A125E OPU - IVF and ET

Nobiletin supplementation in maturation media enhances in vitro oocyte maturation and subsequent embryo development

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Keywords: nobiletin, mitochondria, cortical granule.

Nobiletin is a polymethoxylated flavonoid isolated from citrus fruits exhibiting a wide biological effect in cell adhesion, cell migration, cell cycle regulation and inhibition of reactive oxygen species (ROS) production (Huang et al. Evid Based Complement Alternat Med. 2016); important factors for oocyte *in vitro* maturation (IVM). However, there is no information available on the effect of nobiletin in *in vitro* embryo production (IVP). The aim of this study was to evaluate the effect of nobiletin supplementation in IVM of bovine oocytes on nuclear and cytoplasmatic maturation and their developmental competence. Immature cumulus oocytes complexes (COCs) were aspirated from ovaries of slaughtered heifers. Selected COCs were *in vitro* matured in TCM-199+10% FCS and 10 ng/mL epidermal growth factor (Control) supplemented either with 10, 25, 50 and 100 µM of nobiletin (MedChemExpress, MCE, Sweden) (Nob10, Nob25, Nob50 and Nob100 respectively) or 0.01% dimethyl sulfoxide (DMSO), vehicle for nobiletin dilution. After 24 h of IVM at 5% CO_2 in air at 38.5°C, a representative number of oocytes from each group were fixed and stained with Hoëscht-LCA-FITC or Hoëscht-MitoTracker DeepRed to evaluate nuclear and cytoplasmic maturation (n = 60/group/treatment). Also, 50 oocytes/group were stained with CellROX Deep Red Reagent and CellTracker Fluorescent to measure oocyte metabolism in terms of ROS and glutathione (GSH) content. The remaining oocytes were fertilized (D0) and cultured *in vitro* to evaluate their developmental competence by cleavage rate (D2) and blastocyst yield (D7-8). Data from eight replicates were analyzed by one-way ANOVA. Significantly higher percentage of matured oocytes ($P < 0.05$) were observed in metaphase II when Nob25 (87 ± 0.6%) or Nob50 (89.3 ± 0.3%) were added to the IVM medium compared to Nob10 (72.9 ± 0.3%), Nob100 (71.5 ± 0.8%), control (71.7 ± 0.7%) and DMSO (70.5 ± 0.5%) groups. Furthermore, Nob25 and Nob50 showed higher rate of oocytes with peripheral migration of cortical granules (85.7 ± 0.3% and 89.9 ± 2.2% respectively) and mitochondria (86.7 ± 0.6% and 88.9 ± 1.2% respectively) compared to the remaining groups ($P < 0.05$). In addition, the supplementation of Nob25 and Nob50 showed a significant reduction ($P < 0.05$) in the ROS (2.53 ± 0.8; 2.62 ± 1.2 a.u. respectively), and GSH (2.84 ± 0.4; 3.09 ± 0.1 a.u. respectively) content in comparison with all other groups. Cleavage rate was significantly higher ($P < 0.05$) for Nob25 (89.9 ± 0.3%) and Nob50 (91.3 ± 0.3%) compared to all other groups (Nob10: 75.6 ± 0.3%; Nob100: 74.0 ± 0.6%; control: 74.2 ± 0.4%; and DMSO: 73.6 ± 0.4%). Similarly, cumulative blastocyst yield at D8 was significantly higher ($P < 0.05$) for Nob25 (32.1 ± 0.8%) and Nob50 (35.5 ± 0.8%) compared to Nob10 (23.1 ± 0.7%), Nob100 (24.5 ± 0.9%), control (25.9 ± 0.4%) and DMSO (26.1 ± 0.6%) groups. In conclusion, supplementation of 25 µM or 50 µM of nobiletin to the IVM medium improves oocyte nuclear and cytoplasmic maturation, reduces oxidative stress and improve embryo development.

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A126E OPU - IVF and ET

Nuclear maturation rate of caprine oocytes after *in vitro* maturation in three different media with base of TCM 199.

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Keywords: polar body, antioxidant, EGF.

