Animal Reproduction

THEMATIC SECTION: 39TH ANNUAL MEETING OF THE ASSOCIATION OF EMBRYO TECHNOLOGY IN EUROPE (AETE)

OPU - IVF AND ET

Single administration of FSH delivered in hyaluronic acid for ovarian stimulation prior to ovum pick-up in Italian Mediterranean buffalo

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Keywords: buffalo, OPU, FSH

Buffalo is a livestock species that has undergone a massive increase in number of heads and milk production in the last decade; moreover, the Italian Mediterranean buffalo (IMB) is a breed greatly requested around the world because of its high milk production. Ovum pick-up (OPU) and in vitro embryo production (IVEP) undoubtedly is the best tool to rapidly speed up the propagation of the breed and the genetic progress through the maternal lineage. The aim of the work was to evaluate the efficiency of hyaluronic acid (HA) as delivery vehicle for FSH compared to the standard FSH administration protocol (CTRL, Petrovas G. et al., Animals 30;10(11):1997. 2020) to support the growth of a homogeneous population of medium-sized follicles and oocyte developmental competence, in terms of embryo yields. HA is a suitable and safe vehicle for FSH in prepubertal buffalo (Currin L. et al., Theriogenology, 197, 84-93, 2023). Briefly CTRL (40 mg of FSH - Folltropin, Vétoquinol S.A., Magny-Vernois, France - administered intramuscularly 2 times/day for 3 consecutive days after the removal of the dominant follicle, with OPU performed after 28-32 hours of coasting) was compared with HA protocol involving a single intramuscular administration of the entire dose of FSH (240 mg) in 15 ml of 1% HA solution. IMBs (8/group, over 4 replicates) underwent OPU as previously described (Petrovas G. et al. Animals 30;10(11):1997. 2020). Follicular and oocyte population were recorded, good quality oocytes were in vitro matured, fertilized and cultured to the blastocyst stage according to standard procedures (Di Francesco S. Theriogenology 77.1: 148-154. 2012). The CTRL and HA treatments gave similar results (mean±SE) in terms of total number of aspirated follicles per animal (15.2±1.4 vs 14.4±1.1; respectively), as well as that of small (7.2±0.8 vs 6.6±0.9, respectively) medium (3.7±0.7 vs 3.8±0.5, respectively) and large follicles (4.76±0.6 vs 3.8±0.4, respectively). Similarly, the oocyte population, both in terms of total number of oocytes recovered per animal (10.3±1.1 vs 10.0±1.3, respectively in the CTRL and HA) and of oocytes suitable for IVEP (7.9±0.9 vs 7.6±1.0, respectively in CTRL and HA) is similar in the two groups. Finally, also with regard to embryo yields, the two protocols gave similar results (36.1% vs 35.1%, respectively in CTRL and HA). These preliminary results demonstrated that a single administration of FSH delivered in HA is as efficient as the standard FSH treatment on follicular and oocyte population as well as embryo yields, and hence can be used for ovarian stimulation in IMB. The possibility of replacing the six FSH administrations of CTRL protocol with a single administration conveyed by HA allows to minimize animal handling with a reduction of stress for them and risks for operators, especially in contexts of wild and semi-wild breeding in which the animals are often unaccustomed to be handled.

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OPU - IVF AND ET

The use of a brief synchronization treatment after weaning, combined with superovulation, affects the gene expression of surviving pig blastocysts

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Keywords: blastocysts, synchronization, pig

The combined use of estrus synchronization and superovulation (SS) treatments affects the expression of genes in the ovaries and endometrium, impairing follicle and oocyte growth, as well fertilization and embryo development. However, the effects of such treatments on embryo transcriptome remain unknown. This study aimed to analyze alterations in gene expression of day 6 blastocysts that survived a brief synchronization treatment followed by superovulation. The sows were assigned to one of the three groups: SS7 sows (N=6), which received altrenogest (ALT) treatment for 7 days starting from the day of weaning, and were superovulated with eCG 24 hours after the end of ALT treatment and hCG at the onset of estrus; SO sows (N=6), consisting of sows not treated with ALT but superovulated with eCG 24 hours after weaning and hCG at the onset of estrus; Control sows (N=6) comprising of weaned sows that showed natural estrus. Surgical embryo collection was carried out on day 6 of the cycle (day 0 = onset of estrus). The number of viable embryos to the total number of embryos and oocytes/degenerated embryos collected was lower ($p<0.05$) in the SS7 sows (140/187; 75%) than in the SO (174/186; 94%) and control (105/114; 92%) sows. Microarray analysis (5 embryos per sow) were conducted on blastocysts with good morphology, following the IETS criteria. To identify differentially expressed genes (DEGs) between groups, ANOVA with an unadjusted P-value < 0.05 and a fold change </> 1.5 was used. Compared to controls, SO treatment had minimal impact on blastocyst gene expression. Only 4 pathways were disturbed, with 4 modified transcripts, which were unrelated to reproductive functions or embryonic development. In contrast, SS7 blastocysts exhibited moderate gene expression alterations, including both DEGs and fold changes, compared to controls. Seven pathways were disrupted, affecting 10 transcripts in total. Upregulation of certain pathways, such as the metabolic pathway, involved two upregulated genes (RDH10 and SPTLC2) related to reproductive functions, which notably may indicate suboptimal embryo quality. The downregulation of other pathways, such as the glutathione metabolism pathway, with downregulated genes (GSTK1 and GSTO1) involved in cellular detoxification of reactive oxygen species, could hinder the embryos' response to oxidative stress, potentially impairing subsequent embryo development. The gene expression changes observed in the present study in SS7 embryos, and the results of previous reports indicating SS7 treatment negatively affect fertilization, embryo production, and reproductive tract gene expression, led us to consider its use in embryo transfer (ET) programs inconvenient. These findings have implications for the swine ET industry, as they prevent the simultaneous use of two hormonal treatments (synchronization and superovulation) that function effectively when applied separately.

