It seems that depleted media delay embryo development since at day 7 there were more early blastocysts (54% vs 48% for GTc and GTd, respectively; $P > 0.05$). However, no statistical differences were observed for blastocyst stage and total cell count at day 8. However, morphological quality of blastocysts derived from GTd was significantly higher at day 8 ($P = 0.03$) and the expression of CASP3 was significantly lower in these embryos ($P = 0.0024$). The results indicate that the absence of EVs from protein sources added to culture media improves bovine *in vitro* embryo development by increasing blastocyst quality and perhaps reducing apoptosis at day 8. Supported by FONDECYT, Chile (1170310).



124 Assisted Reproductive Technologies

Interference of dairy production on oocyte recovery and embryo conversion rate of donors from Gyr and crossbred (Holstein x Gyr) dairy cows

V. Miquelanti¹, C. Guaitolini², J. Silva¹, E. Trevisol³, R. Maziero*¹

¹Universidade Paranaense - UNIPAR, Umuarama, Paraná, Brazil; ²Universidade Estadual Paulista - UNESP, Faculdade de Medicina Veterinária e Zootecnia - FMVZ, Botucatu, São Paulo, Brazil; ³In Vitro Brasil, Mogi Mirim, São Paulo, Brazil.

Considering the impact of cattle farming in the country's economy, it is important to emphasize the relevance of research aimed at improving the existing techniques and the development of new technologies for the industry. This study was developed at In Vitro Brasil S/A®, a company headquartered in the city of Mogi Mirim, in the state of São Paulo, in Brazil, where data from follicular aspirations performed in a farm in the city of Passos, in the state of Minas Gerais, were collected. A total of 118 oocyte donor cows were used, selected according to their genetic merit. The animals belonged to the following breeds: 43 Gyr (*Bos taurus indicus*), 60 half-blood Gyr-Holstein (1/2 *Bos taurus taurus* x 1/2 *Bos taurus indicus*) and 15 5/8 Gyr-Holstein (5/8 *Bos taurus taurus* x 3/8 *Bos taurus indicus*). Data were analyzed using the Kruskal-Wallis test (Statistics9®), a non-parametric analysis of variance for samples that are non-dependent on oocyte recovery and embryo conversion data. The ANOVA test was also used, which is an analysis of variance for different dairy production rates and its interference in the oocyte recovery and embryo conversion. Data related to oocyte recovery, embryo production and percentage of oocyte-embryo conversion are reported, comparing the milk production of those animals. According to the statistical analysis, the Gyr breed presented lower oocyte recovery than the other breeds ($P < 0.05$); however, the blastocyst conversion rate was higher, despite not presenting statistical difference in the amount of produced embryos ($P > 0.05$). There was no statistical difference in relation to milk production among the animals, despite the numerical variation ($P > 0.05$). Nevertheless, a progression in the oocyte recovery and embryo production was observed, according to the increase in the lactation rate in the Gyr and half-blood Gyr-Holstein. On the other hand, the 5/8 Gyr-Holstein donors presented a regression in the oocyte recovery and embryo conversion according to the increase in milk production. This study provided unpublished data on the lactation interference in oocyte and embryo production in the assessed breeds. Universidade Paranaense - Unipar and In Vitro Brasil®.



125 Assisted Reproductive Technologies

Contribution of breed and equine chorionic gonadotropin on the collection of oocytes and *in vitro* embryo production in young goats during the breeding season

**G.L. Montes-Quiroz¹, H. Bernal-Barragán¹, R.A. Ledezma-Torres¹, D. Dominguez-Diaz²,
R. Cervantes-Vega¹, A.S. del Bosque-Gonzalez¹, F. Sánchez-Dávila*¹**

¹Universidad Autónoma de Nuevo León. Posgrado Conjunto Facultad Agronomía-Facultad de Medicina Veterinaria y Zootecnia, General Escobedo, Nuevo León, Mexico; ²Union Ganadera Regional de Nuevo Leon, General Bravo, Nuevo León, México.

The gonadotropins which have been used in the treatment of superovulation in goats are porcine or ovine follicle-stimulating hormone (FSH), equine chorionic gonadotropin (eCG) or a combination between FSH and eCG in regime known as "one-shot" (FSH + eCG). The use of eCG in goats, opens the possibility of collecting and maturing oocytes from superior does to accelerate genetic progress in this species. The objective of this study was to determine the effect of breed and eCG on ovarian response and *in vitro* embryo production from young goats. Twenty-nine goats (15 Alpine, 6 Nubian and 8 Saanen) were assigned into 3 treatments of eCG (T1: 0 IU; T2: 500 IU and T3: 1000 IU). The cumulus-oocyte-complexes (COC) were classified in three categories (grade I, II and III) according to their homogeneity morphology of the cytoplasm and compaction of the cumulus cells. Number of follicles before and after ultrasound was analyzed with a linear model that included the effect of breed, dose of eCG and their interaction. The retrieved oocytes, quality and the embryos production were analyzed using the Chi-square test (χ^2) to test significant differences between breeds and eCG doses. Alpine goats showed the largest amount and size of follicles ($P = 0.003$). An effect of eCG dose 24 hours post treatment ($P < 0.05$) was presented, being superior for the goats of T2. The aspiration rate of COC was 34%. The COC quality was zero or low results of Grade I and II, increasing the rate of nudes, obtaining the highest number ($P = 0.003$) in the Saanen goats; the same difference was found ($P = 0.02$) in oocytes grade III in the T2 and T3, with a 42.5 and 37.9% respectively. *In vitro* embryo production was in the Alpine goats (80.0 % of IVF/segmentation; $P = 0.003$). Embryo production was greatest for T2 (69.2%; $P = 0.004$). T3 goats had higher percentage of morula stage (66.6; $P = 0.030$). Therefore, it is concluded that treatment with eCG affected the ovarian status, quality and quantity of embryos according to the breed evaluated. To CONACYT, for the support of scholarship granted to the first author within the program of masters in animal science Agronomy-Veterinary Graduate of the UANL. Also, for the support program for scientific and technological research, PAICYT-UANL-CT249-15, for the financing of the project. To Dr. Juan Francisco Villarreal Arredondo, for his infinite support to carry out this project.



Is the laterality of the corpus luteum and the dominant follicle of Wave 1, and the GnRH administration 5 d after insemination, relevant for pregnancy establishment in heifers?

R. Núñez Olivera*, F. Cuadro, D. Bosolasco, A. Menchaca

Instituto de Reproducción Animal Uruguay, Fundación IRAUy, Montevideo, Uruguay.

Laterality between corpus luteum (CL) and dominant follicle (DF) of Wave 1 may affect pregnancy establishment in cattle (Miura *et al.*, 2015). Development of the first follicular wave dominant follicle on the ovary ipsilateral to the corpus luteum is associated with decreased conception rate in dairy cattle. *J Dairy Sci*, 98:318-321), with lower fertility reported when CL and DF were in the same ovary (ipsilateral). In addition, although the positive effect of GnRH administration during Wave 1 to improve pregnancy rate is contradictory, the interaction of this treatment with the laterality of CL/DF has not been yet evaluated. This experiment was carried out to evaluate the relationship among the laterality of the CL and the DF of Wave 1, and its interaction with the administration of GnRH 5 d after FTAI, with pregnancy rate in heifers. Hereford and Angus crossbreed heifers ($n = 467$) with body condition score 4.4 ± 0.1 (Mean \pm SEM, 1 to 8 scale) received a hormonal treatment for FTAI. The program consisted of the administration of an intravaginal device containing progesterone (DIB 0.5 g, Zoetis, Argentina) for 6 d and 2 mg of estradiol benzoate (Gonadiol, Zoetis) at device insertion. One dose of 500 μ g of cloprostenol (Ciclase DL, Zoetis) and 300 IU of eCG (Novormon, Zoetis) administered im at device removal, and 100 μ g of gonadorelin acetate (GnRH; Gonasyn GDR, Zoetis) administered im at FTAI performed 48 to 72 h after device removal. The laterality of the CL was determined by ultrasonography (WED-9618V, Well.D) at the moment of insemination to define the ovary containing the preovulatory follicle, and confirmed by the presence of the subsequent CL 5 d later. Laterality of the DF of Wave 1 was determined 5 d after insemination, and heifers were allocated into four experimental groups according to the laterality of the CL and the DF of Wave 1 (ipsi or contralateral when both were or not in the same ovary). Half of the herd received one dose of GnRH in a 2 X 2 factorial design. Pregnancy diagnosis was determined by ultrasonography 30 d after FTAI. Data were analyzed by GLMM and presented as mean \pm SEM. Ovulation rate (heifers ovulated/treated) was 97.4% (455/467). Pregnancy rate from ovulated females (70.3%, 320/455) was not different between heifers with CL/DF ipsi or contralateral (70.3%, 175/249 and 70.4%, 145/206; respectively, $P = \text{NS}$), and between heifers with or without GnRH (68.7%, 158/230 and 72.0%, 162/225; respectively, $P = \text{NS}$). No interaction between laterality of CL/DF and GnRH treatment 5 d after FTAI was found ($P = \text{NS}$). In conclusion, the presence of DF of Wave 1 in the ipsi or contralateral ovary to the CL does not affect pregnancy establishment, and the administration of GnRH 5 d after insemination does not improve fertility in *Bos taurus* beef heifers.



