A142E OPU - IVF and ET

Addition of seminal plasma reduces binding of stallion spermatozoa to bovine oocytes

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Keywords: seminal plasma, stallion, binding assay.

Equine *in vitro* fertilization (IVF) is currently not a repeatable process; a heterologous zona binding assay (HZBA) using *in vitro* matured (IVM) bovine oocytes could be an alternative to test the fertilizing capacity of stallion spermatozoa. Survival of stallion spermatozoa during cryopreservation varies considerably between individuals. Seminal plasma (SP) has been shown to repair cryodamage to sperm membranes (Bernardini et al., Theriogenology 76:436-447; 2011) and therefore may influence binding to the zona pellucida. Objective: To investigate the effect of adding SP from "good" (GF) or "bad" (BF) freezer stallions on sperm binding capacity. Ejaculates (one from each of six stallions) were processed by Single Layer Centrifugation (SLC) to remove SP and were frozen using the standard protocol at a commercial stud (Schober et al., Theriogenology 68:745-754; 2007). Straws were thawed at 37°C for 30s; the contents were gently layered on a low density colloid and were centrifuged to separate spermatozoa from cryoprotectant. The pellet was harvested and resuspended in modified Whitten's capacitation medium (MW) containing sodium bicarbonate and BSA. Salt-stored IVM bovine oocytes with intact zona pellucida, were obtained from several batches of ovaries, pooled and stored until needed. They were washed several times in prewarmed PBS/PVA, equilibrated for one hour in 37°C and transferred in groups of 25-27 to four-well plates containing: i) control (C) 500 µL MW (n=152); ii) 500 µL MW supplemented with 5% pooled BF-SP (BF) (n=161); or iii) 500 µL MW containing 5% pooled GF-SP (GF); (n=164). Sperm samples (final concentration 5×10^6 spermatozoa/mL) were added to the drops; the plates were incubated for 14-18 h in 38°C in 5% CO_2 incubator, 95% humidity atmosphere. The sperm-oocyte complexes were pipetted several times then rinsed gently three times to remove loosely attached spermatozoa. They were fixed in 2% (V/V) paraformaldehyde in PBS/PVA overnight at 4°C, washed, stained with Hoechst 33342 (5 µg/mL) and mounted under anti-fade medium (Vectashield) on glass slides. The coverslip was sealed with nail polish and allowed to dry. The number of spermatozoa bound to the zona pellucida (ZP) was assessed using confocal microscopy at 200x. Data were analyzed by General Linear Model using the SAS® software (version 9.3); significance was set to $P \leq 0.05$. All values are LSMEAN ± S.E. The number of spermatozoa bound to ZP was higher in C than in BF or GF (C 21.89±0.67; BF 2.86±0.65; GF 2.50±0.64; C vs. BF $P \leq 0.0001$; C vs GF P $P \leq 0.0001$). No differences were found between BF and GF. In conclusion, addition of SP impaired stallion sperm binding to the zona pellucida of bovine oocytes, independently of whether the SP came from a good freezer or a bad freezer. This effect may be due to the presence of sperm decapacitation factors in the SP.

Acknowledgements: EM Al-Essawe is financed by the Iraqi Ministry of Higher Education and Scientific Research, Baghdad.

A143E OPU - IVF and ET

Biobanking the first collection of oviductal and uterine fluid from hysterectomised patients

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Keywords: biobank, human reproductive fluids, surgery patients.