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OPU - IVF AND ET

Birthweight data of IVP calves

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Keywords: IVP, Birth weight, imprinting

Since the introduction of IVP in the 1990s, it has been observed that IVP calves have a higher birthweight, often referred to as large-offspring syndrome (or LOS), as compared to in vivo derived AI and flush (or MOET) calves. This increased birthweight was reduced using serum-free IVC culture media (Wagtendonk de Leeuw *et al.*, Theriogenology. 2000 Jan 15;53(2):575-97), but it was not completely normalized to the level of AI. To improve animal welfare and further increase the use of IVP embryos in cattle breeding, the increased birth weights need to be normalized. However, this problem is difficult to tackle due to the lack of solid data on reported calf weights. In most countries, no or only estimated data on birth weight are available. Therefore, we started collecting real birth weight data of Holstein cattle on 10 different farms in The Netherlands, where all IVP, flush and AI calves were weighed just after birth. In addition, gestation length, parturition ease (scored on a scale from 1-5, with 1 being the easiest), sex of the calf, colostrum intake within 2 h after birth (in liters) and afterbirth were registered. All embryos were collected, cultured and processed in standard CRV media (Mullaart E *et al.*, 37th AETE, vol. 19; 2022: e22223). Data from 171 IVP, 83 flush and 855 AI calves were registered. The results confirmed that IVP calves were significantly heavier than the flush and AI calves (43.7 ± 6.2, 40.2 ± 4.7 and 40.0 ± 5.8 kg, respectively). Within the AI and flush group, the male calves where significantly heavier than the female calves (+4.1 and +3.4 kg, with p=0.0001 and 0.001, respectively, by t-test), which was expected. However, the weight of the male and the female IVP calves did not differ significantly (1.1 kg heavier males, p=0.23 by t-test). This indicates a disproportionate increase in the birth weight of female over male IVP calves, obscuring the sexual dimorphism in body weight that normally occurs in newborn calves. Using a 97th percentile as a cut-off for LOS (i.e., 47 and 52 kg for female and male, respectively, based on birth weight data from AI calves), 10% of IVP males and 24% of female calves would be categorised as displaying LOS. No differences were observed between gestation length, parturition, sex ratio, colostrum intake, and afterbirth for the 3 groups. It is not clear why females are relatively heavier than male IVP calves. Aberrant DNA methylation and genomic imprinting frequently occur during in vitro embryo development and are often discussed as causative for the increased birth weight of male and female IVP calves. In addition, only female embryos carry an imprinted, inactive X chromosome that undergoes transient reactivation and random inactivation at the blastocyst stage. It is possible that the female-specific X-inactivation dynamics is perturbed in IVP embryos, making them more sensitive for imprinting deviations and higher birth weights.

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OPU - IVF AND ET

HCG application at the time of embryo transfer in Brangus cattle recipients: Effects on pregnancy rate under tropical heat-stress conditions

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Keywords: HCG, pregnancy rate, heat-stress

The use of Human Chorionic Gonadotropin (HCG) has been carried out occasionally in embryo recipient protocols in *Bos indicus* cattle. It has been suggested that HCG stimulates ovulation, accessory corpora lutea formation, and therefore, a progesterone increase (Cunha, *J. Dairy Sci*., 105, 8401-8410, 2022). The main aim of the present research was to assess the potential effects of HCG at the time of embryo transfer in Brangus cattle recipients on seasonal pregnancy rates under subtropical heat-stress conditions. A total of 1,810 embryo transfers (blastocyst stage) were carried out in Brangus cattle (*Bos indicus x Bos taurus*; Age: 4-8 y.o.; body condition score: 3.0-3.5) maintained under the same nutritional, management, and environmental conditions [Köppen-Geiger (Aw), tropical savanna climate, Paraguay; Coord.:S~24°6´0”; W~57°04´60”; Prec.:~1,500 mm; R.H.:~90%; M.T.:~23.4°C (summer: ~30°C; winter: ~15°C); Alt.:~60 m.a.s.l.]. Four groups were considered for the study: control winter (CW; n = 430), control summer (CS; n= 750), HCG winter (HW; n= 210), and HCG summer (HS; n= 420). All recipients were synchronized following a conventional protocol: Day 0: intravaginal progesterone (P4) device (CIDR: 1.38 g) + 2.5 mg intramuscular (IM) estradiol benzoate E2B + 50 mg P4 (IM). Day 7 fresh embryos were transferred and at the time of embryo transfer, 2,000 IU of HCG (IM) were administered in HW and HS groups. The diagnosis of pregnancy was confirmed by ultrasonography on day 35 after embryo transfer. The data were analyzed by GLMM/ Chi-square ($\chi 2$) (SPSS® 25, IBM Corp., USA). Significant differences were observed in pregnancy rates when just seasons were compared [524/1,170 (44.8%) vs. 427/640 (66.7%) for summer and winter, respectively; $P \leq 0.05$)]. Moreover, significant differences were detected regarding CS compared to HS [291/750 (38.8%) vs. 233/420 (55.4%); $P < 0.05$)] and when CW was compared to HW [269/430 (62.5%) vs. 158/210 (75.2%); $P < 0.05$)]. No differences were observed when HS and CW groups were compared ($P > 0.05$). In conclusion, HCG application at the time of embryo transfer improved the pregnancy rate irrespective of the season considered. This effect may be due to the formation of accessory corpora lutea, and, as a consequence, increased levels of progesterone concentration. The differential effects of HCG were more pronounced during the summer. The significant increase in the pregnancy rate observed derived from HCG treatment could be interesting for mitigating the heat-stress-derived detrimental effects during the summer season; however, the use of HCG at the time of embryo transfer would be recommended in winter season as well since the pregnancy rate improvement during this season was demonstrated in Brangus cattle recipients maintained in tropical environments.

