Animal Reproduction

OPU/IVF and ET

The impact of progesterone releasing device on ovarian response and IVP success in stimulated cycles of transvaginal follicular aspiration (OPU) in cattle

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Keywords: progesterone, ovum pick-up, IVP.

Two intravaginal progesterone (P4)-releasing devices are currently available for use in cattle in the UK. We previously established that the PRID Delta 1.55g (Ceva Animal Health Ltd, Amersham, UK) was suitable for use in peri-pubertal OPU donors, but could not confirm any IVP benefits relative to the CIDR 1.38g (Zoetis UK Ltd, Leatherhead, UK) (Black & Sinclair, *Cattle Practice* 25: 276, 2017). The current study, therefore, sought to compare the ovarian response and IVP outcome for stimulated cycles of OPU (Nivet et al. Reprod 143: 165, 2012) that used either a PRID or a CIDR to provide P4 support.

Following establishment of a reference oestrus (Day 0), eight sexually mature Holstein heifers underwent five stimulated cycles of OPU-IVP using established protocols (Nivet et al. Reprod 143: 165, 2012). Briefly, each cycle consisted ablating all ovarian follicles ≥ 5 mm (dominant follicle removal; DFR) and insertion of a PRID or CIDR (Day 3), and FSH stimulation (6 x 70IU Folltropin (Vetoquinol UK Ltd, Towcester, UK) i.m. at 12 h intervals) commenced 48 h later.

Cumulus-oocyte complexes (COCs) were aspirated 38-42 h following final FSH injection (Day 9). A replacement P4 implant was inserted at OPU and the process repeated. Donor animals used the same type of device (i.e. PRID or CIDR; n=4) throughout. Blood samples were collected at DFR and OPU for P4 analysis by ELISA. All proportions were analysed using generalized linear mixed models that assumed binomial errors and used logit-link functions. Follicles aspirated and oocytes retrieved assumed Poison errors and used log-link functions. P4 concentrations were analysed by repeated-measures ANOVA.

The first two cycles of OPU were undertaken in the presence of a visible (by ultrasound) *corpus luteum* (CL) (P4 = 7.26 and 9.25 ng/mL (SED=2.13) for CIDR and PRID treatment groups respectively). The final three cycles of OPU were undertaken in the absence of a visible CL (P4 = 2.34 and 3.18 ng/mL (SED=0.537)). Number of follicles aspirated were 18.5±1.83 and 18.6±1.96 for CL present (CIDR vs PRID), and 16.7±1.42 and 22.3±1.64 (P=0.068) for CL absent (CIDR v PRID) respectively. Mean number of COCs retrieved were 12.1±1.69, 11.9±1.78, 9.8±1.23 and 14.4±1.50 (P=0.052) for the same respective combinations. Proportions of transferrable quality (IETS stages 7-9) blastocysts of matured were 0.443±0.0504, 0.410±0.0540, 0.171±0.0348 and 0.422±0.0376 (P=0.018) also for the same respective combinations.

These observations indicate that a PRID rather than a CIDR increases transferable embryo yields when a visible CL is absent. This may be due to increased P4 in PRID v CIDR devices, although the timing of P4 sampling (at P4 device changeover) prohibited us from confirming this. It will be necessary, therefore, to corroborate these findings to confirm elevated plasma P4 concentrations in the presence of a PRID than a CIDR with more frequent sampling.

Funded by: BBSRC-LINK (BB/R007985/1), Ceva Animal Health Ltd, GG-A received support from Turkish Ministry of Education.

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Effect of exogenous progesterone on the follicular dynamics, recovery, quality, and in-vitro developmental competence of embryos in Sahiwal cattle undergoing repeated ovum pick-up (OPU) sessions

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Keywords: sahiwal cattle, progesterone, *in-vitro* embryo production.