The safety of procedures in assisted reproductive technologies (ART) and the effect of culture conditions on embryo and fetal development, it is raising a great deal of concerns, mainly due to the lack of information about the formulations of commercially available culture media used in human IVF/ICSI treatments. As it was already described, the different IVF culture media influence the rates of successful implantation, pregnancy and birth weights (Kleijkers, Human Reproduction, Vol.31, No.10 pp. 2219–2230, 2016). It has been recently shown that culture media supplemented with natural female reproductive fluids have improved IVF efficiency, morphological embryo quality and epigenetic reprogramming profiles in pig blastocysts, compared with culture media without these supplements (Canovas, Elife, 6: e23670, 2017). This has encouraged the development of strategies that allow a noninvasive collection of reproductive fluids in humans, in order to validate them as supplements in the future. The first objective of this study was the development of a method to collect human oviductal and uterine fluids. A second objective was the initial characterization of reproductive fluids by measuring volume, protein concentration (Bradford Reagent, Sigma, Madrid, Spain), osmolality (Wescor Vapro 5520 Vapor Pressure Osmometer) and pH (pH OxyMini FOR PRESENS, Germany). The fluids were collected from 33 premenopausal women undergoing a total abdominal hysterectomy in the scheduled gynecological surgery of 'Virgen de la Arrixaca' University Clinical Hospital, whose indication was a benign uterine pathology. The oviductal fluid was collected according to the method previously described in Carrasco et al. (Reproduction, 136: 833–842, 2008). The collection of uterine fluid was carried out with a device normally used for mucus sampling. Once collected, the fluids were centrifuged at 7000 g for 10 min at 4°C to remove cellular debris and stored at −80°C in BIOBANC-MUR IMIB. It was possible to collect a mean volume of 23.9 ± 14.6 µl (n=22) of oviductal fluid and 62.8 ± 33.0 µl (n=26) of uterine fluid. The mean total protein concentration was 30.9 ± 14 µg/µl (n=22) for oviductal fluid and 48.9 ± 17.9 µg/µl (n=26) for uterine fluid. Mean value of osmolality was 316.6 ± 35.9 mmol/kg (n=22) for the oviductal fluid and 283.8 ± 69.5 mmol/kg (n=22) for the uterine fluid. Finally, mean pH values for oviductal and uterine fluids were 7.4 ±0.7 (n=22) and 7.8 ±0.3 (n=22), respectively. Although the selected methods allowed the reproductive fluids collection, they should be improved in order to obtain higher volumes without endometrial damage, to perform clinical trials that could validate their use as a supplement in culture media for ART. Besides the volume limitations, we can conclude that it is possible to establish a biobank of reproductive fluids, which meets sanitary conditions and legal requirements for research and future medical applications.

Supported by MINECO-FEDER (AGL 2015-66341-R), 20040/GERM/16 and European Union, Horizon 2020 Marie Sklodowska-Curie Action, REPBIOTECH 675526

A144E OPU - IVF and ET

Seminal plasma proteins increase *in vitro* fertility rate of frozen-thawed ram semen

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Keywords: apoptosis, capacitation, fertility.