127 Assisted Reproductive Technologies

Influence of estrus expression in a fixed-time AI protocol on reproductive performance

G.A. Perry*¹, R.A. Cushman², E.J. Northrop¹, J.J.J. Rich¹, S.D. Perkins¹

¹South Dakota State University, Department of Animal Science, Brookings, South Dakota, USA; ²USDA, ARS, Roman L. Hruska US Meat Animal Research Center, Clay Center, Nebraska, USA.

The ability to induce ovulation of a dominant follicle with an injection of GnRH facilitated the development of fixed-time AI protocols. However, it has been established that cattle need to experience elevated concentrations of progesterone, a drop in progesterone, and a rise in estradiol to establish timing for changes in uterine gene expression. Animals that exhibit estrus prior to fixed-time AI have greater pregnancy success compared to animals that are induced to ovulate without expressing estrus. Thus, the objective of this study was to determine the impact of estrus expression prior to fixed-time AI on timing of when pregnancy occurred. Data were collected on 4,499 cows (*Bos taurus* beef animals ages 13 months to 13 years) in 31 different herds. All animals were synchronized using an injection of GnRH at insertion of an intravaginal progesterone device, an injection of prostaglandin F2 α at device removal, and an injection of GnRH at time of AI. Estrus expression was determined at time of AI based on activation of estrus detection patches. Bulls remained separated for 10 days after AI, and fetal age was determined by transrectal ultrasonography. Animals were grouped as having conceived to AI, or into each of the possible return estrous cycles (cycle 1-d 10 to 31, cycle 2-d 32 to 53, or cycle 3-d 54 to 75, and cycle 4 after d 75). Data were analyzed using the GLIMMIX procedure of SAS with herd as a random variable. Animals that exhibited estrus before AI had increased ($P < 0.01$) pregnancy success to AI ($64 \pm 1.3\%$ vs $45 \pm 1.6\%$, respectively) and during the entire breeding season ($93 \pm 0.7\%$ vs $89 \pm 1.1\%$, respectively) compared to animals that did not exhibit estrus. Of the animals that did not conceive to AI but did conceive during the breeding season, more animals that exhibited estrus before AI conceived during cycle 1 ($P < 0.01$; $46 \pm 2\%$ vs $34 \pm 3\%$). However, more animals that did not exhibit estrus conceived in cycle 2 ($P = 0.02$; $46 \pm 2\%$ vs $39 \pm 2\%$) and 4 ($P = 0.01$; $6 \pm 1\%$ vs $3 \pm 1\%$). There was no difference in cycle 3 ($P = 0.15$; $11 \pm 1\%$ and $13 \pm 2\%$ for estrus and no estrus, respectively). Overall, animals that exhibited estrus before AI but did not conceive to AI conceived earlier in the breeding season compared to animals that did not exhibit estrus ($P < 0.01$; cycle 1.71 ± 0.04 vs cycle 1.93 ± 0.04). There was no difference ($P = 0.74$) in embryonic loss between groups ($2.3 \pm 0.3\%$ and $2.1 \pm 0.4\%$ for estrus and no estrus, respectively). Thus, the ability to induce ovulation is critical for fixed-time AI, but animals that do not exhibit estrus prior to fixed-time AI had decreased AI conception rates, decreased breeding season pregnancy success, and the animals that did conceive did so later in the breeding season compared to animals that did exhibit estrus. USDA is an equal opportunity provider and employer.



128 Assisted Reproductive Technologies

Efficacy of Doppler ultrasonography to detect non-pregnant Nelore cows and heifers submitted to three timed-AI in 48 days

G. Pugliesi¹, E. Moraes², I. Claro-Júnior³, D. Zago Bisinotto¹, A. Kloster⁴, M. Meneghetti³, E. Hara², L. Souza², J.L.M. Vasconcelos⁴

¹Department of Animal Reproduction, FMVZ, University of São Paulo, Pirassununga, SP, Brazil; ²Qualitas Melhoramento Genético, Goiânia, GO, Brazil; ³Zoetis, São Paulo, SP, Brazil; ⁴Department of Animal Production, UNESP, Botucatu, SP, Brazil.

The present study aimed: 1) to evaluate reproductive performance using Color Doppler ultrasonography for detection of luteolysis in non-pregnant cows and heifers 22 days after timed-AI (TAI); and 2) to compare the use of Doppler and B (brightness; gray scale) modes for detection of pregnancy. Suckling Nelore cows (n = 174) and heifers (n = 161) were submitted to a TAI progesterone/estradiol-based protocol. Animals received one progesterone intravaginal device (CIDR, Zoetis) on D13 (D0 = first TAI). On D22, CIDR were withdrawn and animals were evaluated by transrectal ultrasonography (Z5, Mindray), using B and color Doppler modes to measure the luteal area and blood perfusion. Animals with a corpus luteum (CL) = 25% of color signals indicating blood perfusion in the luteal area were diagnosed as non-pregnant (n = 141). Non-pregnant animals received estradiol cypionate (1 mg; ECP, Zoetis), dinoprost tromethamine (25 mg; Lutalyse, Zoetis) and eCG (200 IU for heifers and 300 IU for cows; Novormon, Zoetis) to induce ovulation, and a second TAI was performed on D24. On D37, inseminated animals were resynchronized as previously described. A third TAI was done on D48 in non-pregnant cows detected by Doppler ultrasonography on D46 (n = 80), and in animals where pregnancy loss was observed after first TAI (n = 30). For B-mode evaluation, animals with a CL < 2cm² were considered non-pregnant and pregnancy predictions were compared with the Doppler mode (gold standard) in each category (heifer or cow). Pregnancy was confirmed 37 days after first (D37), second (D61) and third (D85) TAI by detection of an embryo vesicle. Binomial data was evaluated by Fisher's Exact test using PROC FREQ and by logistic regression using PROC GLIMMIX of SAS. Parametric data was evaluated by ANOVA using PROC GLM. Considering all females, pregnancy diagnoses on D22 were in agreement between B and Doppler modes in 91.7% (410/447) of the cases. Accuracy, sensitivity, specificity, negative predictive value and positive predictive value were, respectively, 94.9%, 91.7%, 99.1%, 90.1%, and 99.3% for cows, and 88.3%, 78.2%, 99.1%, 81.2%, and 98.8% for heifers. The majority of incorrect results resulted from 8.3% and 21.8% of false-negatives in cows and heifers, respectively. The calculated kappa value indicated very good (0.897) and good (0.768) agreements between both methods, respectively, for cows and heifers. Luteal area (cm²) was greater (P < 0.05) in cows (2.79 ± 0.05) than in heifers (2.47 ± 0.03) diagnosed as true-positive. Pregnancy loss at day 37 was greater (P < 0.05) for heifers (19.5%, 23/119) than cows (9.6%, 13/135). Accumulative pregnancy rate after three TAI was 85.1% (148/174) for cows and 72.7% (117/161) for heifers. In conclusion, the resynchronization on D13 associated with use of early detection of luteolysis on D22 by Doppler ultrasonography results in desirable pregnancy rates after three TAI in 48 days. The single use of CL size accessed by B-mode ultrasonography for detection of luteolysis results in a high rate of false-negative results and lower accuracy compared to Doppler imaging, especially in heifers. Authors thank: FAPESP (2015/10606-9; 2016/23964-3); Zoetis; Qualitas Melhoramento Genético; DPS Mindray (Adriane), Fazenda Bela Vista (Silvio).



Effect of supplementation of α -tocopherol in superovulated Dorper ewes

R. Rangel-Santos*, A. Lorenzo-Torres, R. Rodríguez-De Lara

Universidad Autónoma Chapingo, Chapingo, Estado de México, México.