The objective of present study was to evaluate the effect of exogenous progesterone (P4) on ovarian follicular population, oocyte recovery, oocyte quality and *in-vitro* developmental competence in Sahiwal cows (*Bos taurus indicus*). After follicle ablation (day 0), twenty (n=20) wave synchronized Sahiwal cows were randomly divided into high-progesterone (Treatment) and low-progesterone (Control) groups. The animals in the treatment group received a progesterone device (CIDR) inserted into the vagina for four days on day 0 while the animals in the control group received no treatment at all. In both groups, animals were subjected to transvaginal ultrasound guided oocyte aspiration on day 4 following dominant follicle ablation and after every 96 hours, seven consecutive OPU's were performed in both groups. Transrectal ultrasonic scanning for follicular dynamics was performed after every 12 hours between the OPU intervals. At the time of each OPU, blood sampling for serum progesterone was performed. The OPU aspirates were searched in the laboratory for the COCs and, under optimized culture conditions, viable oocytes (Grade A, B and C) were processed for IVC (4 replicates) following IVM and IVF until day 7. The COCs (3 replicates) were denuded and treated with Hoechst (Sigma 33342) to estimate oocyte nuclear maturation after 24 hours of IVM at 38.5^{o}C, 5% CO2 and 95% humidity. The data were analyzed by independent t-test and chi-square test using SPSS. The results revealed that the mean growth (mm / day) of F1 (1.47 ± 0.11 vs. 1.71 ± 0.09) and F2 (0.96 ± 0.07 vs. 1.05 ± 0.09) was lower ($P > 0.05$) in the treatment group compared with control group, respectively. The mean concentration of serum P4 in the treatment group (2.31 ± 0.15 ng / ml) increased significantly ($P < 0.05$) by exogenous progesterone (CIDR) compared to control group (0.315 ± 0.03ng / ml). The mean number of medium-sized follicles (0.89 ± 0.13 vs. 1.58 ± 0.19) was significantly lower ($P < 0.05$) in the treatment group compared with control group, respectively. While the mean number of small-sized follicles (91.48 vs. 83.38 %) was significantly higher ($P < 0.05$) in the treatment group as compared to control group. Similarly, the oocyte recovery rate (54.22% compared with 42.53%; $P < 0.05$) and grade I and II oocytes per session (3.37 ± 0.49 compared with 2.21 ± 0.33; $P < 0.05$) were also higher in the treatment group compared with control group, respectively. However, the nuclear maturation rate (71.43 vs. 68%), cleavage rate (52.87 vs. 53.06%) and blastocyst rate (27.54 vs. 25%) did not differ ($P > 0.05$) between the groups. Taken together, exogenous progesterone (CIDR) has improved oocyte recovery and quality, but in both groups the *in-vitro* developmental competence of oocytes in terms of nuclear maturation, cleavage rate, and blastocyst rate remained the same.

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Lycopene improves blastocyst development and quality in a bovine *in vitro* model

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Keywords: antioxidant, embryo production, embryo quality.