Ram seminal plasma proteins (SPP) have an antiapoptotic effect (Mendoza et al., FEBS J 279: 62-63, 2012) and can protect spermatozoa from the cryopreservation damage (Barrios et al., Biol. Reprod. 63: 1531-1537, 2000). Therefore, their use in frozen-thawed seminal doses might increase fertility results. In order to test this hypothesis, ram semen was frozen after adding SPP. SPP were obtained by semen centrifugation at 12000 x g for 5 min at 4 °C, the supernatant was loaded in >3 kDa filters (Filtron Tech, Northborough, MA, USA) and centrifuged for 6 h at 3000 x g at 4 °C. Protease and phosphate inhibitors (Sigma Chemical Co, St. Louis, MO, USA) were added and SPP were stored at -20 ºC until use. Ram semen collected from nine Rasa Aragonesa rams using an artificial vagina was frozen in plastic straws with (P) or without (NP) 40 mg/ml SPP, in a Tris-glucose-citric acid-egg yolk based medium (Evans, Aust. J. Biol. Sci. 41: 103-116, 1988), following the Fiser's et al. method (Theriogenology 28: 599-607, 1987). After thawing at 37ºC for 30 sec in a water bath, seminal parameters of frozen-thawed samples (P and NP) and a fresh semen sample (control, C) were analyzed (n=4). Viability (by the double staining with carboxyfluorescein diacetate/propidium iodide (Sigma Aldrich; Harrison and Vickers, J Reprod Fertil 88: 343-352, 1990)) and apoptotic markers (phosphatidylserine translocation by FITC-Annexin V (Thermo Fisher Scientific, Waltham, MA, USA) combined propidium iodide, and DNA damage by TUNEL assay (Sigma Aldrich, San Luis, MO, USA)) were measured by flow cytometry. The capacitation state was assessed by the chlortetracycline staining (Grasa et al., Reproduction 132: 721-732, 2006), and fertility by IVF of ewes' oocytes (n=103, 99 and 98 for P, NP and C, respectively in 4 replicates) and subsequent embryo development (Forcada et al., Span J Agric Res 11: 366-370, 2013). Obtained results were analyzed by chi-square test (SPSS Statistics, IBM analytics, Armonk, NY, USA). The frozen-thawed processes lowered ($P<0.05$ when P and NP are compared to C) both sperm viability (21.6±7.6% in P, 22.8±7.4% in NP and 61.6±4.3% in C) and the rate of viable spermatozoa without phosphatidylserine translocation (15.1±8.4%, 15.3±9.5% and 27.0±8.1% for P, NP and C, respectively). No differences were found in DNA damage (9.2±2.6%, 12.2±5.8% and 7.2±0.9% for groups P, NP and C, respectively). The addition of SPP resulted in significant differences in the rate of non-capacitated spermatozoa (11.5±0.5% in P, 5.0±1.1% in NP, and 58.0±7.3% in C; $P<0.05$ for all groups), which was reflected in a higher *in vitro* fertility rate (88.5%, 72.2% and 98.7% for P, NP and C, respectively; $P<0.05$) and embryo cleavage (67.0%, 51.5% and 76.5% for P, NP and C, respectively; $P<0.05$ when NP is compared with P and C). Furthermore, blastocyst rate was also higher in groups P and C when compared with group NP (53.6%, 33.3% and 51.3% for P, NP and C, respectively; $P<0.05$). In conclusion, ram SPP can increase fertility results after frozen-thawed procedures. Grants: DGA A26.

A145E OPU - IVF and ET

Effect of reproductive tract O2 levels during *in vitro* fertilization and porcine embryo culture

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Keywords: oxygen, *in vitro* fertilization, embryo culture.

Oxygen concentration is a key factor for many physiological reactions in cells. However, most of the *in vitro* processes during pig embryo production are performed under environmental O_2 conditions (20%). These levels are far away from those recently measured in oviduct and uterus of sows and gilts (7 and 10%, respectively) (López Albors *et al*., Society for Reproduction and Fertility, vol. 2, P045, 2015). Indeed, 20% O_2 have a negative impact on embryo development in several species (Mantikou *et al*., Hum Reprod Update, vol. 19 (3), p. 209, 2013). Therefore, the effect of atmospheric vs. reproductive tract O_2 concentration during IVF and embryo culture (EC) in pig was compared. Porcine oocytes collected from gilts at slaughterhouse were *in vitro* matured and, 44 hours later, *in vitro* fertilized with sperm selected by swim up (Cánovas *et al*., eLife, vol.6, p. e23670, 2017). Gametes were co-cultured (2000 spz/oocyte) in TALP medium with 1% oviductal fluid from the late follicular phase (NaturARTs® PIG OF-LF, Embryocloud, Murcia, Spain), from 0 to 8 hours post insemination (hpi). Putative zygotes were cultured until blastocyst stage in NCSU23 medium with 1% oviductal fluid from the early luteal phase (NaturARTs® PIG OF-EL) from 8 to 48 hpi and 1% uterine fluid (NaturARTs® PIG UF-EL) from 48 to 180 hpi. Two groups were distinguished depending on whether 20% O_2 or 7% O_2 was used during IVF and subsequent EC. After 18-20 hpi, putative zygotes (182/653 from the 20% O_2 and 174/641 from the 7% groups) were fixed and Hoechst stained to evaluate IVF by fluorescence microscopy. After 48 hpi, cleavage rate was assessed. After 180 hpi, kinetic of development was evaluated classifying blastocysts as early, late, hatching or hatched. Later, they were fixed and Hoechst stained to quantify the number of nuclei in each blastocyst by fluorescence microscopy. Data were analysed by one-way ANOVA. A P-value <0.05 was considered to denote statistical significance. Oocytes fertilized under 7% O_2 showed the same penetration and monospermy rates, mean number of spermatozoa inside oocytes, and attached to the zona pellucida than oocytes fertilized under atmospheric O_2 levels. However, embryos cultured under 7% O_2 showed a significant increase in cleavage rate (60.0 ± 2.3%) compared with those cultured under 20% O_2 (32.0 ± 2.2%). Embryos cultured under 7% O_2 showed also a higher mean number of cells per blastocyst (88.9 ± 5.9) compared with those cultured under 20% O_2 (59.0 ± 5.0). Although no significant differences were observed for different embryo developmental stages between the groups due to the limited number of blastocysts (25 from the 20% O_2 and 50 from the 7% groups), absolute values for hatching and hatched blastocysts were larger in embryos cultured under 7% O_2 than under 20%. Overall, O_2 is an important factor to take in consideration during ART. The use of O_2 levels closer to those found in the reproductive tract not only enhances embryonic development but also improves the quality of the blastocysts produced.