The goats are economically important animals as a source of meat and milk especially in the Northeast of Brazil where most of the national herd is reared. The necessity of In vitro production of caprine embryo (IVP) increases due to the importance of genetic selection for high milk production and the importance of the goat as transgenic pattern. IVP results have not yet met neither the scientific objective nor the commercial one. The low IVP rates are mostly caused by the low efficiency of in vitro maturation (IVM). Objective: Verify three different IVM media for caprine oocytes; Materials and Methods: Groups of caprine oocytes that were aspirated from the ovaries of recently slaughtered goats were matured in vitro in three different media for 26 hours in a CO2 incubator (38.5Cº, 5% CO2, and Saturated humidity) as follows: T1 [Tissue culture medium 199 (TCM199) + LH (50µg/ml) + FSH (1µg/ml) + Pyruvate (0.2 mM) + 10% FBS (Fetal Bovine Serum)], T2: [TCM 199 + EGF (Epidermal growth factor) (10ng/ml) + Cysteamine (1 mM/ml)], and T3 [TCM 199 + EGF (10 ng/ml) + Cysteamine (1 mM) + Cysteine (1 mM) + Ascorbic Acid (1 mM)]; 64, 62, and 64 oocytes were matured in T1, T2, and T3 respectively. After the maturation, the oocytes of each group were denuded in 100 µl of PBS (phosphate saline buffering solution) and the first polar body was observed under a phase-contrast microscopy (*40); the oocytes that showed the first polar body were considered matured (Metaphase II oocytes); the results were analyzed with One Way ANOVA with P-Value = 0.05. Results: There was no significant difference in maturation rate ($P > 0.05$) among the groups (T1, T2, and T3 were 35.9%, 48.38%, and 50%, respectively). Conclusion: The nuclear maturation rate was not affected by the different media with TCM 199 base. The presence of the exogenous hormones (FSH and LH) did not improve the nuclear maturation rate, as well as the addition of an antioxidant (Ascorbic Acid), and the precursor of glutathione (Cysteamine) did not improve the nuclear maturation rate of caprine oocytes too, however we can conclude that a simple in vitro maturation medium of caprine oocytes may be enough for the nuclear maturation of caprine oocytes, and the in vitro maturation of goat oocytes can be developed in the absence of a protein source in TCM-199 medium. The maturation can be performed in a medium with absence of gonadotrophins, FSH and LH, reaching stages of nuclear maturation (Metaphase II) and finally; the maturation of goat oocytes can occur in the absence of antioxidants, but more researches have to be conducted to improve the nuclear maturation rate of caprine oocytes. Polar Body, Antioxidant, EGF.

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A127E OPU - IVF and ET

Effects of an enriched n-3 polyunsaturated fatty acid diet on in vitro embryo production in dairy cowsEffects of an enriched n-3 polyunsaturated fatty acid diet on in vitro embryo production in dairy cows

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Keywords: OPU, oocyte, n-3 PUFA.

High-yielding dairy cows fertility remains relatively low, with first service mean success rate between 35 and 40%. In a previous study (Elis s. Animal Reproduction Science 164: 121-132, 2016), long chain n-3 polyunsaturated fatty acids (n-3 PUFA LC) supplementation of the diet of dairy cows tended to decrease the non-fertilization – early embryo mortality rate after first service (n3 PUFA LC: 13.5% (n = 22) vs control 38.8% (n = 23), P = 0.09), suggesting a potential effect on oocyte quality. In this study, we evaluated the effects of n-3 PUFA supplementation on in vitro embryo production in dairy cows, after hormonal ovarian stimulation. 37 primiparous Holstein cows were supplemented with n-3 PUFA (n = 18, micro encapsulated fish oil, 1% DM, OMG750®, Kemin) or n-6 PUFA (n = 19, micro encapsulated soy oil, 1% DM, OMG Soy®, Kemin). Three ovum-pick up sessions were performed on cows every two weeks (5 groups of 6 to 9 cows), after 2, 5 or 7 weeks of supplemented diet (between 92.0 0 ± 2.4 and 127.0 ± 2.4 day postpartum). Fatty acid composition in plasma was measured to assess the efficiency of the diet. Plasma anti mullerian hormone (AMH) assay was performed on the first day of diet supplementation to evaluate potential response of cows to hormonal ovarian stimulation. After follicular puncture, oocyte-cumulus complex (OCC) underwent in vitro maturation, fertilization (IVF) and development. Fertilization rate was determined 48 hours after IVF by counting cleaved embryos. Embryo development rate and embryo quality were determined 7 days after IVF by counting blastocysts. To compare n-3 and n-6 cows, multifactorial linear repression (quantitative parameters) or logistic regression (rates) models were used (fixed effects: diet, supplementation duration and interaction, cow as a random effect). Fatty acid composition showed a significant 1.62-fold increase in plasma EPA after 2 weeks of n-3 supplementation ($P < 0.0001$) while the increase in plasma DHA became significant (1.46-fold, $P < 0.0001$) only after 7 weeks diet. A total of 1462 follicles were punctured on n-3 cows (54 puncture sessions) and 1538 follicles on n-6 cows (57 puncture sessions). OCC recover rate was significantly increased in n-3 cows (41.6% vs 36.2% in n-6 cows, P = 0.0035). No significant difference was reported on cleavage rate (P = 0.1033) between n-3 cows (77%) and n-6 cows (85%). Nevertheless, blastocyst rate (relative to cleaved embryo) tended to increase (P = 0.0865) in n-3 cows (48.2%) compared to n-6 cows (38.7%). A significant increase in good quality blastocysts (grades 1 and 2, relative to cleaved embryo) was observed (P = 0.0217) in n-3 cows (42.7%) compared to n-6 cows (33.3%). The number of total blastocysts and of grade 1 and 2 blastocysts produced per OPU session was 2.87 ± 0.34 and 2.48 ± 0.31 in n-3 cows versus 2.28 ± 0.31 and 1.88 ± 0.28, respectively, in n-6 cows. These results suggest that n-3 supplementation in the diet could improve embryo quality.