Oxidative stress associated with excessive production and accumulation of reactive oxygen species (ROS) reduces embryo viability by interfering with essential cellular processes. Antioxidant supplementation may guard embryonic cells against ROS detrimental effects. Among antioxidants, lycopene is a carotenoid that has the ability to quench singlet oxygen and scavenge free radicals. This study aims to evaluate the effects of supplementation of lycopene (antioxidant), menadione (prooxidant), and their combination during *in vitro* oocyte maturation on subsequent embryo development and quality in a bovine model. Cumulus oocyte complexes, collected from slaughterhouse (n = 806), were matured in 4 groups of 60 in 500 µl of maturation medium (TCM199 medium + 50 mg/ml gentamycin (Life Technologies, Ghent, Belgium) + 20 ng/ml of epidermal growth factor (Sigma-Aldrich, Diegem, Belgium)) and supplemented with 0.2 µM lycopene, 5 µM menadione (Sigma-Aldrich, Diegem, Belgium), 0.2 µM lycopene + 5 µM menadione (L+M), or were not supplemented (control). Maturation and Fertilization were standardly performed in 5%CO2 in air, and embryos were cultured in serum-free medium with 5%CO2 and 5%O2 in all the groups. The effects of pro and antioxidant supplementation on *cleavage,* day 8 blastocyst, and embryo quality parameters were fitted in generalized and linear mixed-effects models, and results are expressed as least squares means and standard errors. Lesser cleavage rates ($P < 0.05$) were found in menadione supplemented oocytes (74 ± 3.7) than in lycopene (92 ± 1.9), L+M (83 ± 3.0), and control (87 ± 2.5). Lycopene supplementation resulted in greater ($P < 0.01$) day 8 blastocyst rates (56 ± 3.4) in comparison to the other groups (33 ± 3.4 for menadione, 40 ± 3.5 for L+M, and 43 ± 3.3 for control). In the lycopene group, total cell number (TCN), inner cell mass (ICM), and TCN/ICM ratio were higher, and numbers of apoptotic cells (AC) and AC/TCN ratio were lower than in menadione, L+M, and control groups ($P < 0.05$). Apoptotic cells and AC/TCN ratio were similar between L+M and control groups ($P > 0.05$). However, blastocysts from menadione supplemented oocytes presented greater AC and AC/TCN ratio than in all the other groups ($P > 0.05$). In conclusion, lycopene supplementation during *in vitro* oocyte maturation improves embryo development and quality and could have protective effects against oxidative stress (menadione supplementation). Further experiments should be conducted to study the molecular basis underlying the effects of lycopene supplementation on subsequent embryo development and quality.

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Follicular fluid supplementation during bovine oocyte maturation *in vitro* improves blastocyst development and quality in an individual culture system

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Keywords: cow, embryo, IVM.

Follicular fluid (FF) provides the natural environment for oocyte maturation. Therefore, we hypothesized that supplementation of FF during *in vitro* maturation (IVM) would enhance the bovine oocytes' developmental capacity. In this study, the effects of FF supplementation during IVM on embryo development and quality were assessed in a group and individual culture system. Follicular fluid was collected from slaughterhouse ovaries (follicles between 12 to 20 mm in diameter) and pooled in heparinized tubes (BD Vacutainer Precision Glide, Becton Dickinson, Franklin Lakes, NJ), centrifuged, and stored at – 80°C until usage. *In vitro* maturation medium with 50 µg/ml gentamycin (Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) and 20 ng/ml epidermal growth factor was supplemented with 0 (control), 1, 5, or 10% of FF. In Experiment 1, IVM, fertilization (IVF) and culture (IVC) were performed in groups (n = 1,056 oocytes in 5 replicates). In Experiment 2, oocytes and embryos were subjected to individual IVM, IVF and IVC (n = 567 oocytes in 7 replicates). After 22 h of maturation oocytes were co-incubated with 1×10^6 spermatozoa/mL for 21 h at 38.5°C in 5% CO_2 in humidified air in 500 µL IVF-TALP (Tyrode's Albumin Lactate Pyruvate) supplemented with bovine serum albumin (BSA) (6 mg/ml; Sigma A8806) and heparin (25 mg/ml) for group culture and in droplets of 20 µL for individual culture. After fertilization, presumed zygotes were transferred in groups of 25 to 50 µL droplets (group culture) and individually to 20 µL droplets (individual culture) of synthetic oviduct fluid (SOF), 0.4% BSA, and ITS (5 µg/ml Insulin + 5 µg/ml transferrin + 5 ng/ml selenium). Day 8 blastocysts were fixed and differentially stained as described by Wydooghe *et al*. (2011). Generalized mixed-effects models were used to test the effects of treatment on day 8 blastocyst rates and mixed linear regression models were used to test the treatment effects on differential and apoptotic staining parameters (cell numbers and apoptotic cell index). For both models, the replicate was set as a random effect and results are expressed as least square means and standard errors. In group culture, supplementation of FF did not affect the day 8 blastocyst rate (46.3 ± 3, 35.5 ± 2.9, 44.3 ± 3, and 45.5 ± 2.9 for control, 1, 5, and 10% FF supplementation, respectively) nor quality ($P > 0.05$). In the individual culture system, 5% FF supplementation increased day 8 blastocyst rate (37 ± 4.1%) in comparison to control (18.7 ± 3.3%; $P = 0.003$) and 1% FF supplementation (18.4 ± 3.2%; $P = 0.003$) but was not different from 10 % FF supplementation (28.8 ± 3.9%; $P = 0.4$). Moreover, 5% FF supplementation during individual culture resulted in (>10%) greater total cells number (110.9 ± 2.7) and (>20%) a higher proportion of inner cell mass cells (51 ± 1.9) than in all the other groups ($P < 0.05$), but apoptotic cell index was not affected ($P > 0.05$). Supplementation of IVM medium with 5% FF significantly increased blastocyst rate and embryo quality in a bovine individual embryo production system. However, the characteristics of slaughterhouse-derived FF are plausibly variable. Several FF batches should be tested to draft definitive conclusions.