Funded by MINECO-FEDER (AGL 2015-66341-R and 20040/GERM/16.

A146E OPU - IVF and ET

The effect of the presence or absence of a cavity in the corpus luteum on progesterone concentrations and pregnancy rate in heifers following embryo transfer

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Keywords: corpus luteum, recipients, pregnancy rate.

The aim of the study was to compare pregnancy rate and concentration of progesterone (P4) in embryo recipients in which the presence of compact corpus luteum (CL_{com}) or corpus luteum with a cavity (CL_{cav}) was observed at the day of embryo transfer. 79 heifers recipients were used in the study. Oestrus was synchronized with two i.m. injections of 25 mg of dinoprost tromethamine (5 ml of Dinolytic, Zoetis, Warsaw, Poland) administered at 14 day intervals. On Day 7 after oestrus, the ovaries were examined with the use of ultrasound (linear probe, 7,5 Mhz, iScan, Draminski). Corpora lutea were divided based on the presence or absence of a cavity into CL_{com} (n=187) and CL_{cav} (n=92). With the use of ultrasonography, the diameter, area and volume of CL and cavities (where present) were measured. Simultaneously, blood samples were taken from 41 heifers (25 recipients with CL_{com} and 16 recipients with CL_{cav}). Serum concentrations of P4 in the samples were evaluated by RIA. Fresh embryos (one embryo per recipient) were placed into the ipsilateral horn of the uterus. Pregnancy was diagnosed by ultrasonography 2 months after embryo transfer. Data were analysed by ANOVA and logistic regression using the STATISTICA 9,0 software PL. The mean diameter, area and volume of CL_{com} and CL_{cav} were 21.7 ± 2.57 mm vs. 23,0±2,56 ($p<0,001$), 384.6 ± 94.5 mm^2 vs. 458.8 ± 98.4 mm^2 ($p<0,0001$) and 7301.7 ± 2416 mm^3 vs. 8849.5 ± 2579 mm^3 ($p<0,0001$), respectively. The mean cavity diameter, area and volume were 9.4 ± 2.91 mm, 140.6 ± 50.4 mm^2 and 1177.5 ± 296 mm^3, respectively. The area and volume of luteal tissue were greater in CL_{cav} compared to CL_{com}. Mean concentrations of P4 12.1 ± 3.58 and 8.1 ± 3.96 ng/ml in CL_{cav} and CL_{com}, respectively ($p<0.0001$). Pregnancy rate two months following embryo transfer were 51.1% and 34.7% for CL_{cav} and CL_{com}, respectively ($p<0.02$). In recipients with CL_{cav}, transfer to the right uterine horn resulted in a pregnancy rate of 41.8% compared to 59,5% for the left uterine horn ($p>0,05$). For recipients with CL_{com} pregnancy rate following transfer to the right horn was 30.4% compared to 37.3% for transfers to the left horn ($p>0,05$). Moreover, when P4 concentration was higher than 10.88 ng/ml in 87.5% of CL there was a cavity, whereas when P4 concentration was lower or equal to 10.88 ng/ml in 88% CL was compact ($p<0.01$). The presence of cavities in the CL 7 days after ovulation appears to have a beneficial effect on the results of fresh embryo tranfer in recipients. Regardless of the type of CL, the placement of the embryo in the left horn of the uterus provided a higher percentage of pregnancies. However, in the case of CL_{cav} placing the embryo in the left horn resulted in higher pregnancy rate than CL_{com}. It seems possible to predict the occurrence of cavity inside the CL basing on the P4 concentration in the blood.