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OPU - IVF AND ET

Ovum pick-up (OPU) in Bos indicus dairy cattle breeds: Oocyte recovery rates and in vitro embryo production using unsorted and sex-sorted sperm

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Keywords: OPU, sex-sorted sperm, embryo production

The ovum pick-up (OPU) technique improves the cattle's genetic gain, shortening the generation interval and increasing the genetic progress. The main objective was to assess the efficiency of OPU in *Bos indicus* dairy cattle breeds evaluating the potential oocyte recovery rates and *in vitro* embryo production using unsorted and sex-sorted sperm. A total of 656 OPU sessions (one per cow) were carried out in two *Bos indicus* cattle breeds [Gyr (GY): 537 and Guzerat (GZ): 119; Age: 3-6 y.o.; body condition score: 3.0-3.5]. *In vitro* embryo production procedure was carried out using unsorted and sex-sorted semen (same breed sire for each group; 1 x 10^6 spz/mL in both types). Four experimental groups were randomly performed: Gyr-unsorted (GYU; n = 173), Gyr-sorted (GYS; n= 374), Guzerat-unsorted (GZU; n= 54), and Guzerat-sorted (GZS; n= 65). All cows were super-stimulated as follows: Day 0: intravaginal CIDR (1.38g progesterone/cow) + intramuscular progesterone (100 mg/cow) + intramuscular 17-beta-estradiol (2 mg/cow); Day 1: Intramuscular eCG (2500 IU/cow); Day 4: OPU. The oocyte-derived parameters assessed were: oocyte yield (OY; total number of oocytes) and quality (GI-II and GIII; based on the number/presentation of COCs layers and ooplasm homogeneity), viable oocyte/donor (OD; viable oocytes per donor), matured oocytes (MO; matured oocytes based on COCs expansion, perivitelline space, 1st polar body extrusion, cytoplasmic color, and zona pellucida shape), viable MO/donor (MOD; viable matured oocytes per donor). The embryo-derived parameters evaluated were: cleaved-embryos/MO (CLMO; cleaved embryos obtained from the total number of matured oocytes), cleaved-embryos/donor (CLD; cleaved embryos obtained from the number of viable matured oocytes per donor), total embryos (TE), viable TE/donor (TED; viable embryos per donor), TE/GI-II (TEG; grade I and II embryos), TE/MO (TEMO; embryos obtained from the total number of matured oocytes), and DE/donor (DED; degenerated embryos per donor). The data were analyzed by GLMM (SPSS® 25, IBM Corp., USA). Differences were observed in OD (29.14±3.89 vs. 17.12±0.85), MOD (26.80±3.53 vs. 14.14±0.72), CLMO (18.93±8.69 vs. 7.89±3.72), CLD (18.63±2.90 vs. 7.26±0.55), TE (10.74±3.73 vs. 6.28±1.35), TED (6.49±1.35 vs. 3.59±0.27), TEG (5.20±1.26 vs. 2.97±0.25), and DED (1.28±0.50 vs. 0.61±0.10) when both breeds were compared being greater in GZ ($P \leq 0.05$). Moreover, differences were detected in TED (5.53±0.74 vs. 3.10±0.23) and TEG (4.70±0.68 vs. 2.39±0.20) when both types of semen were compared being greater using unsorted semen ($P < 0.05$). Differences were observed in CLD, TE, TED, TEG, and DED being greater in GZU compared to GYU, GYS, and GZS ($p<0.05$). No differences were observed among GYU, GYS, and GZS regarding the same parameters ($P > 0.05$). In conclusion, Guzerat breed was superior in most of oocyte-derived and embryo-derived parameters. TE and TEG were improved by using unsorted semen. Finally, the differential results obtained in GZU regarding oocyte and embryo production performance per donor suggest that OPU technique showed better results using Guzerat breed and unsorted semen.

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OPU - IVF AND ET

Embryo transfer in Brangus cattle recipients: Effects of transfer side on pregnancy rate and embryonic/fetal loss under subtropical conditions

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Keywords: Embryo transfer, pregnancy rate, uterine horn