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Crossbreeding effect of double-muscled cattle on *in vitro* embryo development and quality

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Keywords: double-muscled cattle, embryo production, embryo quality.

In the past years, several developing countries have started to breed double-muscled cattle to their native cattle to improve beef quality. However, the developmental competence of the resultant crossbreeding embryos is unknown. The objective of this study was to evaluate the effect of crossbreeding double-muscled (Belgian Blue; BB) semen with beef (Limousin; LIM) and dairy (Holstein-Friesian; HF) derived oocytes on embryo development and quality. As purebred BB control, BB oocytes fertilized with BB sperm was used. A single ejaculate of a single BB bull located in the breeding center of AWE in Ciney (Belgium; 50˚29 N, 5˚11 E) was used for all the experiments. Motility parameters of frozen-thawed sperm samples were evaluated using computer-assisted sperm analysis before used for *in vitro* fertilization. Ovaries were collected at the local slaughterhouse from each breed and transferred to the lab allocated in different bags without medium transport and all placed in a safety closed box. *In vitro* maturation and fertilization were performed (as described by Wydooghe E, Reprod Fertil Dev 26:717, 2014) and embryos were cultured in serum-free medium in three replicates (n = 1,720 oocytes). Basic Eagle's Medium amino acids, minimal essential medium non-essential amino acids (100 x), TCM-199-medium, kanamycin, and gentamycin were purchased from Life Technologies Europe (Ghent, Belgium). All other components were obtained from Sigma (Schnelldorf, Germany) unless otherwise stated. Cleavage was evaluated at 48 h post insemination and blastocyst development at day 8 post insemination. Embryo quality was evaluated via differential-apoptotic staining of day 8 blastocysts (as performed by Wydooghe E, Anal Biochem 416: 228–230, 2011). The effects of breed on developmental and differential-apoptotic staining parameters were fitted in mixed effects and mixed linear effects models, respectively. The replicate was set as a random effect for all the models and results are expressed as least square means with standard errors. Cleavage and day 8 blastocyst rates were greater ($P < 0.05$) for LIM (82.9 ± 6 and 27 ± 4.3%, respectively) than for BB (69.8 ± 8.5 and 19.6 ± 3.1%, respectively) and HF (45.1 ± 10 and 12.3 ± 2.2%, respectively). Holstein-Friesian presented lower cleavage and day 8 blastocyst rates than BB ($P < 0.05$). Limousin blastocysts presented a higher number ($P < 0.05$) of inner cell mass cells (ICM; 68 ± 7.8) than HF (40.4 ± 8.2). No other differential-apoptotic staining parameter differed among breeds ($P > 0.05$). In conclusion, crossbreeding double-muscled cattle by *in vitro* fertilization with LIM oocytes yields better embryo development and quality (ICM number) compared with the purebred combination, while the combination with HF oocytes produced the lowest rate of blastocysts. This finding might be due to one of the facts that culled HF cows are typically older than BB or LIM or are culled for infertility. This experiment consisted of a preliminary study to mimic what maybe happens in aspects of potential fertility in a BB breeding program. However, more studies need to be conducted to draw definitive conclusions.