The efficiency of a multiple ovulation and embryo transfer (MOET) program depends on the number of good quality embryos obtained, and some studies suggest that supplementation with antioxidants can help to improve embryo quality. The objective of the study was to evaluate the effect of α -tocopherol supplementation on embryo quality of a MOET program in Dorper ewes. In total, 20 Dorper ewes were superovulated, from which 10 ewes were treated with 1000 IU of α -tocopherol given im 60 h before sponge removal, while the other 10 Dorper ewes were not treated (0 IU). The ewes were synchronized with intravaginal sponges containing 20 mg fluorogestone acetate (FGA) for 12 d and on the 10th d they were superovulated with a purified source of follicle stimulating hormone. Estrus was detected with teaser rams, and ewes in estrus were inseminated by laparoscopy 18 h after estrus onset with 4 doses of fresh semen containing 100×10^6 spermatozoa each. Only semen from a single ram and of proven fertility was used. Embryo recovery was attempted 7 d after estrus by laparotomy. Ovulation rate, recovery rate, fertilization rate and embryo quality were measured. The results were analyzed using the least-squares procedures (Proc GLM of SAS) in case of continuous variables (ovulation rate, mean number of transferable embryos, mean number of unfertilized eggs). In the case of fertilization rate and recovery rate, data were analysed as binomial events using a generalized linear model (Proc GENMOD of SAS) with a logit link function, with ewe considered as the experimental unit. Fertilization rate was higher ($P < 0.05$) in ewes treated with α -tocopherol (59.26%) compared to non-treated ewes (40.74%). However, there was no effect ($P > 0.05$) of α -tocopherol administration on ovulation rate (15.40 vs. 11.20), embryo recovery rate (58.62 vs. 41.38%), mean number of transferable embryos (4.60 vs. 2.85) or unfertilized eggs (1.80 vs. 3.43) for α -tocopherol and vs non-treated ewes, respectively. In conclusion, the administration of α -tocopherol only improved the fertilization rate of superovulated ewes under the conditions of the study. The authors acknowledge the support of Univesidad Autónoma Chapingo for the conduction of the study.



130 Assisted Reproductive Technologies

Characterization and control of the oocyte population for ovum pick up in Nellore donors

J.N. Sakoda*¹, A.C.S. Soares¹, I.L. Gama¹, C.L. Martins¹, V. Lodde², A.M. Luciano², J. Buratini¹

¹São Paulo State University, Botucatu, São Paulo, Brazil; ²University of Milan, Milan, Lombardy, Italy.

Current strategies for *in vitro* embryo production (IVP) include OPU on random day of estrous cycle. Oocytes recovered are heterogeneous in chromatin configuration, oocyte-cumulus coupling, transcriptional activity and developmental competence. Since oocyte *in vitro* maturation (IVM) should promote synchrony between nuclear and cytoplasmic maturation, this heterogeneity may underlie the low efficiency of current IVP strategies. This study aimed to characterize oocyte population obtained by OPU and to develop a protocol to synchronize oocytes destined to IVP in Nellore. In experiment 1, 14 Nellore cows were subjected to two treatments in crossover design: 1) OPU at random day (Control); 2) aspiration of follicles >2mm at random day (D0), two IM injections of FSH (Folltropin; 24+16mg) 10h apart on D2, OPU from follicles >2mm on D5 (ASP/FSH-40). In experiment 2, 12 Nellore cows were subjected to the same treatments as in experiment 1, except for reduction of FSH dose (Folltropin; 12+8mg) (ASP-FSH/20). At OPU, antral follicles were counted and classified as small (3-5mm), medium (6-8mm) and large (>8mm). Oocytes were counted, fixed and stained with Hoechst 33342 then examined by fluorescence microscopy to be classified according with chromatin condensation and germinal vesicle configuration in GV0, GV1, GV2, GV3 and GVBD (Lodde *et al.*, 2007). Data were arcsine transformed and groups compared by paired T test (P<0.05). Total number of follicles aspirated at OPU was not affected by treatment in any experiments. In experiment 1, 285 follicles were aspirated in Control group and 306 in Asp/FSH-40 group, while in experiment 2, total numbers 468 and 463 follicles were aspirated in Control and ASP-FSH/20 groups, respectively. However, in experiment 1, ASP-FSH/40 treatment reduced percentage of small follicles (P=0.02). Percentages of small, medium and large follicles were 67.6*, 24.1 and 8.3 in Control, and 50*, 35.4 and 14.6 in ASP-FSH/40, respectively. Distribution of oocytes in different categories of chromatin compaction was only affected by treatment in experiment 2, with ASP-FSH/20 reducing the percentage of GV3 oocytes (P=0.01). In experiment 1, percentages of GV0, GV1, GV2, GV3, GVBD and degenerated oocytes in Control group were 1.34, 0.25, 39.06, 46.37, 0.12 and 12.86 respectively (n=177), while they were 0, 4.14, 40.49, 38.94, 0 and 16.42 in ASP-FSH/40 respectively (n=195). In experiment 2, percentages of GV0, GV1, GV2, GV3, GVBD and degenerated oocytes were 0, 1.95, 47.55, 40.47*, 0 and 10.03 in Control group respectively (n=267), while they were 0, 4.22, 49.53, 30.50*, 0 and 15.75 in ASP-FSH/40 respectively (n=265). In conclusion, oocytes recovered by OPU at a random day in Nellore were mostly at intermediate (GV2) or advanced (GV3) stage of chromatin compaction. Since these stages have been associated with higher developmental competence, this finding may help to explain the suitability of the Nellore breed to IVP. Protocols combining antral follicle aspiration and low doses of FSH to stimulate follicle recruitment may further homogenize the oocyte population for OPU in Nellore. However, further improvement of ASP-FSH protocols and fine-tuning with IVM culture system are needed before their real impact on IVP outcomes can be determined. FAPESP, CAPES.



131 Assisted Reproductive Technologies

Differences in the endometrial response to age-matched long and short conceptuses during early pregnancy in cattle

J.M. Sánchez*¹, D.J. Mathew¹, C. Passaro¹, S.K. Behura², S.T. Butler³, T.E. Spencer², P. Lonergan¹

¹School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland; ²Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA; ³Animal Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, County Cork, Ireland.

Bovine embryos undergo a prolonged post-hatching development during which they undergo dramatic morphological change (elongation process), passing sequentially from a spherical to a filamentous-shaped structure. Inadequate elongation of the conceptus results in lower production of interferon tau (IFNT), maybe contributing to the inability to maintain the corpus luteum and thus pregnancy loss. Significant gaps in our knowledge of the complex biological mechanisms governing conceptus elongation remain. We have previously reported significant variation in conceptus length amongst conceptuses recovered from the same uterine environment following multiple embryo transfer that may be related to an inherent lack of developmental competency. Others have reported differences in gene expression between conceptuses recovered on Day 15 of gestation, based on their length. Here, we combined in vitro production of bovine blastocysts, multiple embryo transfer techniques and a conceptus-endometrial explant co-culture system to study the dialogue between an advanced (long) and retarded (short) conceptus and the maternal endometrium on Day 15 of pregnancy. We hypothesized that differences in endometrial response between long and short age-matched conceptuses could be either dependent or independent of IFNT. Seven long (mean length \pm SEM 25.4 ± 5.7 mm) and six short (1.8 ± 0.3 mm) Day 15 conceptuses recovered from recipient heifers following the transfer of Day 7 blastocysts (10 per recipient), were individually placed on top of endometrial explants (from uteri recovered from heifers in the late luteal phase) and then co-cultured for 6 h in one mL of RPMI medium. Additional explants were cultured with medium containing 100 ng/mL of recombinant ovine IFNT (IFNT; n = 6) or in media alone (Control; n = 6). Total RNA was isolated from explant cultures and analyzed by RNA-Seq. Exposure of endometrium to IFNT, a large conceptus or a small conceptus altered ($P < 0.05$) the expression of 491, 498 and 230 transcripts, respectively, compared with control endometrium. Further analysis revealed three categories of differentially expressed genes (DEGs) compared to the control: (i) genes commonly responsive to exposure to IFNT and conceptuses irrespective of size (n = 223); (ii) genes commonly responsive to IFNT and long conceptuses only (n = 168) that are over-represented by metabolism, cell-cell adhesion, receptor-mediated endocytosis, and mitochondrion organization functions, and (iii) genes induced by the presence of a conceptus but independent of IFNT (n = 108) - of these genes, the vast majority (n = 101) were exclusively induced by long conceptuses. Functional analysis revealed that regulation of molecular function, magnesium ion transmembrane transport, clathrin coat assembly, and beta-amyloid metabolism were the principal gene ontologies associated with these DEG. In conclusion, bovine endometrium responds differently in terms of its gene expression signature to embryos of varying size, in both an IFNT-dependent and independent manner, suggesting that these differences in communication between a long and short conceptus and the endometrium may be critical for embryo survival. Funded by Irish Department of Agriculture, Food and The Marine through the Research Stimulus Fund (Grant number: 13/S/528).