Embryo transfer (ET) practice increases the number of offspring per cow; however, the ET efficiency depends on several factors. The main objective of the present study was to evaluate the potential effects of the embryo transfer side (right and left uterine horn) on the pregnancy rate (PR) on Day 30 and 60 post-transfer in Brangus cattle in subtropical conditions. A total of 426 embryo transfers were carried out in Brangus cows (Bos indicus x Bos taurus; Age: 4-8 y.o.; body condition score: 3.0-3.5) maintained under the same nutritional, management, and environmental conditions [Köppen-Geiger (Aw), tropical savanna climate, Paraguay; Coord.:S~24°6´0"; W~57°04´60"; Prec.:~1,500 mm; R.H.:~90%; M.T.:~23.4°C (summer: ~30°C; winter: ~15°C); Alt.:~60 m.a.s.l.]. OPU sessions were carried out in Brangus donors, which were super-stimulated as follows: Day 0: intravaginal CIDR (1.38g/ cow) + intramuscular progesterone (100 mg/ cow) + intramuscular 17-beta-estradiol (2 mg/cow); Day 1: Intramuscular eCG (2500 IU/cow); Day 4: OPU. The embryos were produced following a standard IVP protocol. OPU-derived COCs were subjected to standard in vitro maturation and fertilization using conventional unsorted semen (1 x 10^6 spz/mL). The embryos were cultured for 7 days post-fertilization before embryo transfer. The resulting embryos were transferred fresh to estrous synchronized cows. Recipients were synchronized following a conventional protocol: Day 0: intravaginal progesterone (P4) device (CIDR: 1.38 g) + 2.5 mg intramuscular (IM) estradiol benzoate E2B + 50 mg P4 (IM) and subjected to embryo transfer. The transfer was randomly performed to the right (n= 263) or left (n= 163) uterine horn. Pregnancy diagnosis was carried out using ultrasonography on Day 30 and 60 following embryo transfers. The data were analyzed by GLMM/ Chi-square ($\chi 2$) (SPSS® 25, IBM Corp., USA). No differences were observed in PR when the right and left uterine horns were compared on Day 30 (53.6%, 141/263 vs. 52.1%, 85/163, $p>0.05$). Moreover, no differences were detected in PR between uterine horns on Day 60 (40.7%, 107/263 vs. 36.2%, 59/163 for right and left uterine horns, respectively, $p>0.05$). Finally, differences were observed when PR was diagnosed on Day 30 and Day 60 regarding both right (53.6% vs. 40.7%, $p< 0.05$) and left (52.1% vs. 36.2%, $p< 0.05$) uterine horn. No differences were observed regarding embryo loss rates in the right (13.1%) and in the left (15.9%) uterine horns when Day 30 was compared to Day 60 ($p>0.05$). In conclusion, PR was greater on Day 30 compared to Day 60 post-embryo transfer in Brangus recipients. PR was not associated with the right or left uterine horn after embryo transfer irrespective of the day of pregnancy. Important differences were observed regarding embryo loss rate in the 30-day period between PR diagnoses. The embryo loss rate increased over time regardless of the uterine horn considered.

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OPU - IVF AND ET

Are there any differences between blastocysts derived from prepubertal and pubertal heifers in the aspect of mitochondrial function ?

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Keywords: pre- pubertal heifers, cows, mitochondria, blastocysts, transcriptome

In the cattle-breeding industry, there is increasing commercial demand for in vitro embryo production from young and even prepubertal heifers. Therefore, the goal of the present study was to assess the difference between mitochondrial DNA content, oxidative stress, and developmental competence in blastocysts derived from prepubertal vs. pubertal heifers. We also compared transcriptomic profiles of those embryos. Two groups of OPU derived blastocysts were analysed in the study (n=120 from 24 prepubertal and 12 pubertal heifers). Mitochondrial content was determined based on mitochondrial DNA copy number evaluation (qPCR). Oxidative stress was measured by intracellular glutathione concentration, reactive oxygen species levels and mRNA expression of antioxidant-associated genes. The developmental competence of embryos was evaluated by mRNA expression of blastocyst quality markers (OCT4, SOX2, NANOG, PLAC8, IGF1R, IGF2R, 342 PLAU, SSLP1, DSC2, DNMT3A, and AQP3). Total RNA was isolated from examined embryos (n=15 embryos in each group) and RNA-seq was performed. Analysis of the transcriptome profile was determined by DAVID. The rate of oocytes developing to blastocysts in vitro was significantly lower when oocytes originated from prepubertal vs pubertal animals (12% vs 32%, $P<0.05$). Blastocysts from two groups did not differ in terms of morphological quality. Morphologically appropriate blastocysts derived from prepubertal heifers had higher concentrations of reactive oxygen species and glutathione ($P<0.05$) compared to blastocysts from pubertal heifers. In the blastocysts produced from prepubertal heifers we found higher mitochondrial DNA copy number (453667 ± 17243 vs 403667 ± 7371, $P<0.05$) and also alterations in expression of developmental competence gene markers ($P<0.05$). The total of 436 differentially expressed genes (DEGS) were identified in the examined embryos. KEGG pathway analysis confirmed that many DEGS were involved in mitochondrial function via the influence on oxidative phosphorylation, expressed by significant differences of the level of genes such as: ATP synthases (ATP5MF, ATP5PD, ATP12A), NADH dehydrogenases (NDUFS3, NDUFA13, NDUFA3) and cytochrome c oxidase (COX17) ($P<0.05$). The impaired oxidative phosphorylation in blastocysts derived from prepubertal heifers, in addition to higher mtDNA copy number and altered gene expression of developmental competence markers, suggest lower quality of the blastocysts derived from prepubertal animals, despite their unaltered morphology.