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Follicular wave synchronization and superstimulation prior to ovum pick-up for improving *in vitro* embryo production in non-lactating Holstein cows

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Keywords: OPU/IVP, synchronization, FSH stimulation.

Transvaginal ultrasound needle-guided ovum pick-up (OPU) and IVP represent powerful tools to multiply selected females in a short period of time. Maximizing the quantity and quality of retrieved oocytes is key for obtaining the best embryo development and implantation rates. The aim of this study was to compare OPU yields and developmental competence of oocyte-cumulus complexes (COCs). Holstein (>5 year-old) pluriparous open dry cows (n = 25), handled under the same feeding and environmental conditions, were used for OPU as oocyte donors. Control and treatment groups were organized as follows: G1: no synchronization (Synch) (n = 5), G2: Synch with no superstimulation (SOV) (n = 5), G3: Synch with SOV (n = 5) and OPU 36 h after the last FSH injection, G4: Synch with SOV (n = 5) and OPU 48 h after the last FSH injection, and G5: Synch with SOV (n = 5) and OPU 72 h after the last FSH injection. G1 received saline solution intramuscularly (i.m.). Follicular waves in all groups were synchronized by GnRH, PGF, and CIDR followed by SOV treatments 48 h later. FSH injections (pFSH = 180 mg, Folltropin, Bioniche) were performed i.m. twice a day, for three days. The OPU procedures were performed using an ultrasound device (Mindray DP-30 Vet) equipped with a micro-convex transducer 5.0-8.5 MHz probe, disposable 20G needle and a flow rate of 15 mL/min. The 50 mL collection tube with aspiration media (PBS + BSA + heparin) was maintained at 36°C. Retrieved oocytes were classified according to IETS guidelines. Only viable COCs containing compact and complete cumulus cell layers were selected and matured. The IVP protocol followed Ferré *et al*., Reproduction, Fertility and Development 29, 132-132, 2017. On Days 3 and 7 cleaved zygotes and blastocysts, respectively, were evaluated according to IETS standards. Cows were arranged in a crossover design and data analyzed using ANOVA and logistic regression with $\alpha = 0.05$. Fisher's LSD test with Bonferroni correction was used to determine treatment differences. Synch versus no Synch, respectively, resulted in significantly more follicles/OPU (12.88 ± 0.96 vs. 9.60 ± 0.96), oocytes/OPU (10.92 ± 0.80 vs. 7.72 ± 0.80) and embryos/OPU (2.24 ± 0.20 vs. 1.40 ± 0.20) but not for cleavage rate (65.39 ± 3.13% vs. 63.03 ± 3.76%) and embryo rate (24.14 ± 2.81% vs. 21.21 ± 3.18%). Synch combined with SOV showed: follicles/OPU (18.00 ± 0.96*, 19.44 ± 0.96* and 22.12 ± 0.9**), oocytes/OPU (15.24 ± 0.80*, 17.44 ± 0.80** and 14.32 ± 0.80*), cleavage rate (67.38 ± 2.60%*, 72.70 ± 2.25%** and 75.25 ± 2.48%***), embryo rate (26.46 ± 2.45%*, 29.85 ± 2.31%** and 33.66 ± 2.71%***) and embryos/OPU (3.44 ± 0.20*, 4.68 ± 0.20** and 4.08 ± 0.20***) for 36 h, 48 h and 72 h FSH coasting, respectively (different superscripts = $P < 0.05$). In conclusion, Synch and FSH stimulation prior to OPU in non-lactating Holstein cows increased the number of collected and viable oocytes, cleavage, and embryo development rates. More transferrable embryos were obtained using 48 h FSH SOV coasting which may justify the extra cost associated with a Synch protocol and FSH treatment.

A129E OPU - IVF and ET

Risk of *Coxiella burnetii* transmission via embryo transfer using *in vitro* early caprine embryos