132 Assisted Reproductive Technologies

Effect of time of gamete co-incubation on *in vitro* production of bovine embryos

A.S. Santos Filho^{*1}, C. Coutinho², S. Guido¹, W. Cristo³, J. Oliveira¹, W. El Shible

¹Instituto Agronômico de Pernambuco, Arcoverde, PE, Brasil; ²Universidade Federal Rural de Pernambuco, Recife, PE, Brasil; ³Faculdades Integradas Aparício Carvalho, Porto Velho, RO, Brasil, ⁴Universidade Estadual do Ceará, Fortaleza, CE, Brasil.

In vitro production of bovine embryos has become a widespread technology of assisted reproduction implemented in cattle breeding in Brazil. The aim of this study was to evaluate the effect of duration of gamete co-incubation on embryo production. The experiment was conducted at the laboratory of animal breeding and reproduction in the experimental station of the Agronomic Institute of Pernambuco (IPA) in the city of Arcoverde. For the study, 319 oocytes were aspirated from antral follicles (3-8 mm) of ovaries from recently slaughtered cows, and then each 20-25 oocytes were matured for 24 hours in drops of 100 microliters of TCM199 medium. After maturation, the oocytes were divided into three groups according to the co-incubation time: T1 (10 hours-105 oocytes); T2 (14 hours -113 oocytes) and T3 (18 hours-101 oocytes) co-incubation, respectively, and were fertilized by 2×10^6 spermatozoa. Spermatozoa were obtained from frozen-thawed commercial semen collected from one bull (Alta Genetics Brazil). Thawed spermatozoa were washed in a discontinuous gradient of 45/90% Percoll using centrifugation at 700 g for 10 min. The pellet was resuspended with washing medium TALP and centrifuged once again at 700 g for 5 min. The final pellet was resuspended with *in vitro* fertilization (IVF) medium. After the IVF, drops of 100 μ L of IVF medium were covered with mineral oil. After IVF, the presumptive zygotes were washed and removed from fertilization wells at 10, 14 or 18 hours post-IVF and then cultured in SOF medium. The incubation was at 38.5°C, with 5% CO₂, and 95% of humidity. The cleavage rate was observed 48 hours after IVF; and 7 days after IVF, the blastocyst rate was observed. Statistical analyses were performed using the SAS software package version 9.1.3, using the PROC FREQ procedure and the Chi-square test. The cleavage rates were 38.0%; 41.5%; 45.6% in T1, T2 and T3 respectively, and the co-incubation time of the gametes did not influence the cleavage rate ($P > 0.05$). Similarly, no differences were observed in relation to the number of initial blastocysts, blastocysts, expanded blastocysts and hatched blastocysts ($P > 0.05$). Although studies of other research groups have shown good results with a short period of co-incubation of the gametes, in this study the lowest blastocyst rate was produced after 10 hours of co-incubation. The rates of embryos produced were 17,14, 23,89, and 28,71% ($P < 0.005$). Although co-incubation for only 10 hours presented a smaller number of embryos produced, we believe that more studies must be done, because there is a strong influence of the semen on IVF of cattle. Our results have shown that in commercial production of embryos, it is possible to maximize the use of semen, since we have an interval of 4 hours of co-incubation without negatively affecting the production of embryos. References: Effect of spermatozoa concentration on *in vitro* production of bovine embryos. Al Shebli Wasim¹, Santos Filho A.S.², Bartolomeu C.C.³, Guido S.I.² Anim. Reprod., v.14, n.3, p.734, Jul./Sept. 2017.



133 Assisted Reproductive Technologies

Improvement on the composition of sequential culture media based on the bovine content of oviductal and uterine fluids

E.C. Santos^{*1}, A.M. Fonseca Junior¹, C.B. Lima², J. Ispada², M.P. Milazzotto¹

¹Universidade Federal do ABC, Santo Andre, São Paulo, Brasil; ²Universidade de São Paulo, São Paulo, São Paulo, Brasil.

The *in vitro* production (IVP) of bovine embryos aims to mimic the female reproductive tract in order to generate blastocysts more similar to those produced *in vivo*. However, the concentration of nutrients available in a dynamic system such as the *in vivo* may be greater than that required for a static *in vitro* system. In fact, in rats, the development of zygotes at reduced amounts of nutrients in the culture medium increased blastocyst quality and rates. The aim of this work is to develop a sequential culture media – named embryonic culture supplementation (ECS) – based on the content of energy substrates and amino acids present in the composition of bovine oviduct (ECS1) and uterus (ECS2) fluids. Also, we intend to verify if, *in vitro*, a reduced concentration of such substrates could be beneficial to embryo development and quality. For this, we used the salt-based composition of SOF (Synthetic Oviduct Fluid) supplemented with 8 mg/mL of Bovine Serum Albumin (BSA) as the base for *Conventional control group* (SOFaa: supplemented with 2% essential amino acids, 1% nonessential amino acids, 1.5 mM glucose) and both culture media (*ECS1 and ECS2* supplemented with glucose, pyruvate, lactate and amino acids according to the composition of oviductal and uterus fluid). We prepared ECS1 and ECS2 supplemented with 100% (ECS 100), 75% (ECS 75), 50% (ECS 50) and 25% (ECS 25) of substrates concentration to evaluate the effect of reduced supplementation on *in vitro* culture (IVC). To determine the efficiency of these new culture media, blastocysts were produced *in vitro* by conventional protocols. At the time of IVC, presumptive zygotes were transferred to *ECS1 100, ECS1 75, ECS1 50, ECS1 25* and *SOFaa*. At Day 4 (D4), cleavage rates were assessed and embryos from all ECS1 groups were transferred to their correspondent ECS2 where they remained until blastocyst (D8) and hatching (D9) evaluation. All data were analyzed by Student t test (n = 469 oocytes/3 replicates). Preliminary data show that the reduction of nutrients availability does not affect cleavage and blastocysts rates when compared to control group, respectively in ECS 25 (75.8 ± 6.6 and 27.4 ± 6.7), ECS 50 (85.7 ± 4.5 and 34.8 ± 10.5), ECS 75 (82.1 ± 4.8 and 25.8 ± 7.5), ECS 100 (84.2 ± 4.5 and 20 ± 1.2) and SOFaa (80.6 ± 2.8 and 22 ± 4.7). However, the hatching rate is significantly higher in groups ECS50 (48.2 ± 5.7; P = 0.05) and ECS75 (73.5 ± 8.5; P = 0.01) when compared to SOFaa (12.1 ± 12.1). Blastocysts from the ECS 50 presented higher speed of development and better morphology (subjective evaluation). In conclusion, the formulated sequential culture media is not only able to support embryo development to blastocyst, but the reduction in energy substrates and amino acids concentration seems to be beneficial for blastocyst quality. Despite these promising results, the molecular and biochemical analysis are being conducted to verify a possible improvement in viability. Center of Natural and Human Sciences, Universidade Federal do ABC, Santo André, SP, Brazil.



134 Assisted Reproductive Technologies

Transcervical is more efficient than surgical embryo collection in Brazilian hair sheep

**J. Santos¹, M. Balaro¹, I. Cosentino¹, V. Brair², P. Pinto¹, J. Souza-Fabjan¹, E. Arashiro¹,
J.F. Fonseca³, F.Z. Brandão^{*1}**

¹Universidade Federal Fluminense, Niterói, RJ, Brazil; ²Unigranrio, Duque de Caxias, RJ, Brazil; ³Embrapa Caprinos e Ovinos, Coronel Pacheco, MG, Brazil.

This study compared the efficiency of either transcervical (TC) or surgical (laparotomy; LP) embryo collection in hair sheep. Santa Inês ewes (n = 27) were subjected to a short-term protocol for estrus synchronization and superovulation. Both cervix passage attempt and laparoscopic *corpora lutea* (CL) count were performed 12 to 24 h before embryo collection. Depending on the success of cervical passage, the ewes were collected by either TC (n = 16) or LP (n = 11). The cervical dilation protocol was applied in all ewes and consisted of 120 µg cloprostenol i.m and 100 µg estradiol benzoate i.v. (diluted in 2.5 mL of saline + 2.5 mL of ethanol), both given 12 h before, and 100 IU oxytocin i.v. 15 min prior to cervical passage attempt. All ewes were sedated with 0.1 mg.kg⁻¹ acepromazine and 0.2 mg.kg⁻¹ diazepam i.v. and then received an epidural injection with 2.0 mg.kg⁻¹ ketamine. The TC collection was performed using a circuit closed system (Circuito Embrapa for goats/sheep embryo recovery). LP collection was carried out using the same sedation procedures but with anesthetic induction with 4 mg.kg⁻¹ propofol and 0.1 mg.kg⁻¹ diazepam i.v. and maintenance with 3% isoflurane. Uterine flushing recovery was aided by a foley catheter and an urethral probe. Behavioral aspects such as time to standing and to eat after each procedure were recorded. Heart rate (HR) and rectal temperature (RT) were measured at 10 moments: before fasting, before sedation, during the procedure, immediately after the procedure, and 1, 3, 6, 12, 24 and 48 h after the procedure. Normal data were compared by one-way ANOVA followed by Student's t-test or Tukey's test. Non-normal data were analyzed by Kruskal-Wallis test followed by Student Newman-Keuls. the LP procedure took longer than TC (31.6 ± 14.3 vs 24.5 ± 6.5 min; P < 0.05). The uterine flushing recovery (99.2 vs 91.9%) and embryo recovery (60.5 vs 37.1%) were greater for the TC than LP method (P < 0.05). Compared with the LP group, TC ewes had a higher RT during (37.0 vs 36.4°C; P < 0.05) and immediately after (36.9 vs 35.7°C; P < 0.05) the procedures. HR was higher in TC when compared to LP group immediately after collection (101.8 vs 88.0 bpm; P < 0.05). However, HR was higher (P < 0.05) in the LP than TC group at 12 h (94.2 vs 79.8 bpm) and 24 h (108.7 vs 88.3 bpm) after the procedures. Behavioral aspects were not different between techniques (P > 0.05). This study demonstrated the overall superior efficiency of TC embryo collection in Brazilian hair sheep. FAPERJ, CNPq (400785/2016-1) and Embrapa (02.13.06.026.00.02).