A147E OPU - IVF and ET

Bovine *in vitro* maturation medium with different protein supplementation influences the maturation and fertilization rates

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Keywords: bovine follicular fluid, IVM, IVF.

While some studies show beneficial outcomes on the use of bovine follicular fluid (bFF) in in vitro maturation (IVM), others display neutral or even detrimental effects. The main problem is related to the inhibitory effect on the meiosis resumption when high concentrations are used (Kim, Theriogenology, 45, 798, 1996). We hypothesized that inactivation of bFF might avoid this feature, thus we conducted 2 experiments (Exp) to evaluate the effect of bFF (either heat-inactivated or not) on the oocyte competence assessed by different parameters related to the nuclear and cytoplasmic maturation (Exp1) and the IVF efficiency (Exp2). Cumulus-oocyte complex's (COC) were obtained from slaughterhouse ovaries and IVM was performed using TCM-199 with 10% of either Fetal Bovine Serum (Control), bFF or bFF heat-inactivated (bFFin – 30' 56ºC). COC's were incubated for 22-24h and either denuded for Exp1, or submitted to IVF for Exp2. Frozen semen was used for IVF in TALP medium and incubated for 20-22h with oocytes. After fixation and Hoechst staining, oocytes and zygotes were evaluated under a fluorescence microscopy to assess nuclear status or fertilization parameters. In addition, cumulus cell expansion was measured in fresh oocytes before and after IVM. Total number of oocytes and replicates were as follows: 387 within 4 replicates for nuclear status; 432 within 3 replicates for cumulus expansion; 691 within 5 replicates for IVF. Data were analysed by one-way analysis of variance (ANOVA) and Tukey test with a level of significance $p<0.05$. The software used was IBM SPSS Statistics (v22.0). Values are percentages ± S.E.M. In Exp1, the nº of oocytes reaching metaphase II and showing a clear polar body were not significantly different among groups (69.29±3.91 for control, 68.29±4.21 for bFF and 68.55±4.19 for bFFin). Cumulus cell expansion showed no statistical difference between groups. In Exp2, the sperm penetration rate wasn't significantly different between control and bFF (91.29±1.9 and 83.37±2.5 respectively) but it was between control and bFFin (78.41±2.7). Monospermy, mean nº of penetrated sperm per oocyte (S/O) and male pronucleus formation (MPN) showed no significant differences among groups. Mean nº of sperm bound to the zona pellucida (S/ZP) was different between groups, with bFF showing the lowest value (1.98 S/ZP) and control the highest (4.5 S/ZP). The efficiency of the IVF was also different with the highest value for control 73.45±3.0 and the lowest for bFFin 59.49±3.2. However, there were no significant differences between bFF (63.48±3.2) and the other groups. In conclusion, adding bFF/bFFin to the IVM medium did not improve nor decreased maturation rates. However, IVF efficiency was lower when using bFFin but not when untreated bFF was used. Most likely, as others studies have shown (Collins, Theriogenology, 43, 1, 189, 1995), heating might inactivate some crucial heat-labile proteins that will further influence the ability to form a viable embryo.

Supported by European Union, Horizon 2020 Marie Sklodowska-Curie Action REPBIOTECH 675526

A148E OPU - IVF and ET

In vitro viability and developmental competence of porcine morulae stored in liquid state for up to three days

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Keywords: embryo storage, porcine, morulae.