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Subjectivity in the morphological selection of bovine immature cumulus-oocyte complexes

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Keywords: COC morphology, visual examination, kappa statistics.

Successful *in vitro* production (IVP) strongly depends upon the quality of the cumulus-oocyte complex (COC). The COC's morphological characteristics are often used to predict developmental competence. Whenever antral follicles are punctured for embryo production, COCs of different morphological characteristics are harvested and selection is performed by visual examination. This method is simple and non-invasive but it is also highly subjective. This study aims to determine the agreement of morphological evaluation of bovine immature COCs among IVP researchers. A non-random set of 29 pictures of immature bovine COCs was presented in duplicates to eight bovine IVP researchers, with different institutional backgrounds. Pictures were selected to balance for oocyte category (assessed by an experienced researcher who served here as referent) and were presented in random order. The observers were asked to categorize the oocytes to one out of four categories: A) compact cumulus of > 5 layers of granulosa cells that are completely surrounding the oocyte with homogenous ooplasm, B) cumulus is less compact and darker than A and ooplasm is dark and slightly granular, C) cumulus consists of ≤ 5 layers of granulosa cells and/or is not completely surrounding the oocyte with homogenous ooplasm, and D) cumulus cells are expanded, and the ooplasm is granular. The categorization of the most experienced observer was set as the reference value. Responses among and within observers were assessed using kappa statistics and sensitivity (se) and specificity (sp) tests. The referent observer classified 8 COCs in category B and 7 COCs in categories A, C, or D. Kappa values (κ) for inter- and intra-observer agreement were κ = 0.27 and 0.25 respectively, with κ = 1 referring to 100 % agreement. True positive rates (se) for categories A, B, C, and D were 52, 34, 50, and 45%, respectively. True negative rate (sp) was 71% for category A, 81% for category B, and 87% for categories C and D. The inter- and intra-observer agreements were poor. Evaluation of immature bovine oocytes based on their morphological characteristics is highly subjective with weak repeatability among observers. The moderate sp and low se suggest that it is easier to discriminate than to concur in the same oocyte category. There is a need to develop a simplified rating model to determine oocyte quality without losing practical feasibility. The implementation of automatized methods using artificial intelligence could significantly objectify this task.

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Trypsin treatment of porcine oocytes impairs *in vitro* fertilization output

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Keywords: zona pellucida, fertilization, porcine.

Polyspermy is the main limitation of porcine *in vitro* fertilization (IVF) success. The extracellular matrix or Zona Pellucida (ZP) of oocytes have a fundamental implication on sperm penetration. Since ZP is sensitive to protease digestion a soft treatment with trypsin was evaluated in order to evaluate porcine IVF output. *In vitro* matured oocytes-cumulus complexes were mechanically decumulated by soft pipetting until all cumulus cells were removed and washed twice in Tyrode's albumin-lactate-pyruvate (TALP) medium previously equilibrated at 38.5°C under 5% CO_2. DO (decumulated-oocytes) were then divided into two groups: trypsin and control. Both groups were transferred to a new 4-well Nunc plate with 500 µl TALP for 30 minutes. Trypsin group was supplemented with 0.5% trypsin. Photographs of DO from both groups were taken at time 30 min for ZP thickness measurement using image-J software. Afterwards, DO were incubated in TALP with fresh boar spermatozoa selected by a Percoll® density gradient. At 18h after IVF, putative zygotes were fixed and stained for penetration rate, monospermy and efficiency (percentage of monospermic oocytes from total inseminated) evaluation. Five replicates with a total of 289 DO were used for the IVF assessment (143 control; 146 trypsin) and 45 DO for ZP thickness evaluation (22 control; 23 trypsin). The statistical analyses were performed using the software IBM SPSS statistics *vs.*24. Normal distribution (Shapiro-Wilks) of data and equality of variances (Levene`s test) were evaluated. IVF parameters were assessed by chi-square analysis and the ZP thickness was analyzed by Student`s t-test. It was observed that the penetration rate and the efficiency were significantly higher in the control group than in the trypsin group (80.4% *vs.* 6.2%, and 20.3% *vs.* 2.7%, respectively, P<0.0001). No differences were observed on monospermy (44.4% and 25.2%, respectively, P=0.209). Moreover, no differences were observed on ZP thickness on DO from control and trypsin group after 30 minutes (19.05±1.77 µm and 19.94±1.54 µm respectively, P=0.282). In conclusion, a soft trypsin digestion of the ZP impaired IVF performance without altering ZP thickness.