135 Assisted Reproductive Technologies

Factors that affect embryo transfer conception rate in lactating dairy cows

R.M. Santos*¹, B.G. Alves¹, M. Oliveira², D. Demetrio²

¹Universidade Federal de Uberlândia, Uberlândia, MG, Brazil; ²Ruann and Maddox Dairy, Riverdale, CA, USA.

Embryo transfer could be used to produce more calves from high genetic merit cows and also could increase fertility of cows with high milk production. The objective of this study was to evaluate the effects of donor category (dry or milking cow); embryo category (*in vivo* produced fresh or frozen); and synchronism (6, 7 or 8) on conception rate following embryo transfer (ET) in lactating Holstein cows. Data from 8241 ET performed from 2007 until 2017, in a single commercial dairy farm were analyzed. Cycling lactating Holstein cows, producing 42.64 ± 7.2 kg milk/d, treated with Presynch-Ovsynch or after natural heat received ET 6 to 8 days later. The embryos were produced *in vivo* in non-lactating or lactating dairy cows and were transferred fresh or frozen. Only embryos grade 1 or 2 were transferred fresh or frozen. Pregnancy was diagnosed 35 days after estrus. The variable conception rate was analyzed by multiple logistic regression at SAS program and the model included effects of donor category, embryo category, synchronism, and interactions. The effect of donor category was not detected ($P = 0.395$). The conception rate for embryos produced in non-lactating cows was 50.92% (3122/6131) and in lactating cows 49.62% (1047/2110). But the conception rate was affected by embryo category ($P < 0.001$). Transfer of fresh embryos resulted in 56.08% (2311/4121) of conception rate and frozen embryos 45.10% (1858/4120). Effects of synchronism were detected in conception rate ($P < 0.001$). The ET at 6 days after estrus resulted in 48.10% (695/1445) of conception rate, at 7 days 49.85% (2748/5513) and at 8 days 56.59% (726/1283). No interaction between the variables was detected. The transfer of fresh embryos is an important tool to increase the probability of conception of lactating Holstein cows. Despite of lower conception rate after transfer of frozen embryos, for the establishment of an embryo transfer program and to achieve a good reproduction efficiency, the farm should have a reserve of frozen embryos, because the cows that will receive embryos are set 7 days before, and sometimes the production of fresh embryo is less than what is needed, and for a more successful program, embryo transfer should be performed at 8 days after estrus. Ruann and Maddox Dairy.



Sertoli cell-mediated differentiation of bovine fetal mesenchymal stem cells into germ cell lineage in an *in vitro* co-culture system

M. Segunda¹, J. Cortez¹, M. De los Reyes¹, J. Palomino¹, C. Torres¹, O. Peralta^{*1,2}

¹Faculty of Veterinary and Animal Sciences, University of Chile, Santiago, Chile; ²Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA.

Due to their abundant source and high differentiation potential, mesenchymal stem cells (MSC) may be suitable candidates for *in vitro* gamete derivation. Nevertheless, germ cell differentiation requires endocrine and auto/paracrine regulation in a specific environment, as well as direct cell-to-cell interactions provided by the somatic cells of the testis. Sertoli cells (SC) play an essential role by forming niches for germ cells providing essential factors for germ cell (GC) differentiation. The aim of the present study was to evaluate the effect of co-culture of SC on bovine fetal MSC (bfMSC) differentiation into germ cell lineage. Sertoli cells were isolated from bull testis and characterized by quantification of biomarker wilms tumor 1 (WT1) expression and androgen binding protein (ABP) mRNA levels using flow-cytometry (FC) and quantitative-PCR (Q-PCR) analyses. bfMSC were isolated from bone marrow (BM-MSC) and adipose (AT-MSC) tissue and were co-cultured with SC for 21 days. Bovine testis samples (positive controls), fibroblasts (negative controls), bfMSC-SC co-cultures, bfMSC and SC were analyzed for expression of housekeeping genes β -ACTIN and GAPDH, pluripotent genes OCT4 and NANOG and male germ cell genes DAZL and PIWIL2 by Q-PCR and FC. High levels of WT1 and ABP mRNA were quantified in bovine SC and testes samples; however, no transcripts of these genes were detected in bovine fibroblasts. Moreover, a high ($P < 0.05$) proportion ($85.9 \pm 3.9\%$) of SC were positive for WT1. OCT4 mRNA levels were higher ($P < 0.05$) in cocultures of SC with BM-MSC and AT-MSC at Day 14 compared to monocultures of SC, BM-MSC and AT-MSC. Moreover, a higher ($P < 0.05$) proportion of cells positive for Oct4 was detected in cocultures of SC and BM-MSC ($71.2 \pm 0.9\%$) compared to SC monoculture ($4.4 \pm 0.7\%$). The proportion of cells positive for Nanog was higher ($P < 0.05$) in cocultures of SC with BM-MSC compared to monocultures of SC and BM-MSC. Levels of mRNA of DAZL were upregulated ($P < 0.05$) in coculture of SC with AT-MSC at Day 14 compared to monocultures of SC and AT-MSC. However, at Day 21, DAZL mRNA levels were reduced ($P < 0.05$) in coculture of SC and AT-MSC. Dazl expression was higher ($P < 0.05$) in monocultures of AT-MSC ($57.2 \pm 0.4\%$) and cocultures of SC with AT-MSC ($54.2 \pm 3.4\%$) compared to monocultures of SC ($36.8 \pm 1.7\%$) and BM-MSC. Levels of mRNA of PIWIL2 were higher in monocultures of SC and AT-MSC, and in cocultures of SC with BM-MSC compared to Day 14. Moreover, PIWIL2 mRNA levels were upregulated in coculture of SC with AT-MSC at Day 14 compared to Day 21 and monocultures of SC and AT-MSC. These data indicate that coculture with SC may increase the proportion of cells expressing Oct4 and Nanog suggesting an advance of BM-MSC into a pluripotent state. Moreover, the cell-to-cell interaction with SC may mediate activation of GC-specific genes inducing differentiation into the GC lineage. Supported by Fondecyt grant 1161251, Government of Chile.



137 Assisted Reproductive Technologies

Nuclear Maturation of bovine oocytes submitted to intrafollicular transfer of immature oocytes (IFIOT)

V.A.O. Silva*¹, O.A. Faria², L.R.O. Dias², F.M.C. Caixeta², J.F.W. Sprícigo³, M.A.N. Dode⁴

¹ICESP Faculty, Brasília, DF, Brazil; ²University of Brasilia, Brasília, DF, Brazil; ³University of Guelph, Guelph, ON, Canada, ⁴Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil.