The use of vitrified porcine embryos for non-surgical embryo transfer (Ns-ET) programs has disadvantages, as complying with strict air regulations for handling and transport of dewars, the risk of devitrification during transport, the need of LN_2 in the recipient farms or the number of embryos needed per Ns-ET. These drawbacks call for alternative procedures for short-term embryo storage in liquid state. This study aimed to evaluate storage of in vivo derived pig morulae in liquid state for up to 72 h on their further in vitro development. In Experiment 1, morulae (N=228) were stored at 25ºC or 37ºC in TL-HEPES-PVA defined medium (DM) or NCSU23-HEPES-BSA semi-defined medium (S-DM) for 48 h. After storage, embryos were assessed for viability (embryos with appropiate morphology according to the International Embryo Transfer Society criteria) and development, and then conventionally cultured (NCSU23-BSA-fetal calf serum, 38.5°C, 5% of CO_2 and 95% humidity) for 48 h to assess their hatching competence. Non-stored morulae (N=44) cultured under conventional conditions were used as controls. Differences among groups were analyzed using Fisher's exact test. At 48 h of storage, DM at 25ºC was detrimental ($P<0.05$) for embryo viability (73.9%) compared to the control (93.2%) and the rest of the experimental groups (90.9% to 98.3%). Following conventional culture, S-DM at 37ºC was the only group able to maintain embryo viability in a percentage similar to the control group (96.7%). Embryo development at 48 h of storage was delayed ($P <0.001$) in all experimental groups compared with the controls, being the delay more severe at 25ºC. Most embryos stored at 37ºC reached blastocyst stage but, unlike controls, none of them hatched at the end of storage. After conventional culture, the hatching rate of embryos stored in S-DM at 37ºC was similar to that of controls (85.0%) but higher ($P<0.01$) than for the other groups (9.1% to 23.8%). In Experiment 2, morulae (N=59) were stored at 37ºC in S-DM for 72 h, assessed for viability and development, and conventionally cultured for 24 h. Non-stored morulae (N=50) cultured under conventional conditions were used as controls. There were no differences in embryo viability between S-DM and controls at the end of storage (98.3% vs 90.0%, respectively). Moreover, all viable embryos from S-DM group remained viable after 24 h of conventional culture. Although there was a development delay in the stored embryos compared with the controls, some stored embryos (6.9%) hatched at the end of storage. The hatching ability after conventional culture was similar for stored and control embryos (65.5% and 70.4%, respectively). In conclusion, morulae stored in S-DM at 37°C for up to 72 h maintain in vitro viability and developmental competence. In addition, most blastocysts derived from stored morulae conserved intact the zona pellucida at the end of storage. These findings open new possibilities for porcine embryo transport in liquid state. Supported by MINECO-FEDER (RTC-2016-5448-2 and AGL2015-69735-R) and Seneca Foundation (19892/GERM/15).

A149E OPU - IVF and ET

Comparison of two culture conditions during maturation on *in vitro* development of sheep embryos

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Keywords: *Ovis aries*, static culture, dynamic culture.