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Previous experiments using *in vitro* infection have shown that *Coxiella burnetii* has a strong tendency to adhere to the zona pellucida (ZP) of *in vivo* derived goat embryos, and the washing procedure recommended by the IETS for bovine embryos failed to remove it (Alsaleh et al., 2013). The aim of this study was, for *in-vitro* produced caprine embryos infected *in-vitro*, to (i) evaluate the ability of *C. burnetii* to adhere to intact *zona pellucida*, (ii) test the efficacy of IETS recommended rules for the washing of bovine embryos to eliminate *C. burnetii*, and (iii) determine by confocal microscopy the bacteria location. One hundred ZP-intact caprine embryos, produced *in vitro*, at the 8 to 16 cell stage, were randomly allocated into 11 batches of eight to nine embryos. Nine batches were incubated for 18 hours with 10^9 *Coxiella*/ml of CbB1 strain (ISP, INRA Val de Loire). The embryos were then recovered and washed in batches in 10 successive baths following the IETS guidelines. In parallel, two batches of embryos were subjected to similar procedures but without exposure to *C. burnetii* to serve as the control group. One of the nine batches of infected embryos and one of the two non-infected control batches were used to perform immunolabeling to localize the bacteria. *C. burnetii* DNA was detected by C-PCR in all eight batches of infected embryos after 10 successive washings. However, bacterial DNA was not detected in the embryos of the control group. The first five washing media of the infected groups were consistently positive and *Coxiella* DNA was detected up to the 10^{th} wash in two batches. After immunolabeling, the observation of embryos under confocal microscopy allowed to localize C. *burnetti* on the external part of the *zona pellucida* without deep penetration. The presence of *C. burnetii* was seen on the surface of the *zona pellucida*, with bacterial loads differing from one embryo to another in the same batch. This study clearly demonstrates that *C. burnetii*, after *in vitro* infection at 10^9 *Coxiella*/mL, stick strongly to the external part of the *zona pellucida* of *in vitro* produced early caprine embryos without profound penetration. The 10 washings protocol recommended by IETS to eliminate the pathogenic agents of bovine embryos is unable to eliminate these bacteria in caprine embryos. Nevertheless, the finding of *C. burnetii* DNA by C-PCR does not imply that the bacteria found are still infective. Further studies are required to investigate whether enzymatic and/or antibiotic treatment of caprine embryos infected by *C. burnetii* would eliminate or inactivate the bacteria from the *zona pellucida* of *in vitro* produced goat embryos.

A130E OPU - IVF and ET

A retrospective study on influence of weight of heifer's donors at 12 months in relation to age at first estrous, age at first embryo flushing and number of viable embryos in a breeding program

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Keywords: MOET, Heifers donnors, Nutrition.

It has been known that calves nutrition plan influence the onset of puberty and the age of first estrous in heifers. In AURIVA Holstein breeding Program heifers come from different farms (in different breeding conditions). They arrive at AURIVA station (Denguin South West, France) with the aim to start in vivo embryo production as early as possible to maximize gain genetic and minimize the costs. This retrospective study from 2010 to 2017 aimed to evaluate the impact of heifers weight at 12 months on age of first estrous, first in vivo embryo collection and number of viable embryos under breeding program.
Holstein heifers animals (n = 174) were used to analyze these parameters under a breeding MOET program. Animals were grouped according to their weight at 12 months of age as Low (L; <320 kg; n = 57), Normal (N; 320 to 370 kg; n = 81) or High (H; >370 kg; n = 36). Superovulation was induced by eight intramuscular injections of follicle-stimulating hormone (FSH), Folltropin (Bioniche Teo, Inverin, Co., Galway, Ireland) or Stimufol (Stimufol; Reprobiol, Liège, Belgium), at 12-hour intervals over 4 days, involving decreasing doses, 500 IU (Folltropin) or 350 mg (Stimufol) on 9 to 12 days after the onset of standing estrus. Our unpublished data showed no difference in superovulatory response to these two FSH preparations. The donors were treated with 500µg of cloprostenol (PGF) with the 5th FSH treatment. First insemination was performed 12 hours after the onset of standing estrus. The donors were inseminated twice 12 hours apart with conventional semen. Embryo flushing was performed 7 days after AI. Recovered ova/embryos were evaluated according to the International Embryo Transfer Society classification system. Age of first estrous, first embryo flushing and number of viable embryos were analyzed by ANOVA test. First heat (days ± SD) was observed significantly earlier ($P < 0.05$) in H animals (391.2 ± 47.7) compared to L animals (417.5 ± 41.18), $P < 0.05$. Not differences were observed between H vs N (404.3 ± 42.17) or L vs N animals ($P > 0.05$). Moreover, age at first collection (days ± SD) was lower in H animals (424.7 ± 47.6) compared to L animals (451.5 ± 37.43), $P < 0.05$. Not differences were observed between H vs N (438.5 ± 42.21) or L vs N animals ($P > 0.05$). The average number of viable embryos collected during first flush was not different between L (5.4 ± 4.8), N (6.9 ± 4.8) or H (5.3 ± 4.0) animals ($P > 0.05$). In conclusion this study confirms the importance of the nutrition of heifers before entering a MOET breeding program. Animals with a weight higher than 370 kg at 12 months will start in vivo embryo production earlier than animals weighting less than 320 kg. Reduced time of heifers lodging represents an economic and genetic gain.

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Effects of melatonin on bovine embryonic developmental competence and kinetics *in vitro*

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Keywords: IVF, bovine, melatonin.