The objective of the present study was to evaluate nuclear maturation status of bovine cumulus oocyte complexes (COCs) submitted to different periods of *in vivo* maturation after intrafollicular transfer of immature oocytes (IFIOT). For IFIOT, animals were synchronized by administering an intramuscular injection of 2.0 mg estradiol benzoate and inserting an intravaginal progesterone-releasing device on Day 0. On D8 Progesterone device was removed and an intramuscular injection of PGF2 α was administered. On Day 9 those heifers presenting one dominant follicle (≥ 10 mm) received an injection of estradiol benzoate and the IFIOT. COCs were aspirated from slaughterhouse ovaries (2-8 mm diameter follicles), selected and injected into the dominant follicle in groups of 50. To evaluate maturation, the injected follicles were aspirated at 16 h (n = 171), 24 h (n = 104) and 30 h (n = 144) after IFIOT. For control, COCs were *in vitro* matured for 0 h (CONT0, n = 125), 16 h (CONT16, n = 122), 24 h (CONT24, n = 126) or 30 h (CONT30, n = 133). During the handling period all COCs were kept in follicular fluid. After maturation period, oocytes from all groups were denuded, fixed and stained with lacmoid. Oocytes were classified according to meiotic stage as germinal vesicle (GV), germinal vesicle break down (GVBD), metaphase I (MI), anaphase I (AI), telophase I (TI) and metaphase II (MII). Data of nuclear maturation were analyzed by Chi-square test ($P < 0.05$). At 0 h all of the COC were at in GV stage. On the CONT group the majority of the oocytes were in MI at 16 h (45.6%) and in MII at 24 h (96.8%) and 36 h (94.05). However, a different kinetics ($P < 0.05$) was observed for all IFIOT groups in every maturation time. Higher percentage ($P < 0.05$) of oocytes were at GVBD stage at 16 h (45.6%) than at 24 (10.6%) and 30 h (13.2%) after IFIOT. In contrast, MI stage was observed in a lower percentage ($P < 0.05$) of oocytes in the IFIOT16 (24.6%) compared to IFIOT 24 (57.6%) and 30 (57.6%). The percentage of oocytes that completed maturation and were at MI was different ($P < 0.05$) for all IFIOT groups being 0.6% for ITFOI16, 3.8% for IFIOT 24 and 12.5% for IFIOT30. These results suggest that IFIOT induces a delay in nuclear maturation in at least 8 h. In addition, 30 h of *in vivo* maturation is not sufficient for the oocyte to reach MII stage, if the IFOIT is performed 24 h after the removal of progesterone device. Acknowledgements: CNPq, FAPDF, Embrapa.



138 Assisted Reproductive Technologies

Effects of ovarian synchronization associated with a two-step IVM strategy on *in vitro* embryo production in cattle

A.C.S. Soares^{*1}, V. Lodde², L.G.M. Bragança³, K.N.G. Marques³, A.M. Luciano², J. Buratini¹

¹Sao Paulo State University, Botucatu, São Paulo, Brazil; ²University of Milan, Milan, Lombardy, Italy; ³Neogen Assisted Reproduction, Ribeirão Preto, São Paulo, Brazil.

Suboptimal *in vitro* conditions for oocyte maturation uncoupling nuclear and cytoplasmic maturation is a major challenge to increased efficiency of *in vitro* fertilization (IVF) in cattle. In addition, oocytes obtained by ovum pick up (OPU) are heterogeneous with respect to chromatin configuration, transcriptional profile and developmental competence. We have recently developed a synchronizing protocol including ultrasound-guided follicle aspiration and FSH treatment that allows the recovery of a more homogenous population of oocytes, most of them at an intermediate stage of chromatin compaction. This study aimed to assess the association of this synchronizing protocol with a two-step *in vitro* maturation (IVM) strategy previously tested in our laboratory on *in vitro* embryo production in cattle. Twenty-four non-lactating Holstein cows were subjected to two treatments in a “cross-over” design with a 30-d interval between replicates. The Control treatment was OPU at a random day followed by the farm IVM protocol with culture for 24 h in a medium containing 0.5 mg/mL FSH and 10% bovine fetal serum. Cows in the Treatment group had all follicles larger than 3 mm aspirated at a random day considered as day 0, received two IM injections of FSH (Folltropin; 56mg) 12 h apart on day 2, and were subjected to OPU on day 5. Cumulus-oocyte complexes (COCs) were cultured for 9 h in a pre-IVM medium containing 100 ng/mL NPPC and intrafollicular levels of FSH and steroids, and then 24 h in a serum-free medium containing intrafollicular concentrations of FSH and 100 ng/mL AREG (compositions described in Soares *et al.* 2017). After IVM, COCs from both groups were submitted to the farm protocol for IVF and *in vitro* culture (IVC). After 8 d of IVC, embryo production was assessed by total blastocyst rate in relation to the number of oocytes subjected to IVM and the percentage of viable embryos (those morphologically selected for freezing and later transfer) in relation to total blastocysts. Data were arcsine transformed and groups compared with the Student's t-test. Control and Treatment groups did not significantly differ for either blastocyst rate or percentage of viable embryos. Blastocyst rates were $28.9 \pm 3.95\%$ (n = 416 oocytes) and $17.84 \pm 2.15\%$ (n = 428 oocytes) for Control and Treatment groups, respectively, whereas rates of viable blastocysts were $61.87 \pm 7.18\%$ and $69.85 \pm 7.06\%$, respectively. In conclusion, protocols for ovarian synchronization still need to be fine-tuned with embryo culture methods to significantly impact on *in vitro* embryo production in cattle. Nevertheless, the association of ovarian synchronization with a serum-free two step IVM system provided embryo outcomes comparable to those provided by a regular IVM protocol using serum and a supraphysiological concentration of FSH. CAPES, FAPESP, Prof. José Luiz Moraes Vasconcelos, União Química.



139 Assisted Reproductive Technologies

Superstimulation prior to *ovum pick-up* to improve *in vitro* embryo production in buffalo donors

J.G. Soares¹, N.A.T. Carvalho², A.C. Basso³, Y.F. Watanabe⁴, O.Y. Watanabe⁵, B.M. Bayeux⁶, R.D. Mingoti⁶, P.S. Baruselli⁶

¹Universidade Federal de São Paulo, Escola Paulista de Medicina, Centro de Pesquisa em Urologia, São Paulo, SP, Brazil; ²Unidade de Pesquisa e Desenvolvimento de Registro/Centro de Zootecnia Diversificada/Instituto de Zootecnia, Registro, SP, Brazil; ³In Vitro Brasil Ltda, Mogi Mirim, SP, Brazil, ⁴Vitrogen – YVF Biotech, Cravinhos, SP, Brazil; ⁵WTA - Watanabe Tecnologia Aplicada, Cravinhos, SP, Brazil; ⁶Departamento de Reprodução Animal, Universidade de São Paulo, São Paulo, SP, Brazil.

The aim of this study was to evaluate follicular population and oocyte and embryo production of buffalo donors submitted to superstimulation with FSH prior to *ovum pick-up* (OPU) and *in vitro* embryo production (IVEP). A total of 54 buffalo donors (18 heifers, 15 primiparous and 21 multiparous) was randomly allocated to one of two groups (Control or FSH), in a *cross-over* experimental design. All animals received an intravaginal P4 device (1.0 g) plus EB (2.0 mg, intramuscular [im]) at a random stage of the estrous cycle (Day 0). Buffalo donors in the Control group received no further treatment, whereas buffalo donors in the FSH group received a total dosage of 200 mg of p-FSH on Days 4 and 5 in four decreasing doses 12 h apart (57, 57, 43, and 43 mg). On Day 7, the progesterone device was removed, and OPU was conducted in both groups. Data were analyzed by the GLIMMIX procedure of SAS 9.3[®]. There was no difference between groups ($P = 0.53$) regarding the total follicles aspirated, however, the FSH treatment increased ($P < 0.001$) the proportion of large (> 10 mm; FSH = 16.2% and Control = 2.0%) and medium-sized (6-10 mm; FSH = 36.3% and Control=6.1%) follicles available for the OPU procedure. The viable oocyte rate was greater in buffalo donors treated with FSH compared to Control group (Heifers = 58 vs. 50%; Primiparous = 56 vs. 47%; Multiparous = 57 vs. 50% respectively; $P = 0.03$). Also, buffalo donors treated with FSH had a higher blastocyst rate (Heifers = 34 vs. 17%; Primiparous = 28 vs. 27%; Multiparous = 32 vs. 24%; $P = 0.03$) and embryo yield per OPU-IVEP session (Heifers = 3.7 ± 0.7 vs. 1.8 ± 0.5 ; Primiparous= 2.7 ± 0.8 vs. 2.4 ± 0.6 ; Multiparous= 2.6 ± 0.7 vs. 2.0 ± 0.5 ; $P = 0.07$). These results provide evidence that superstimulation with FSH increased the proportion of medium-sized follicles available for the OPU procedure. Consequently, the treatment also enhanced the proportion of viable oocytes for culture and resulted in greater blastocyst rates and embryo yield per OPU-IVEP session in buffalo. It was concluded that superstimulation with FSH prior to OPU increased the IVEP efficiency in buffalo donors.



140 Assisted Reproductive Technologies

A pre-maturation with C-type natriuretic peptide plus estradiol prolongs meiotic arrest in juvenile goat oocytes

S. Soto-Heras*¹, J. Thompson², I. Menéndez-Blanco¹, M-G. Catalá¹, D. Izquierdo¹, M-T. Paramio¹

¹Departament de Ciència Animal i dels Aliments, Facultat de Veterinària, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain; ²The Robinson Research Institute, ARC Centre for Nanoscale Biophotonics, Adelaide Medical School, The University of Adelaide, South Australia, Australia.