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Inhibiting Diacylglycerol Acyltransferase-1 enzyme in bovine embryos produced *in vitro* reduces their lipid content and improves cryotolerance

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Keywords: *triacylglycerol*, cryopreservation, cattle.

Diacylglycerol acyltransferase-1 (DGAT1) is an enzyme that catalyzes the final step in triglyceride synthesis, which is a major component of the lipid droplets in embryos. Intracellular lipids accumulated in embryos produced *in vitro* have been associated with reduced cryotolerance and quality. We have evidenced that inhibiting DGAT1 synthesis in culture media with 10 or 50 µM DGAT1 inhibitor (A922500® Sigma-Aldrich) improves bovine embryo quality in terms of mitochondrial activity and total cell number (Giraldo-Giraldo J. et al., Reprod Dom Anim, 54:122 (2019)). Thus, in the present study we assessed if DGAT1 inhibition in *in vitro* culture of bovine embryos reduces lipid content and improves post-vitrification survival. Zygotes were cultured in groups of 25 in 25µL drops of synthetic oviduct fluid (SOF) supplemented with 5% fetal calf serum (FCS) alone (control) or with 10 or 50 µM DGAT1 inhibitor (T10 and T50, respectively) or 0.1% dimethyl sulfoxide (T_{DMSO}: vehicle for DGAT1 dilution), from 54 hours post-insemination (hpi) until Day 8 at 38.5ºC, 5% CO_2, 5% O_2 and 90% N_2. A representative number of blastocysts on day 7-8 (grade 1 and 2 according to IETS manual) from each group was used for quality evaluation through (i) lipid content (n≈30/group) stained with Bodipy (lipid droplet area in μm^2) and (ii) survival after vitrification/warming (n≈70/group). Survival was defined as re-expansion of the blastocoel and its maintenance for 72 h after warming. Data obtained were analyzed using one-way ANOVA. No differences were found in cleavage rate at 54 hpi (control: 90.5 ± 1.1%, TDMSO: 88.28 ± 1.0%, T10: 88.8 ± 1.2% and T50: 89.8 ± 1.2%) or in blastocyst yield on Days 7 and 8 (control: 29.1 ± 1.3% - 33.3 ± 1.1%, T_{DMSO}: 26.1 ± 1.2% - 31.6 ± 1.2%, T10: 29.2 ± 1.3% - 35.7 ± 1.2% and T50: 29.2 ± 1.0% - 34.7 ± 1.0% Day 7-8, respectively). Lipid droplets area was significantly reduced ($P<0.05$) in T10 (0.08 ± 0.0 μm^2) and T50 (0.09 ± 0.0 μm^2) groups compared with control (0.39 ± 0.02 μm^2) and T_{DMSO} (0.36 ± 0.02 μm^2) groups. In terms of blastocyst cryotolerance, during the first 24 h after warming, there were no differences in survival between the groups, which ranged from 82.9 ± 1.4% to 88.6 ± 2.1%. However, 48 h after warming, the survival rates of blastocysts obtained from T10 (83.3 ± 1.9%) was significantly higher ($P<0.001$) than those of the T50 (75.1 ± 1.3%), T_{DMSO} (72.5 ± 1.2%) and control group (75.5 ± 2.0%). At 72 h after warming, those differences were even more marked (T10: 73.8 ± 0.8% vs T50: 56.1 ± 1.2%, T_{DMSO}: 55.9 ± 1.6% and control: 57.1 ± 2.0%; $P<0.001$). Hatching rate was also higher in T10: 57.2 ± 2.8% vs T50: 39.6 ± 2.0%, T_{DMSO}: 38.4 ± 3.5% and control: 40.7 ± 2.1% ($P<0.001$). In conclusion, inhibition of DGAT1-synthesis in bovine embryos produced *in vitro* contributes to reverse the negative effect of serum by decreasing their lipid content and the lowest dose improve embryo cryotolerance.