Oxidative stress has been identified as a major factor affecting embryo development *in vitro*. Melatonin is a well-known potent free radical scavenger and broad-spectrum antioxidant and could thus protect early embryos from oxidative damage. The goal of the current study was to evaluate the effects of melatonin on developmental competence and kinetics of bovine embryos derived from *in vitro* fertilization (IVF). A total of 1131 oocytes were collected by slicing of ovaries obtained from a local abattoir and were cultured in the presence or not of melatonin (MT) to two different concentrations [MT-10^{-9} (0.0002328 mg/mL) and MT-10^{-11} (0.000002328 mg/mL)] during maturation, fertilization, and *in vitro* culture). As melatonin is a lipophilic hormone, it has to be dissolved in ethanol, thus an ethanol (ETH) as a "sham" group and a standard control group (without any supplements) were included in the experimental setting for a total of four experimental groups (Control: N = 260, ETH: N = 304, MT-10^{-9}: N = 277, and MT-10^{-11}: N = 290). Final concentration of ethanol in ETH and MT groups was 0.01%. Variables evaluated included cleavage rate (CR) 72 hours post-insemination (72 hpi), blastocyst rate (BR) (186 hpi), and hatching rates (HR) (210 hpi). Additionally, the embryonic developmental kinetics were analyzed. Data were statistically analyzed using the SAS/STAT® software (SAS. version 9.3) with the logistic procedure (PROC LOGISTIC). Significant differences were defined as $P < 0.05$. A statistical tendency was considered at $P = 0.08$. There were no differences ($P > 0.05$) for CR in the control group when compared with the two melatonin concentrations. Ethanol supplementation reduced significantly ($P < 0.05$) CR in comparison with all other groups. The blastocyst rates for control, ETH, MT-10^{-9} and M-10^{-11} were 20.8 %, 23.4%, 27.1%, and 25.5%, respectively. The addition of melatonin at the 10^{-9} concentration revealed a statistical tendency ($P = 0.08$) towards improved BR compared with the control group. Furthermore, the groups supplemented with ethanol and melatonin showed higher ($P = 0.002$) hatching rates than the control group. The hatching rates at 210 hpi (Day 9) for control, ETH, MT-10^{-9} and M-10^{-11} were 20.4 %, 52.1%, 53.3%, and 50.0%, respectively. No differences ($P > 0.05$) were observed for HR in the ethanol and different melatonin concentrations. Supplementation with ethanol and/or melatonin accelerated embryo development kinetics. The percentage of blastocysts reaching the hatching stage at 186 hpi (Day 8) was lower ($P < 0.05$) in the control Group (14.8%), compared with ETH, MT-10^{-9} and MT-10^{-11} (36.6%, 32.0%, and 33.8%), respectively. Likewise, the proportion of blastocysts which reached the advanced blastocyst stage (expanded and hatching) at 186 hpi was higher in MT-10^{-11} compared with the control group (68.98% vs 51.85%, respectively). In conclusion, the presence of melatonin and ethanol (0.01% v/v) during early embryo development *in vitro* affects the kinetics of embryo development and increased hatching of bovine oocytes fertilized *in vitro*.

A132E OPU - IVF and ET

Detection of cell-free DNA in embryo culture medium: its potential application as a noninvasive method for sex determination in cattle

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Keywords: cell-free DNA, culture media, blastocyst.

The application of noninvasive sex determination methods of embryos is believed to be crucial in assisted reproduction procedures. The aim of this study was to detect cell-free genomic DNA (gDNA) in the embryo surrounding spent media for sex determination of individually cultured bovine embryos. For this, bovine ovaries were collected from slaughter house. Immature oocytes aspirated from follicles of 3-5 mm in diameter, were matured and fertilized in vitro. Embryos were cultured individually starting from eight-cell stage until day 7 blastocyst in 10 µl drops of culture media under oil (SOFaa-medium). To verify the effect of different media on the release of cell-free DNA, individual embryos were cultured either in SOFaa supplemented with 5% exosome-free serum (SOF-EXO) media (n = 15) or SOFaa supplemented with 0.1% hyaluronic acid (SOF+HA) media (n = 9). Parthenogenetically activated oocytes and the corresponding embryos were cultured in SOF-EXO medium (n = 6) to be used as controls. Individual blastocysts and the corresponding spent media were collected at day 7. Cell-free DNA was isolated from each spent media using QIAamp®Circulating Nucleic Acid kit followed by whole genome amplification using REPLI-g single cell kit (Qiagen, Hilden, Germany). In parallel, extraction of DNA from individual blastocyst was performed using blastocyst Lysis buffer. Multiplex PCR amplification was done to detect sex related fragments in DNA samples from both individual blastocysts and amplified cell-free DNA recovered from corresponding spent media. For this, two different primers; bovine Y-chromosome specific primer and bovine autosomal centromere-specific were used for sex specific PCR amplification. DNA of female and male animal tissues was used as control. The results of the present study revealed that, cell-free DNA was detected in 53.3% of embryos cultured in SOF-EXO spent media. Of these, the sex of 87.5% of individually cultured blastocysts was accurately determined using the cell free DNA isolated from the corresponding spent media. Similarly, cell-free DNA was detected in 55.6 % when embryos were cultured individually in SOF+HA spent media. Among these, 60% of the sex determinations were in accordance between spent media analysis and determination using the blastocyst itself. Moreover, cell-free DNA was detected in 66.7% of culture media drops when harbouring individual parthenogenetic embryos. Noteworthy, sex determination using cell-free DNA in parthenogenetic embryos was achieved with an accuracy of 100%. Lack of any Y-chromosome specific DNA in these samples could therefore demonstrate absence of genetic pollution. Inaccurate sex determination in some samples could be due to lower amount of cell-free DNA. In conclusion, cell-free DNA released from embryos to their surrounding culture medium can potentially be used as a noninvasive sex determination method prior to embryo transfer in cattle. Further studies should be conducted to improve efficiencies with respect to DNA isolation and amplification in spent media.