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Optimization of heterologous IVF with Iberian ibex (Capra pyrenaica) sperm and domestic goat (Capra hircus) oocytes

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Keywords: ibex, goat, heterologous IVF

Assisted reproductive technologies are key to maintain genetic stocks of wild species, such as the Iberian ibex (*Capra pyrenaica*). *In vitro* fertilization (IVF) could be used to test the fertilization ability of stored semen, but optimal IVF conditions remain largely unexplored. Heterologous *in vitro* fertilization (IVF) of domestic goat (*Capra* hircus) oocytes constitutes an excellent platform to optimize IVF procedures for Iberian ibex. Previous studies showed that supplementation of SOF medium with 2% estrus sheep serum (ESS) during heterologous IVF with prepubertal goat oocytes improved blastocyst rates. The aim of this study was to optimize IVF procedures for Iberian ibex and to analyze whether ESS is required for fertilization. Cumulus-oocyte complexes obtained from adult domestic goat ovaries were matured *in vitro* and then incubated with $2x10^6$ frozen-thawed ibex epididymal spermatozoa/ml from the same male in four different media: TALP medium supplemented with 10 µg/ml heparin (TALP, n=141) or synthetic oviductal fluid (SOF) supplemented with 10 µg/ml heparin alone (SOF-0; n=193) or combined with 2% (SOF-2; n=121) or 20% (SOF-20; n=131) of estrus sheep serum (ESS), at 38.5 ºC under an atmosphere of 5% CO_2 with maximum humidity (3 experimental replicates). Presumptive zygotes were cultured in SOF supplemented with 0.4% BSA up to day (D) 4 and in SOF supplemented with FBS from D4, at 38.5 ºC under an atmosphere of 5% CO_2 and 5% O_2 with maximum humidity. Cleavage rate was recorded at day (D) 2 and blastocyst rates were recorded at D8. Blastocysts were fixed and the development of specific lineages was assessed by immunostaining for SOX2 (epiblast), SOX17 (hypoblast) and CDX2 (trophectoderm). No significant differences were found in cleavage (91.1±2.5 vs. 90.9±0.4 vs. 91.3±3.3 vs. 87.2±3.2 mean±s.e.m. for TALP vs. SOF-0 vs. SOF-2 vs. SOF-20) or blastocyst rates (52.4±4 vs. 49±3.1 vs. 36±8 vs. 37.1±4.9%; mean±s.e.m. for TALP vs. SOF-0 vs. SOF-2 vs. SOF-20). Total, SOX2+ and SOX17+ cell number was similar between groups (Total: 170.9±16.4 vs. 132.2±10.1 vs. 141.1±23 vs. 179±22.2; SOX2+: 17.6±1.4 vs. 24.1±3.5 vs. 13.1±2 vs. 16.4±2.1; SOX17+: 21.9±4.6 vs. 19.7±3.4 vs. 21.1±5.6 vs. 30±7.4; mean±s.e.m. for TALP vs. SOF-0 vs. SOF-2 vs. SOF-20), but CDX2+ cell number was significantly higher in TALP and SOF-20 than in SOF-2 (120.7±15.8 vs. 71.2±11.8 vs. 67.6±19.6 vs. 123.2 ± 22; for TALP vs. SOF-0 vs. SOF-2 vs. SOF-20; ANOVA $p<0.05$). In conclusion, heterologous IVF with Iberian ibex (*Capra pyrenaica*) sperm and domestic goat (*Capra hircus*) oocytes does not require ESS.

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Animal Reproduction

THEMATIC SECTION: 39TH ANNUAL MEETING OF THE ASSOCIATION OF EMBRYO TECHNOLOGY IN EUROPE (AETE)

OPU - IVF AND ET

Correlation between sperm traits and in vitro fertilization outcomes in X-sexed and non-sexed sperm

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Keywords: cattle, sperm motility, cleavage

Cattle sperm characteristics play an important role in the outcomes of *in vitro* fertilization (IVF). Utilization of gender selected semen allows production of male and female offspring to take advantage of sex-influenced characteristics (Kasimanickam R, 2021, Bovine Reproduction, 20, 1000-10). The aim of this study was to investigate the correlation between sperm motility and velocity characteristics with fertilization and cleavage rate in non-sexed and X-sexed sperm. The heterogeneous cattle ovaries of unknown reproductive status were collected at the local abattoir. A total of 75 oocytes were *in vitro* fertilized per treatment with X sexed and non-sexed frozen-thawed semen of proven fertility collected from the same bull (Angus) purchased from American Breeders Service Global Inc Company. Prior to IVF, the sperm motility was analyzed with the aid of a computer assisted sperm analysis (CASA; Microscopic, S.L, Barcelona, Spain). The frozen-thawed X-sexed and non sexed frozen thawed semen with a sperm concentration of 1 x 10^6 sperm/mL (5000 spermatozoa) were analyzed and the experiment was replicated 5 times. The correlation between sperm motility and velocity characteristics with total fertilization and oocyte cleavage rate was analyzed using the analysis of variance (general linear model) and statistical analysis system (SAS®). Treatment means were separated using Fisher's protected t-test and the significant differences were determined by P-value at a significant level of $P < 0.05$. In X-sexed sperm, the results for total sperm motility (52.61±4.25) and total fertilization percentage which was determined by two and more than two pronuclei (24.66± 3.05) were shown to be negatively correlated ($r=-0.20$; $P < 0.05$). Furthermore, a positive correlation was observed between the total motility of X-sexed sperm (52.61±4.25) and the total cleavage percentage of oocytes which was determined by 2-4 cell of cleaved presumptive zygotes (15.00±5.00; $P < 0.05$), with a correlation coefficient of 0.01. Interestingly, a positive correlation was observed between hyperactive (5.00±3.46) and total cleavage rate (15.00±5.00), with a correlation coefficient of 0.00 ($P < 0.05$). In non-sexed sperm, the results for total sperm motility (61.88±2.73) and total fertilization percentage (29.33±6.10) were shown to be negatively correlated ($r=-0.92$; $P < 0.05$). Moreover, a negative correlation was observed between non-sexed total sperm motility (61.88±2.73) and the total cleavage rate (42.67±1.15), with a correlation coefficient of -0.74 ($P < 0.05$). Additionally, a strong negative correlation was observed between hyperactive (3.00±3.46) and total cleavage rate (42.66±1.15), with a negative correlation coefficient of -0.50 in non-sexed sperm ($P < 0.05$). The negative correlation between sperm motility and total oocyte fertilization rate in both non-sexed and X-sexed sperm suggests that high total sperm motility is essential for successful fertilization and embryonic development. In conclusion, the sperm motility and velocity characteristics are strongly correlated with fertilization and cleavage rate in both non-sexed and X-sexed sperm, indicating that higher sperm motility and velocity characteristics plays a role in early embryo development.