Funding: MINECO-Spain AGL2015-70140-R & RTI2018-093548-B-I00; COLCIENCIAS 727/2015-Colombia; SENESCYT-Ecuador; FAPESP-Brazil 2017/20339-3 & CNPq-Brazil 304276/2018-9.

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Effect of prolactin on developmental competence of bovine OPU-oocytes matured *in vitro*

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Keywords: OPU-oocytes, maturation, prolactin.

In vitro maturation (IVM) of the oocytes recovered through ultrasound-guided transvaginal follicular aspiration (Ovum Pick-Up, OPU) is an important step for *in vitro* embryo production (IVP) in cattle. *In vitro* culture during maturation decreases oocyte quality and therefore, IVM conditions need to be improved. The goal of the present research was to study effects of pituitary hormone, prolactin (PRL) on the nuclear maturation of OPU-derived bovine oocytes and their development competence *in vitro*. Cumulus-oocyte complexes (COC) were selected from non-stimulated Simmental heifers at the age of 17 to 23 months (n=4) twice a week (11 OPU-sessions per animal, 5.3±0.4 COC per session) and classified immediately after OPU. The viable COC (compact cumulus and homogeneous cytoplasm, n=171) were cultured in standard maturation medium (TCM-199 supplemented with 10 % fetal calf serum (FCS), 0.2 mM sodium pyruvate, 10 µg mL-1 porcine FSH, and 10 µg mL-1 ovine LH) for 24 h in the absence (Control) or in the presence of 50 ng/ml bovine PRL (Research Center for Endocrinology, Moscow, Russia). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). After IVM, a subpopulation of oocytes was fixed with 4% paraformaldehyde, and the nuclear state was determined by DAPI staining. The remaining oocytes (62 Control and 52 PRL-treated group) underwent *in vitro* fertilization (IVF) and *in vitro* culture (IVC). Frozen/thawed sperm of the same Simmental bull were prepared in Sperm-TALP medium by swim-up procedure. *In vitro* matured OPU-oocytes were co-incubated for 18 h with prepared sperm in the modified Fert-TALP medium containing 10 µg mL–1 heparin, PHE (20 µM penicillamine, 10 µM hypotaurine, 1 µM epinephrine), and 0.1% MEM nonessential amino acids. Fertilized oocytes were cultured in CR1aa medium until Day 5, transferred to the same medium supplemented with 5 % FCS and cultured up to Day 7. At Days 2 and 7 after fertilization, the cleavage and blastocyst rates were determined. All the cultures were performed in 100 µl droplets of medium covered with mineral oil at 38.5°C and 5% CO2 in humidified air. The data for nuclear state (5 replicates per treatment) and IVF/IVC (10 replicates per treatment) were analyzed by ANOVA. After 24 h of maturation, the rate of M-II oocytes did not differ between non-treated and treated groups and were 83.8±4.6 and 88.3±5.6 respectively. However, after IVF/IVC, the cleavage rates of oocytes matured in the medium supplemented with PRL was significantly higher compared with control medium (82.4±5.4% vs. 69.4±2.6%, ($p<0.05$)). Furthermore, a significant increase in blastocyst rate was observed in the PRL-containing medium (20.5±1.9%; 0.91 blastocysts per OPU-session) compared with the control group (12.3±1.6%, $p<0.01$; 0.56 blastocysts per OPU-session). The findings indicated that PRL supplements during IVM of bovine oocytes recovered from live animal through ultrasound-guided transvaginal follicular aspiration may improve their capacity for the subsequent embryo development *in vitro*.