In vitro embryo production (IVP) could be an important assisted reproductive technology to rapidly disseminate desirable genetic traits in goat breeds. Oocyte competence is a key factor for the success of IVP, which is dependent on synchronizing both nuclear and cytoplasmic maturation. Oocytes retrieved from antral follicles are arrested at the germinal vesicle (GV) stage, but immediately resume meiosis spontaneously. Furthermore, oocytes from juvenile goats have low embryo development due to the small size of the follicles from which they come, with an unknown grade of atresia that make them unpredictable for IVP. Previous studies in other livestock species have reported that a prolonged meiosis inhibition by implementing a 'pre-maturation' period, with IBMX (a general phosphodiesterase inhibitor) or C-type natriuretic peptide (CNP; which physiologically maintains the oocyte in GV), improves oocyte competence. The aim of the present study was to investigate the impact of CNP +/- estradiol on meiosis of oocytes from juvenile goats (2 months old), as the first step to develop a pre-in vitro maturation (pre-IVM) system that could improve their competence. Ovaries from juvenile goats were collected at a local slaughterhouse. Cumulus oocyte complexes (COCs; N = 668) were recovered by slicing follicles in TCM-199 medium with HEPES, NaHCO₃, and heparin plus 500 µM IBMX (to avoid GV breakdown during oocyte recovery). Morphologically selected COCs were incubated for 6 or 8 h in a pre-IVM medium at 38.5°C and 5% CO₂. The pre-IVM medium (control group) was: TCM-199 with 100 µM cysteamine and 0.4 % (w/v) bovine serum albumin. In experiment 1, pre-IVM medium was supplemented with different doses of CNP (50 nM, 100 nM and 200 nM). In experiment 2 pre-IVM medium contained the same doses of CNP plus 10 nM estradiol (E2; 50 nM CNP + E2, 100 nM CNP + E2, 200 nM CNP + E2 and an E2-only group). After pre-IVM oocytes were fixed in ethanol:acetic (3:1) and stained with 1% orcein in 45% acetic acid for nuclear assessment. Twelve oocytes were assessed per group per time point in 3 replicates. Data was analyzed with two-way ANOVA followed by Tukey's post-hoc test. The GV rate between control and experimental treatments were not different in experiment 1. In contrast, in experiment 2, 200 nM CNP plus E2 had a higher GV rate (P < 0.05) at 6 h (75.4%) compared to the control group (27.8%) and the E2 group (31.6%). No significant differences were observed with the other concentrations of CNP (50 nM 48.7%, 100 nM 50.2%). However, the increased GV rate with 200 nM + E2 at 6 h was ameliorated by 8 h (44.4%) compared to the control group (25.4%). In other species, CNP can inhibit GVBD by itself, but the addition of E2 enhances the effect of CNP by increasing the expression of the CNP receptor (NPR2). Our results imply that basal NPR2 expression in juvenile goat oocytes is low but with the addition of E2 enables CNP to transiently block nuclear maturation. In conclusion, we have developed a protocol for blocking the nuclear maturation using CNP plus E2 in juvenile goat oocytes. More studies are required to understand the mechanisms of action of these molecules and to assess if this pre-IVM protocol improves the developmental competence of juvenile goat oocytes. Funding: Spanish MINECO (AGL2017-85837-R) and Spanish MECO (FPU14/00423).



141 Assisted Reproductive Technologies

Effect of CRISPR/Cas9 microinjection on development and mutation rate of sheep embryos

M. Souza-Neves^{*1}, P. Santos-Neto¹, F. Cuadro¹, M. Crispo², A. Menchaca¹

¹Fundación IRAUy, Montevideo, Montevideo, Uruguay; ²Institut Pasteur, Montevideo, Montevideo, Uruguay.

The objective was to evaluate the effect of zygote cytoplasmic microinjection on development and mutation rate of *in vitro* produced sheep embryos. Ovaries were collected from a slaughterhouse and oocytes were recovered and selected for *in vitro* maturation. Only good quality oocytes were incubated for embryo production by *in vitro* maturation, fertilization and culture as previously described (Menchaca *et al.*, 2016. Anim Reprod, 13:273-278). The time of insemination was defined as Day 0. Microinjection of presumptive zygotes was performed into the cytoplasm with CRISPR/Cas9 system 16-18 h after fertilization. A total of 983 presumptive zygotes were divided in different experimental groups: Buffer (n = 323), Protein (n = 347), RNA (n = 313) and a control group without microinjection (n = 146). Microinjection of Buffer group was done with buffer alone (10 mM Tris, 0.1 mM EDTA); in the Protein group it was performed with 100 ng/μL of sgRNA85, 100ng/μL of sgRNA258 and 50 ng/μL of Cas9 protein diluted in injection buffer (20 mM Hepes, 150 mM KCl); while the RNA group was microinjected with 7.5 ng/μL of sgRNA85, 7.5 ng/μL of sgRNA258 and 20ng/μL of Cas9 mRNA diluted in injection buffer (10 mM Tris, 0.1 mM EDTA). After microinjection, the zygotes were maintained under culture conditions with 5% CO₂, 5% O₂, 90% N₂ at 39°C in a humidified atmosphere until Day 8. On Day 6, DNA from morulae/blastocyst obtained from the Protein group (n = 11) and the RNA group (n = 9) was extracted and analyzed by Sanger sequencing to detect mutations at the gene level. Cleavage rate on Day 3 (2 to 8-cell embryos/microinjected zygotes) and development rate on Day 6 (morulae and blastocysts/microinjected zygotes) were evaluated and the results were expressed as Mean±SEM. Statistical analysis was performed by generalized linear mixed models and differences were considered significant at a level of P < 0.05. Cleavage and development rate were lower in microinjected than control zygotes (Buffer 60.7 ± 4.6 and 23.2 ± 2.1, Protein 65.5 ± 3.3 and 28.0 ± 2.5, RNA 62.9 ± 4.0 and 22.3 ± 3.5, Control 79.4 ± 2.9 and 43.9 ± 5.4, respectively; P < 0.05). There was no difference between microinjected groups (Buffer, Protein and RNA; P = NS). As a result of the sequencing analysis, none of the Protein microinjected embryos resulted in mutation whereas 53.85% (7/13) of RNA microinjected embryos analyzed resulted in mutation (P < 0.05). The current experiment demonstrated that microinjection into zygotes negatively affected the cleavage and development rate of *in vitro* produced embryos, independently of the use of Buffer, Protein or RNA. Furthermore, the greater efficiency for mutation induction was obtained with Cas9 RNA microinjection for CRISPR/Cas9 system.



142 Assisted Reproductive Technologies

Relations between plasma anti-muellerian-hormone (AMH) concentrations, fertility performance and influencing factors in Holstein-Friesian heifers

A. Vernunft*¹, A. Boldt², T. Viergutz¹, V. Röttgen¹, J.M. Weitzel¹

¹Institute of Reproductive Biology; Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany; ²Institute of Livestock Farming, Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries, Dummerstorf, Germany.

An independent prognostic biomarker for fertility would be worthwhile in cattle breeding. Some recent publications described the measurement of the ovarian reserve as an interesting candidate. One specific marker of the ovarian reserve is the Anti-Muellerian-Hormone (AMH). However, results between the existing studies in this field are inconsistent. The aim of this study was to investigate, whether plasma AMH levels could be used in German Holstein heifers as a predictive and independent biomarker for their fertility as heifer or later as dairy cow under the conditions of milk production. Therefore, blood samples, animal related data and fertility parameters from 926 German Holstein heifers (age of 16 ± 2.3 month) were collected on five farms. Their plasma AMH concentration was measured by using a human, but for bovines validated AMH-ELISA-Kit (DSL-10-144400). Fertility parameters and performance data of the first lactation could be obtained and analyzed subsequently for 672 of the tested heifers. For statistical analysis correlations and linear regressions were calculated between the AMH levels and fertility/performance data. Differences between animal groups (different farms, numbers of insemination, months of pregnancy) were tested by ANOVA. To validate the applicability of AMH as an independent biomarker, environmental and animal related influencing factors on the AMH concentration were analyzed in a first step. No significant correlation between AMH and farm, age, weight, height, BCS, progesterone level and early pregnancy status at blood sampling was detected. Also the later milk yield, milk fat or milk protein showed no statistical significant correlation to the AMH level as heifer ($r < 0.1$, $P > 0.1$). In a second step relationships between AMH and fertility were examined, but the statistical analysis revealed also no significant correlation between AMH levels and the acquired fertility parameters during the observation period (age at first insemination, calving age, number of inseminations, days to first post-partum insemination, period from first to successful insemination, days open, conception rates; $r < 0.1$, $P > 0.1$). The results support the perception that AMH plasma levels of heifers could be considered as mainly independent of environmental and animal related influencing factors, but the results showed also clearly that a single assessment of the AMH plasma concentration in Holstein-Friesian heifers is not suitable as biomarker to predict fertility under the conditions of German milk production. This does not exclude a causal biological relationship between ovarian reserve and fertility, which may become apparent in other sampling schedules, production systems, breeds or the use of other assessment methods of the ovarian reserve.