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The influence of flunixin meglumine (FM), hCG or a combination of hCG and FM on the conception results in embryo recipient heifers including the passage time through the cervix and the presence of a large follicle

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Keywords: flunixin meglumine, hCG, recipients.

The application of hCG before embryo transfer (ET) causes luteinization of a dominant follicle (Rizos, 2012). Non-steroid anti-inflammatory drugs (NSAIDs) lower the pro-inflammatory action of prostaglandins during cervical manipulation (Scenna, 2005). Some authors, however, do not confirm this effect (Nogueira, 2004, Torres, 2013). It is possible that the administration of hCG increases the likelihood of pregnancy in embryo recipients. It should be higher in cows with both CL and the large ovarian follicle. The probability of pregnancy should also be higher in recipients receiving flunixin meglumine (FM) - especially with the prolonged passage time through the cervix (PT). Consequently the highest pregnancy rate should occur in recipients treated with a combination of FM and hCG. The aim of the study was to assess the probability of pregnancy in cows treated with FM, hCG or a combination of FM and hCG in relation to the PT and the presence of a large ovarian follicle. The conception results of 952 recipients of embryos collected by in vivo delivery (IVD) were included in the analysis. Recipients (CL>15 mm) were selected on day 7 after heat on the base of the ultrasound examination. Each follicle with a diameter of > 5 mm was considered as a large. Follicle of this size is palpable during rectal examination. All embryos were transferred by one experienced veterinarian. The FM was administered intramuscularly (IM) in an amount of 500 mg (Flunimeg 50 ml Fluniksyna –Scanvet Poland Sp. z o.o.), while hCG - IM in an amount of 1500 IU. hCG (Chorulon, Intervet International) was administered 15-2 min. before ET. In order to evaluate the factors determining cows' fertilization the logit model was estimated with the use of maximum likelihood estimation method and STATA software. The average time of passage through the cervix to the embryo deposition site was 70.1s and it had no significant impact on the probability of conception ($P < 0.09$). The average conception rate was 60.7%. In particular groups the conception rate was 61.3%, 63.4%, 58.5% and 58.3%, respectively, in FM, hCG, FM/hCG and control groups. There was no significant effect of FM administration ($P < 0.35$), hCG ($P < 0.32$) or the combination of both FM/hCG on the conception results in embryo recipients. The presence of a large follicle did not affect the conception rate ($P < 0.23$). The different PT did not impact the results of the hormonal application (odds ratio $P < 0.08$). The presence of a large follicle did not significantly change the treatment effects of used hormonal solutions ($P < 0.45$). Administration of hCG, FM and a combination of both FM and hCG did not affect the probability of pregnancy of embryo recipients. Likewise, the results of conception were not improved by administering hCG to the recipients with a large ovarian follicle. FM application in females with prolonged passage time through the cervix does not affect the probability of pregnancy.

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The influence of dominant follicles and corpora lutea location on the conception rate in embryo recipient heifers

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Keywords: dominant follicle, embryo transfer, dairy cattle.

Development of the first follicular wave dominant follicle on the ovary ipsilateral to the corpus luteum is associated with a decreased conception rate in dairy artificially inseminated cows (Miura, 2015). In embryo recipients that phenomenon has not been studied yet. Meanwhile, the position of the dominant follicle (DF) could be an additional criterion for the pre-transfer selection of embryo recipients. The aim of the study was to determine the effect of the DF placed ipsi- or contralateral to the ovary with CL on the conception rate. 967 recipients were examined in the study. Heats were synchronized with the use of prostaglandin (2xPG14). On the day of the transfer, the ultrasound examination of the ovaries was performed. Recipients with CL > 15 mm were qualified for the transfer. Additionally, the DF position and diameter were defined. Each follicle with a diameter > 5 mm was described as dominant. 682 fresh and 287 frozen embryos were used in the study. Pregnancy was examined palpatively 2 months after embryo transfer. Statistical analysis was done with STAPA. A dominant follicle was observed in 928 (95.8%) recipients. The diameter of DFs was between 5 to 22 mm, with the average 10.1 mm. In 443 (47.7%) recipients, the DF was contralateral to the ovary with the CL, in 485 (52.3%) the DF was ipsilateral to the CL. In 39 (4.1%) recipients there was no dominant follicle. The percentage of pregnant recipients, in which embryos were introduced into the uteri horn on the same side as both CL and DF was 60,7% and it was lower (P > 0.05) than in recipients with embryos introduced into the uteri horn on the side of the ovary with the CL, and contralateral to the ovary with the DF (61.04%). If the CL was observed on the left ovary and the DF on the right one, 61.6% of recipients were pregnant, while if the CL was observed on the right ovary and the DF contralateral to it, the percentage of pregnant recipients was 59.5. If there was no DF on the ovaries, the conception rate was 55.5% (P > 0.05). Introducing embryos to the left horn (with the CL on the left ovary, no DF) resulted in 42.8% of pregnant recipients, while introducing them to the right horn (with the CL on the right ovary, no DF) ended with 59.1% of pregnancies. To conclude, the presence of the dominant follicle on the ovary ipsi- or contralateral to the ovary with the CL had no significant effect on the pregnancy probability. It seems that the DF location on the ovary in relation to the CL is an insufficient additional criterion for the selection of embryo recipients.