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OPU - IVF AND ET

Effect of embryo transfer application on pregnancy success in repeat breeder cows and heifers

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Keywords: Repeat breeder, embryo transfer, cow, heifer

In Turkey, like other developing countries, embryo transfer (ET) in dairy cattle is not routinely practiced under field conditions. Many factors such as cost, success rate and technical issues are responsible. However, high replacement cost of milking cows with elite heifers or cows makes ET feasible in Turkey in commercial dairy enterprises and recently demand is increasing for ET. Repeat breeding is one of the major factors for culling. In this study, it was aimed to determine the effect of ET applied to repeat breeder cows and heifers on pregnancy success. For this purpose, 11 holstein heifers (the average age: 22 months) and 14 holstein cows (the average days in milk: 329 days) inseminated at least five times but not pregnant were used as recipients. Holstein donor and recipients (cows and heifers) were superovulated and synchronized, respectively. Briefly, superovulation was induced in six donors by intramuscular FSH (Stimufol, Reprobiol, Belgium) at a dose of 10 ml per cow at 12 h intervals for 4 days commencing on 9th day after estrus. Double AI was done at 12 h intervals in donor cows and flushing was done one week later. Only first quality blastocysts were used for ET. Recipient repeat breeder cows and heifers were synchronized with two injections of PGF2α at a dose of 2 ml per animal at 11 days apart. ET was performed 7 days after estrus detection. Pregnancy was detected in 7 heifers (63.6%) and 7 cows (50.0%). Chi-square test was used for statistical evaluation. Even the sample sizes are small, these ratios are very encouraging for repeat breeders animals. Obtaining preliminary results is very useful for the Turkish dairy industry where culling and maintaining the repeat breeder cows and heifers are difficult and very expensive. It is suggested that further investigations should be warranted to evaluate the viability of ET as a therapeutic measure in valuable repeat breeder cows and heifers as an alternative approach to culling.

Animal Reproduction

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OPU - IVF AND ET

Acylated ghrelin diminishes the superovulatory response in dairy sheep

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Keywords: ghrelin, antimullerian hormone, superovulation

We have previously shown that the stomach derived ghrelin attenuates gonadotrophins' secretion and suppresses in vitro embryo production in ruminants. As ghrelin secretion is positively associated to fasting and to negative energy balance, here we investigated the effects of long-term infusion of ghrelin on the superovulatory response, embryo yield and anti-mullerian hormone (AMH) secretion in dairy sheep. During the breeding season, twelve ewes were fitted for 28 days with either an osmotic minipump that delivered a truncated form of acylated ghrelin molecule, at a dose of 1.5μg/kg/day (treated -T, 28-day ghrelin infusion), or with an empty pump (non-treated controls -C). Ten days after pump insertion the estrus cycle of all animals was synchronized with intravaginal progestogen sponges. Starting 48 hours before sponge removal, the ewes were superovulated with 6 decreasing doses of porcine FSH (133mg/ewe) given twice daily, and the animals were naturally mated to fertile rams. Blood samples were collected at pump insertion and along with the first and the last FSH injection for Antimullerian Hormone (AMH) determination, using a commercial ELISA kit. Embryos were collected six days after estrous/mating by laparotomy. After uterine flushing, the ovaries were exteriorized, the corpora lutea and all visible follicles were counted; the follicles were aspirated and the follicular fluid of small and large follicles was separately stored for AMH assessment. The data between groups were tested by t-test. The superovulatory response (number of CLs) and the collected embryos (morulae and blastocysts) were higher ($p<0.001$) in group C ewes (CL 8.3±1.3, embryos 5.5±1.8) than in group T (CL 2.8± 1.3, embryos 1.3±0.6). More ($p<0.05$)small follicles were found on the ovaries of group C (7.4± 1.5) compared to group T (5.5± 1.0), while the number of large follicles did not differ between groups. At pump insertion, the serum AMH concentration did not differ between groups ($p=0.8$), but it was higher at the first (C, 3.9±1.2pg/ml vs T 2.1± 1.0, $p=0.06$) and the last FSH dose (C 4.3±1.0 pg/ml vs T 2.0± 1.1pg/ml, $p=0.03$) in control ewes. AMH concentrations were higher ($p<0.03$) in group C small follicles (C, 2.01±0.45 ng/ml; T, 0.96±0.45 ng/ml), but not in large ($p>0.05$). These results imply that ghrelin wanes the pool of small follicles and their responsiveness to FSH, and it confirms findings from other species that the determination of AMH concentrations could be used as potential predictor of ovarian response to superovulation in sheep.