143 Assisted Reproductive Technologies

Prostacyclin PGI₂ modulates *in vitro* maturation of bovine cumulus-oocyte complexes

**I. Woclawek-Potocka*, K. Grycmacher, D. Boruszewska, E. Sinderewicz, J. Staszkiwicz-Chodor,
I. Kowalczyk-Zieba**

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences.

Cumulus cells are essential for the proper proceeding of the bovine oocyte maturation. During maturation this germ cell acquires adequate developmental competence, cumulus cells undergo expansion, taking active part in the glucose metabolism, which is the energy substrate for the oocyte. The objective of the present study was to examine the effect of prostaglandin (PG) I₂ on cumulus expansion and glucose uptake as well as expression of genes involved in these processes. Cumulus-oocyte complexes (COCs; n = 10 for control and examined groups, replied 8 times) were obtained by aspiration from subordinate ovarian follicles (of the diameter less than 6 mm) and matured *in vitro* in presence or absence of PGI₂ (500 pg/mL) for 24 h. Following maturation, cumulus cells were separated from oocytes and used for gene expression analysis (amphiregulin - AREG, epiregulin - EREG, betacellulin - BTC, metalloproteinase family member - ADAM, epidermal growth factor receptor - EGFR, tumor necrosis factor alpha induced protein 6 - TNFAIP6, prostaglandin endoperoxide synthase 2 - PTGS2, pentraxin - 3-PTX3, hyaluronan synthase 2 - HAS2, glutamine-fructose-6phosphate transaminase- GFPT, phosphofructokinase - PFK, lactate dehydrogenase- LDH; Real-Time PCR). The data from Real-Time PCR were analysed by Miner PCR Software Algorithm with GAPDH as the reference gene. The obtained results were analyzed using student T test (GraphPad PRISM 6.0. Software). Although cumulus expansion did not vary in the examined groups of COCs, PGI₂ simulated mRNA expression of ADAM, PTGS2, GLUT1 and GLUT4 and reduced transcript abundance of CTSS and CTSK. The data obtained in the present study suggest that supplementation of the maturation medium with PGI₂ might enhance bovine oocyte maturation *in vitro* via the modulation of cumulus expansion and glucose metabolism in cumulus cells. Supported by Grants-in-Aid for Scientific Research from the Polish National Science Centre: 2015/17/B/NZ9/01688 and by the grant of KNOW Consortium "Healthy Animal - Safe Food", MS&HE Decision No. 05-1/KNOW2/2015.



144 Assisted Reproductive Technologies

Effect of successive ovum pick up on follicular development, and oocyte quality and quantity, in a commercial, buffalo (*Bubalus bubalis*) *in vitro*, embryo production program

R. Yuponi*¹, N. Vallejos¹, J.A. Berdugo², J.L. Konrad¹, G. Crudeli¹

¹Facultad de Ciencias Veterinarias, UNNE, Corrientes, Corrientes Argentina; ²BIOGEM, Universidad Nacional de Colombia.

The first buffalo calf born after *in vitro* embryo production (IVP) was in 2004 and despite the fact that many research teams are working to improve buffalo embryo production, there are very few commercial IVP programs for buffalo. In addition, although researchers have attempted applying methods used in cattle to buffaloes, there are species differences in follicular size and number, quantity and quality of oocytes, embryo development, and the need to use specially designed medium that make IVP in buffalo of lower efficiency than in cattle. The aim of this study was to evaluate the effect of short term, repeated ovum pick up (OPU) on follicular number and quality of oocytes in a commercial buffalo IVP program. The study was conducted in Corrientes Argentina during the breeding season of 2017. Twenty mature and fertile Mediterranean and Murrah donors, body condition score 3/5 to 4/5 with no anatomical abnormalities were available for OPU, and were subjected to 3 sequential, weekly transvaginal, ultrasound guided aspirations, using a vacuum pump at 40 mm/Hg pressure attached to an 18-g needle. Antral follicle count (AFC) was performed before aspiration. Oocytes were recovered from follicular fluid and graded from I to IV according number of granulosa cells and cytoplasm. Follicle number, grade and quantity of oocytes were recorded, and data was analyzed using paired Student t test and Pearson correlation coefficients. Statistical significance was established at $P < 0.05$. Fifty-nine OPU were performed, and the time for each averaged 9 min. Five hundred follicles were punctured, and 260 oocytes were recovered (52%); of those, 9%, 20%, 34%, 35% were Grade I, II, III, IV, respectively. There were no differences in AFC (mean 7.2 per buffalo). There were differences in the number of follicles aspirated within the third (7.8) aspiration compared with the first (8.8) and the second (8.4). No correlations were found between number of follicles and recovered oocytes. Our results show that, in buffalos, the number of ovarian follicles declines after 21 days of aspiration, which has also been reported by others. Because buffalo females tend to produce lower quality oocytes than cattle, all oocytes recovered after OPU are used for embryo production. Although it may be necessary to increase the sample size to find correlations between follicle numbers and oocyte recovery, this research contributes with more information for commercial OPU buffalo programs to improve the efficiency of IVP. Rincon del Madregon Cabaña, UNNE, Universidad Nacional de Colombia.



145 Assisted Reproductive Technologies

Birth of fresh or vitrified CRISPR/Cas9 microinjected sheep embryos transferred on Day 3 or Day 6

P.C. Santos Neto^{*1}, F. Cuadro¹, M. Souza-Neves¹, M. Crispo², A. Menchaca¹

¹Instituto de Reproducción Animal Uruguay, Fundación IRAUy, Montevideo, Uruguay; ²Unidad de Animales Transgenicos y Experimentación, Institut Pasteur de Montevideo, Uruguay.

The objective was to evaluate the pregnancy outcomes of fresh or vitrified CRISPR/Cas9 microinjected sheep embryos transferred on Day 3 or Day 6 of *in vitro* development. A total of 501 microinjected embryos were randomly assigned to three experimental groups; fresh embryos on Day 3 (n = 120), fresh embryos on Day 6 (n = 75) or vitrified embryos on Day 6 (n = 306). Embryos were obtained by *in vitro* fertilization following the procedure routinely used in our laboratory (Menchaca *et al.*, 2016. Anim Reprod, 13:273-278). Microinjection of the CRISPR/Cas9 system was performed into the cytoplasm of presumptive zygotes 17 h after fertilization (Day 0) using a specific CRISPR/Cas9 design for a knock out model, and the embryos were *in vitro* cultured until vitrification or transfer. Vitrification/warming were performed using Cryotop method in embryos on Day 6 (Santos-Neto *et al.*, 2015. Cryobiology,70:17-22). Fresh 8 to 12-cell embryos on Day 3 (five embryos per ewe) and fresh or vitrified morulae/blastocysts embryos on Day 6 (two-three embryos per ewe) were transferred into synchronized Corriedale breed recipients. For both stages, embryo transfer was performed by laparoscopy placing embryos into the cranial side of the ipsilateral uterine horn to the corpus luteum. Pregnancy rate (number of pregnant ewes/total transferred ewes) and embryo survival rate on Day 30 of gestation (number of embryos on Day 30/total transferred embryos) were determined by ultrasonography, and birth rate (total born lambs/viable embryos on Day 30) was registered at delivery. Statistical analysis was performed by GLMM. Pregnancy rate was greater for recipients receiving fresh embryos on Day 3 and Day 6 compared with those vitrified on Day 6 (54.2%, 13/24; 48.0%, 12/25; versus 22.0%, 24/109, respectively; P < 0.05). Embryo survival on Day 30 of fresh embryos transferred on Day 3 (14.2%, 17/120) did not differ from those transferred on Day 6 (21.3%, 16/75; P = NS), while it was lower with vitrified embryos on Day 6 (10.5%, 32/306; P < 0.05) if compared with those transferred fresh on Day 6. Birth rate was similar between groups (fresh embryos on Day 3, 88.2%, 15/17; fresh embryos on Day 6, 75.0%, 12/16; vitrified embryos on Day 6, 87.5%, 28/32; P = NS). These preliminary results suggest that the transfer of microinjected fresh embryos allows greater pregnancy rate when compared to vitrified embryos. Since survival rate of embryos transferred on Day 3 and Day 6 was similar, the advantage of a reduced period of *in vitro* culture for early embryo transfer deserves further investigation. This information has implications for the generation of CRISPR/Cas9 edited animals in livestock.