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Reproductive fluids added to embryo culture vs. standard culture in cow: first results on pregnancy rates

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Keywords: reproductive fluids, IVP, ET.

Reproductive fluids (RF), though being part of the natural environment of embryo development, are not yet included in current IVC media. It has been shown in bovine that inclusion of RF in embryo IVC produces blastocysts with higher quality (Hamdi, Rep Fert Dev. RD17286, 2017). In porcine, embryos produced with RF had gene expression and DNA methylation patterns closer to in vivo grown embryos (Cánovas, eLife. 6:e23670, 2017). However, it is still unknown if the transfer of these embryos to recipients can give rise to pregnancies, thus justifying the conduction of this experiment. IVM, IVF and IVC details were already described elsewhere (Hamdi, Rep Fert Dev. RD17286, 2017). In IVC two different culture groups were created, according to the supplementation: RF group (SOF-RF) - 1,25%(v/v) NaturARTs BOF-EL (Embryocloud, Spain) from day 1 to 4 and 1,25%(v/v) BUF-ML from day 4 to 8 - and BSA group (SOF-BSA) - 3 mg mL-1 BSA from day 1 to 8. Vitrification and warming were performed using commercial media (Kitazato-Dibimed, Spain) with an open-system Cryotop, following manufacturer's instructions. Vitrification took place on IVC day 7/8 with embryos on stage 6-7 of development. Warming of embryos was performed less than 4h before transfers, loaded in straws and kept at 38,5ºC. Embryo transfers (ET) were made non-surgically to Holstein multiparous recipients either on day 6 or 7 after oestrus detection. Synchronization was made using Double-Ovsynch protocol. A total of 64 ET (n = 36, SOF-RF; and n = 28, SOF-BSA) were made in a 6 months period, from November 2017 until March 2018. Pregnancies were detected by ultrasound 30 days post-ET. Data were analysed by t-test independent samples with $P < 0,05$ resulting in significant differences (data are means ± SEM). Pregnancy rate (P%) per group did not have a significant difference when comparing recipients of the same day. However, when comparing P% by recipient day there were significant differences: recipients on day 6 had 10,00 ± 5,57 P% (n = 30), while recipients on day 7 had 35,29 ± 8,32 P% (n = 34). Specific values for SOF-RF were 11,08 ± 10,1 and 36,8 ± 9,6; and for SOF-BSA were 7,77 ± 11,6 and 33,3 ± 10,8, respectively. ET's are routinely made to recipients on day 6 to 8 post-oestrus detection. Our data are consistent with other reports for P% of IVP embryos for day 7 recipients, but not for day 6. Additionally, the source of the oocytes is not OPU but rather slaughtered cows, which could decrease our blastocyst development and viability, despite it was maintained for day 7 recipients. In conclusion, the presence of RF in embryo IVC gave rise to pregnancies at a similar level than a control group. Day 6 recipients showed an adverse effect on pregnancy rates, regardless of the group. Further data (calves phenotypes mainly) are necessary to evaluate if the improvements reported at the blastocyst stage by including RF in bovine IVP are also evident after birth. Supported by European Union, Horizon 2020 Marie Sklodowska-Curie Action REPBIOTECH675526; AGL2015-66341-R MINECO-FEDER; 20040/GERM/16 Fundación Seneca.

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Transcervical embryo recovery in Lacaune ewes superovulated with different doses of FSH

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Keywords: cervix, dairy sheep, uterine flushing.