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OPU - IVF AND ET

Comparative study of In-vitro embryo production (IVEP) in Gir & Sahiwal (Bos indicus) Indian cattle breeds

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Keywords: Ovum Pick up, In vitro fertilization, Oocyte, Embryo culture, Gir breed, Sahiwal breed

Gir and Sahiwal (*Bos indicus*) are among the major milk-producing Indian cattle breeds. The study aimed to compare the oocyte recovery rate and embryo production using IVP in Gir (n=17) & Sahiwal (n=10) cattle donors. The study was carried out at Central Research Station, BAIF Development Research Foundation, Uruli Kanchan, Pune, Maharashtra, from October 2019 to October 2022. All these experimental donors were maintained under the same management practices. A total of 698 Ovum Pick Up (OPU) sessions (Gir 354 & Sahiwal 344) were performed. All OPUs were done using ultrasound device with intravaginal micro convex transducer with 7.5 MHz frequency (USG Model: Exago by IMV). OPU procedure was carried out about once every 15 days irrespective of season. In all the OPU sessions during the experimental period, 20-gauge OPU needle was used and the vacuum pump pressure was maintained in between 70 to 90 mm of Hg. Temperature range maintained of vacuum pump was in between 37.0 to 38.5 ^{O}C. OPU was performed without using any pre-stimulation protocols for all these non-lactating donors. The donors were ranged between 1st to 4th parity. All the IVP cycles were randomly processed with media available and no specific media was used for any specific breed. All the recovered oocytes after grading, were further processed in laboratory for IVP with a protocol of 20 to 22 hours of maturation period, 16 to 18 hours for fertilization and 7 days post fertilization for culture. For both breeds, grading of oocytes was done between grade 1 to grade 5 based on presence of COC layers surrounding oocyte and cytoplasm content of oocytes. All the steps of IVP were performed in one laboratory. The culture conditions were same for both breeds like 5% CO2, 5% O2, 90% N2 and the incubation at 38.5 °C with high humidity. On day 7th blastocyst rate was observed. Grading of embryos was done as per IETS embryo grading manual. Both the parameters were analyzed using mixed model with animal (donors) as a random effect while breed as a fixed effect while Tukey test was used to identify the critical differences between the breed. The analysis was carried out using R software version 4.2.3. The oocyte recovery rate was not affected by breed, but the embryo production were influenced by breed variable. The average oocyte recovery in Gir breed was 9.76 ± 1.27 and in Sahiwal 6.90 ± 1.20 per OPU session ($P<0.05$). Gir breed produced 2.75 ± 0.32 viable embryos per OPU session vs 1.72 ± 0.33 in Sahiwal ($P<0.05$). In conclusion, oocyte recovery and embryo production by IVP, were higher in Gir cows compared to Sahiwal ones.

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OPU - IVF AND ET

In vitro maturation of bovine oocytes using a portable CO2 incubator

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Keywords: maturation, incubator

During in vitro maturation (IVM) oocytes are placed in media supplemented with hormones for 22 to 24 hours to complete the meiotic maturation, using incubators with stable temperature and a CO_2 concentration of 5%. These incubators in IVP laboratories differ in size and models but most of them are static. In some cases there is no opportunity to use a static incubator at the place of oocyte collection, and the IVP laboratory is too far away. For example in ovum-pick-up (OPU) programs with different teams working at a distance an alternative to transport of oocytes only in warmed media without CO_2 is needed. The aim of this study was to investigate whether IVM of bovine oocytes could be performed successfully in a portable incubator. Tissue-culture-medium 199 (TCM) supplemented with hCG and eCG was used as maturation medium. Cumulus-oocyte-complexes (COCs) were collected from ovaries of slaughtered cattle and divided into 3 different maturation groups: in the first group 60 COCs were matured in 100 µl droplets TCM in a petri-dish with an oil overlay (lab/oil), 20 COCs per droplet. 60 COCs were placed in 250 µl TCM in 0.5 ml tubes without oil in the second group (lab/free), also 20 COCs per tube. For both groups an incubator (Heracell 150i, Thermo Scientific™, Schwerte, Germany) with 38.0 °C and 5% CO_2 was used. The third group was also matured in tubes without oil, but in a portable CO_2 incubator (CellTrans+, Labotect GmbH, Göttingen, Germany) employing the same maturation conditions (cell/free). After 24 h maturation rates were analyzed via Hoechst staining. Embryos were generated using a standard IVF and IVC protocol up to day 8 (IVF = day 0). At day 7 and 8 cleavage and developmental rates were recorded. Additionally at day 8 blastocysts and expanded blastocysts were stained with Hoechst 33342 and ethidium homodimer to count live and dead cells. Four trials with 240 COCs per group were performed. Data were analyzed with Oneway-Anova followed by a Tukey test. Maturation rates (89.7 ± 1.5%, 78.6 ± 0.6%, and 78.1 ± 0.6%, respectively), cleavage (79.3 ± 8.3%, 74.7 ± 3.6%, 74.6 ± 2.3%) and developmental rates (23.9 ± 6.3%, 22.3 ± 1.4%, 21.8 ± 1.5%) did not differ significantly ($P > 0.05$) between the oocytes and embryos from the 3 groups, nor did the ratio of live and dead cells of the stained embryos (19.5 ± 6.4, 21.4 ± 8.3, 20.0 ± 5.4). This shows that portable incubators could be used instead of static incubators during maturation of COCs without a loss of oocyte quality and developmental competence. It offers an alternative method especially to OPU-IVP teams working at different stations.

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OPU - IVF AND ET

Preliminary results on glycaemic response after oral glucose tolerance test (OGTT) in sows derived from assisted reproductive technologies

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Keywords: Insulin, glucagon, pig.