The study was supported by the Russian Science Foundation (project No. 19-16-00115).

Animal Reproduction

OPU/IVF and ET

Poliovulatory response and embryo recovery rate in beef sheep in Romania, as a possibility for genetic development - A case report

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Keywords: MOET, poliovulation, ET, sheep.

Embryo transfer (ET) technology gained commercial prominence in the international movement of bovine genetics. The development of this reproductive biotechnology in sheep has in the past had a similar development especially in important breeds of sheep and goats (like the Suffolk breed). Small ruminant ET is a well described and yet underexploited animal breeding technology. The size of sheep and goats, aspects of their anatomy and seasonal reproductive behavior, present challenges not common to cattle. Those considerations have not deterred serious breeders and ET practitioners in sheep and goat producing countries. The success of an ET protocol in sheep depends on many factors, but in the end, what matters is the number of embryos obtained, Recovery rates is an essential step in ET. The aim of our experiment was to observe the ovarian reaction to the treatment of Suffolk (UK) sheep polyovulation, and the recovery rate of embryos produced *in vivo*. A number of 6 Suffolk sheep were poliovulated at the beginning of the natural breeding season, using the P4-FSH-PGF protocol. The poliovulate (POV) method was based on the administration of intravaginal sponges containing 20 mg of flugestone acetate (Chronogest®, Intervet, Holland) followed by 500 IU FSH:LH (Pluset®, Calier, Spain) in decreasing doses in the last 4 days, and a cloprostenol (125 µg.IM), (Estrumate®, MDS, Holland) on day 11. The poliovulatory ovarian response was monitored by transrectal ultrasound (Honda HS-1600V®, Japan; 5 MHz) before estrus was detected, and on the day of embryo recovery. When estrus was detected, 3 mounts were performed at intervals of 12 hours, and 7 days later, the embryos were recovered by laparascopic surgical technique. Uterine flushings were made using Vigro complete flush™ (Vetoquinol, USA) a two-way catheter (Vortech 14Ch) and a filter (EmSafe Filter). Examination of the recovered flush fluid is routinely performed under magnification of 20-80X using a microscope. The size, morphology and developmental stages of small ruminant embryos are similar to those of bovine embryos. All POV sheep responded to stimulation and new follicular waves were identified on the ovaries. On the first day after POV, all ovaries had more than 5 dominant follicles, no differences were observed between sheep and between ovaries. But 7 days after estrus, corpus lutea (CL) was observed in only 83.3% (5/6) sheep. At the time of abdominal laparotomy, an average of 9.1 (0,11,12,15,9,8) CL/sheep were identified. The total number of CL observed was 55, 29 on the right ovary, 26 on the left. The distribution right/left of CL was 0/0, 6/5, 7/5, 7/8, 4/5, 5/3. The total number of embryos obtained was 48, and then the recovery rate (number of embryo/number of CL) was 83.3%. Its distribution was 10/11, 8/12, 13/15, 9/9, 8/8. 35 embryos (73%) were transferable. They all were excellent or good early blastocysts (stage 5 - quality 1, acording to the IETS recommended codification). The unviable embryos were degenerated or unfertilized. In conclusion, the POV protocol and the harvesting method applied have a positive effect in the production of *in vivo* embryos in Suffolk sheep and can garantee the success of ET activity of this breed in Romania".