This study assessed the effect of different FSH dosages for superovulation and the feasibility of transcervical embryo recovery in Lacaune ewes. Ewes (n = 2 5) received 60 mg medroxiprogesterone acetate sponge (Progespon®, Syntex, Buenos Aires, Argentina) for nine days, 37.5 µg d-cloprostenol i.m. (Prolise®, Tecnopec, São Paulo, Brazil) 24 h before sponge removal and 50 µg gonadorelin (GnRH analogue, Gestran®, Tecnopec, São Paulo, Brazil) 24 h after sponge removal. Superovulatory treatments consisted of 100 mg (G100, n = 13) or 200 mg (G200, n = 12) of porcine FSH (Folltropin®-V; Bioniche Animal Health, Belleville, Canada), given i.m. (twice daily) for three consecutive days, in decreasing doses (25, 25, 15, 15, 10 and 10%), starting at 60 h before sponge removal. Ewes were checked for estrus twice daily and were naturally mated by fertile rams (4:1 ratio) while in estrus. Transrectal ovarian ultrasonography was performed at the 5th day after estrus, to count the number of corpora lutea (CL) with Doppler mode ultrasound (Mindray M5VET®, Shenzen, China – 8.0 MHz). All ewes received 37.5 µg d-cloprostenol (Prolise®, Tecnopec, São Paulo, Brazil) and 1 mg estradiol benzoate (Sincrodiol®, OuroFino, Cravinhos, Brazil) i.m. 16 h before uterine flushing and 50 IU oxytocin (Ocitocina forte UCB®, São Paulo, Brazil) i.v. 20 min before uterine flushing. Embryo collection was performed at days 5 or 6 after estrus by transcervical technique (Fonseca et al., Theriogenology, 86:144-151, 2016) in all ewes that showed estrus and had more than 2 CL (n = 17). Qualitative data were analyzed by Fisher exact test. Quantitative data were analyzed by generalized linear models, using SAS® software (v 9.3, SAS Institute, Cary, USA). The percentage of ewes that showed estrus and the percentage of responding donors (> 2 CL) did not differ (P > 0.05) between treatments: 77% (10/13) and 62% (8/13) for G100 and 100% (12/12) and 83% (10/12) G200, respectively. The number of CL was higher (P < 0.05) for G200 (10.5 ± 1.5) than G100 (4.2 ± 1.5). Overall, cervical transposition and uterine flushing was possible in 100% (17/17) of ewes. The total time procedure was 32.3 ± 0.1 min for G100 and 27.7 ± 0.1 min for G200 (P > 0.05).The number of recovered structures and viable embryos per ewe collected was higher (P < 0.05) for G200 (7.5 ± 0.1 and 6.2 ± 0.1) than G100 (0.4 ± 0.6 and 0.4 ± 0.6), respectively.The dose of 200 mg of FSH promoted greater superovulatory response and recovery of viable embryos by transcervical technique. Probably, the poor ovulatory response with 100 mg of FSH was also affected by the formation of luteinized unovulated follicles. The protocol for cervical relaxation was efficient to allow the transcervical embryo recovery of Lacaune ewes.
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A137E OPU - IVF and ET

Porcine follicular fluid as chemoattractant improves sperm attraction and *in vitro* fertilization

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Keywords: porcine spermatozoa, follicular fluid, chemotaxis.

Under physiologic conditions, different biofluids (follicular fluid (FF), oviductal fluid (OF), and secretion of cumulus-oocyte complex (COCs)) take part in the spermatozoa attraction previous to fertilization. Despite the progesterone (P4) being part of the composition of these biofluids, it's also considered as the main chemoattractant (Blengini et al., Asian Journal of Andrology, 13, 769-773, 2011). However, there are other components not defined in these media that could attract spermatozoa more efficiently. Thus, the aim of this study was to study the ability of biofluids for sperm attraction and the effect on *in vitro* fertilization (IVF) parameters. Periovulatory OF, FF and conditioned medium (CM) were collected previously described by Soriano et al., 2017. In the present study, a chemotaxis system was designed using a Petri cell culture dishes (35 x 10 mm) with four wells separated by 3 mm (GN627170, Sigma). Six wells were filled with fresh spermatozoa ($20x10^6$/mL) from fertile boars (N = 6) that were capacitated for 45 min in 180 µL of the capacitation media (TALP), previously equilibrated for 3h at 38.5ºC and 5% CO_2. The opposite wells (six) were filled with TALP (control group) and TALP supplemented with the different chemoattractants: FF, OF, CM, P4, and mixture of all chemoattractans (experimental groups). Afterwards, 3-4 mm long capillaries bridges were placed between the wells containing capacitated spermatozoa and the opposite ones for 20 min. After that, the capillaries were removed and 22 (per replicate) denuded *in vitro* maturated oocytes were deposited with spermatozoa previously adjusted to $22x10^3$ in each group (chemoattractans and control groups). The experimental groups were: 1) TALP (control), 2) FF (1%), 3) OF (1%), 4) CM (2%) 5) P4 (28.3 pM), and 6) FF, OF, CM, and P4: Σ. After 18 h the oocytes were fixed and IVF parameters were evaluated in each experimental groups. All the data were analysed by ANOVA followed by Tukey post hoc to compare means and standard error ($P < 0.05$). A total of six replicates were carried out. Follicular fluid alone (FF) showed the highest values for ZP binding, penetration rate, spermatozoa/oocyte and pronuclear formation ($P < 0.05$). This preliminary study suggests that FF is the most important chemoattractant for porcine spermatozoa in *in-vitro* conditions. However, further experiment will be performed on the embryo development and quality.

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