In human, murine and rabbit species, individuals derived from embryos produced in vitro (IVP) may present, among others, disorders in glucose metabolism (Chen et al. 2014, Diabetes 63:3189–3,98; García-Domínguez et al. 2020, Animals 10:1043-1059). In pigs, available information is very scarce and we have reported in 45-days-old piglets differences in the glycaemic response after an oral glucose tolerance test (OGTT) between IVP-produced animals and those conceived in vivo by artificial insemination (AI) (Paris-Oller et al. 2022, JDOHaD 13:593-605). However, it is unknown if these differences are corrected or maintained during adult life. The objective of the present study was to evaluate the glucose tolerance in the same colony of pigs during their adult life by means of an oral glucose tolerance test (OGTT). The animals were obtained from a previous study (Paris-Oller et al. 2021, J Anim Sci Biotech 12:32-44) that were born after artificial insemination (AI group) and surgical transfer of in vitro-produced embryos (IVP group). All animals were kept under same housing and feeding conditions since birth. The OGTT was performed in AI (n=8) and IVP (n=10) sows with 3.5-3.6 years age, and weighing from 227 to 249 kg. For the OGTT, animals were previously fasting for 24h and 2h without drinking water. Sows ingested 1.75 g/kg body weight of glucose solution (100% glucose carbs, Myprotein) and blood samples were collected via auricular vein before OGTT (t=0) and over 240 minutes (15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min) following glucose administration. Blood glucose concentration was immediately measured by a glucometer (Aposan) using test strips, and blood serum was obtained and freeze (-80ºC) until determination of insulin (immunoturbidimetric method) and glucagon (10-1281-01 Mercodia, Uppsala, Sweden). Data were analysed using an ANOVA test with nested design (animal within reproductive treatment group) and reproductive treatment (AI, ET) and time of sampling and interaction treatment and time as the main factors. Data are expressed as mean ± SEM. Values of $p<0.05$ were considered significant. Glycaemia was influenced by time of sampling ($p=0.019$) and was higher in AI-derived animals than in IVP group (66.95±1.29 *vs.* 60.57±1.31 g/dL, $p<0.001$), while the interaction group and time was not significant ($p=0.401$). On the other hand, the insulin concentration was only influenced by the origin of the animals, with higher values in IVP than AI animals (58.66±4.88 *vs.* 75.79±4.27 µUI/ml, $p=0.013$). As for the glucagon concentration, it was similar for all the times of sampling and between groups (AI: 2.84±0.39 *vs.* IVP: 2.75±0.24 pmol/L, $p=0.664$). These observations suggest that, up to some extend, the differences in the response to OGTT in IVP-produced pigs are maintained during their adult life. Moreover, IVP sows challenged with an OGTT show changes in the insulin response. Increasing the number of animals, and determination of complementary biochemical parameters are needed for a better interpretation of the results.

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OPU - IVF AND ET

Laser-Assisted Biopsy is an efficient technique to perform genetic analysis of Bovine Embryos in Selected Breeding Programs

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Keywords: In vivo produced bovine embryos, Laser-Assisted Biopsy, WGA, Embryo genomic selection

Embryo genomic selection is increasingly being used to select the best embryos within cattle breeding programs. However, during the early stages of embryo development, the number of cells, and then the amount of DNA from embryo biopsy, is limited. Whole genome amplification (WGA) is used to amplify this limited amount of DNA for downstream genetic analysis. Although the reliability of this genetic analysis is evaluated by the call rate (CR, proportion of SNPs detected from the chip) to be used in genomic evaluation) and depends for a significant part on the cell sample quality. Since 2021, at Auriva -Elevage, a new laser-assisted biopsy technique has been used to minimize damage to the embryo during the biopsy process. In a recent previous study, we demonstrated that the laser-assisted biopsy method is an efficient and minimally invasive method with a higher in vivo embryo survival rate after 48 h of culture compared with the conventional microblade process. It results in a similar pregnancy rate compared to that obtained for non-biopsied embryos (Gamarra et al., Reprod., Fert. and Develop. 2022, 35(2)). However, with these samples, a significantly lower SNP call rate was observed in the laser method when compared to the microblade technique. The aim of this study was to evaluate on a larger number of embryos, the impact of laser-assisted biopsy of in vivo produced embryos on Single Nucleotide Polymorphism (SNP) call rate in comparison with the conventional microblade method used in Auriva-Elevage breeding programs. For the laser biopsy procedure, a hole in the zona pellucida of Day 7 in vivo produced embryos (n=1486) was created by two laser pulses of 3.7 ms (Octax Laser-Germany), through which a biopsy pipette was inserted to aspirate 3 to 8 trophoblast cells. For microblade biopsy, embryos (n=3028) were immobilized by a holding pipette, and a steel blade was used to cut 3 to 10 trophoblast cells. Collected cells were stored at -20°C until whole genome amplification (WGA). DNA extraction and WGA were performed using the REPLI-g Single Cell Kit (Qiagen, Manchester, UK). WGA-DNA from each group were genotyped on Illumine EUROG MD V4 chip in order to evaluate the CR. Biopsied embryos were slow frozen using Ethylene Glycol (1.5 M) plus sucrose (0.1M) for later direct embryo transfer. Biopsies with a call rate ≥ 80% are considered to allow an accurate estimation of the genomic breeding values. The results show that the percentage of call rate (≥80%) was non-significantly different (Chi-2, p=0.17) for laser-assisted biopsy (89%, 1319/1486) compared to microblade one (90%, 2728/3028). This study confirms that laser-assisted biopsy of in vivo-produced bovine embryos is an efficient and minimally invasive method to obtain genetic material for whole genome amplification. It can be considered as a good alternative to the conventional microblade method to perform genetic analysis of bovine embryos.