Over the last two decades, the most studied variables to improve embryo development in vitro include the chemical composition of culture media. In fact, these approaches have proven to be beneficial and have contributed to improve success rates after assisted reproduction. However, not only the chemical requirements should be considered, but potential physical requirements may also be important factors in the continuous search for improving in vitro conditions. The objective of this study was to evaluate the effect of two culture systems (static and dynamic) during oocyte maturation in early ovine embryonic development. A total of 338 oocytes were obtained by aspiration of ovaries collected from a slaughterhouse. The oocyte control group (T1, n = 165) was placed for 24 h in a static culture, while another group (T2, n = 173) underwent dynamic culture receiving orbital movement with the aid of an electric stirrer agitator (AGO-1016, PRENDO, Mexico), for 5 seconds every 60 minutes for 24 hours. In both treatments the same maturation medium was used (TCM-199; In vitro S.A., Mexico), which was supplemented with 10% fetal bovine serum (Microlab, Mexico), 5 µg mL-1FSH (Folltropin, Vetoquinol, USA), 5 IU mL-1 hCG (Chorulon, Intervet, Colombia), 1 µg mL-1 17-β estradiol (Estrol, Pharmavet Argentina) and 50 IU heparin / mL (PISA, Mexico). The oocytes were fertilized with fresh semen using 55x106 mL-1 spermatozoa in medium TALP-Hepes (In vitro S.A., Mexico) and 18 hours later both groups were placed in Cleavage medium (COOK Medical, Australia), 60 hours later they were placed in Blastocyst medium (COOK Medical, Australia), performing the same management in both treatments. Embryo development was carried out in a CO2 incubator at 38.5 °C, 5% CO2 and 95% humidity. The size and development of the embryos was measured with an inverted microscope and a camera (AmScope) 144 hours after fertilization. The criterion for evaluating maturation in the cumulus-oocyte complexes (COCs) was by identifying the polar corpuscle and the level of expansion of the granulosa cells Fertilization was evaluated by the first cell division at 30 hours after performing Change to Cleavage medium (i.e.: 48 hours post insemination). The percentage of maturation, fertilization and blastocysts yield was calculated based on the initial number of COCs of each treatment. The means were compared by Student's t-test and chi-square according to the type of variable, using SAS. The percentage of maturation rate was higher ($P < 0.05$) in oocytes that underwent dynamic culture compared to static culture (78.3 ± 2.6 vs. 71.3 ± 2.7%). However, fertilization rate (72.8 ± 8.3 vs. 67.3 ± 13.0%), blastocyst yield (39.3 ± 6.8 vs. 36.36 ± 11.5%), and blastocyst diameter (166.5 ± 3.4 vs. 163.8 ± 2 µm) were similar ($P > 0.05$) in T1 and T2 groups. In conclusion, under the conditions of this study the use of dynamic culture for maturing sheep oocytes only improved maturation rate without any effect on embryo development.

A150E OPU - IVF and ET

The addition of ascorbic acid to the vitrification-warming media enhances the cryotolerance of in vitro produced porcine blastocysts

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Keywords: ascorbic acid, blastocysts, vitrification.

It is known that vitrification and warming procedures disturb the oxidation-reduction status increasing intracellular reactive oxygen species levels in porcine blastocyst. This study aimed to assess the effects of adding ascorbic acid (AsA) as antioxidant to vitrification-warming media on the post-warming survival and quality of IVP porcine blastocysts. Immature oocytes (N=3600) collected from prepubertal gilts were cultured in maturation medium supplemented with 10 IU/mL eCG and 10 IU/mL hCG for 22 h and then for an additional 22 h in maturation medium without hormonal supplements. Mature oocytes were inseminated with thawed sperm (1000 spermatozoa per oocyte) in fertilization medium for 5 h. Presumed zygotes were cultured in glucose-free embryo culture medium (supplemented with pyruvate and lactate) for 2 days and in embryo culture medium containing glucose for an additional 4 days. Blastocysts were vitrified and warmed with the superfine open pulled straw method using TL-HEPES as basic medium and ethylene-glycol and dimethyl sulfoxide as cryoprotectants (Sanchez-Osorio et al. Theriogenology, 2010, 73:300-308). We added 50 µg/mL of AsA both vitrification and warming media (VW+ group). Control group media were not supplemented with AsA. After warming, VW+ (N=281) and control (N=307) blastocysts were cultured for 24 h to assess embryo survival (ratio of blastocysts that reformed their blastocoelic cavities at the end of culture to the total number of embryos cultured) and hatching rates. To evaluate the quality of vitrified-warmed blastocysts, the number of inner cell mass (ICM) and trophectoderm (TE) cells was determined in each embryo using a differential staining based on an indirect immunofluorescence reaction. For that, a primary antibody (anti-CDX2), which specifically binds TE cells, and a secondary antibody (anti-Mouse IgG) conjugated with alexa Fluor® 568 that emits red fluorescence were used. Afterwards, all blastocysts cells were counterstained with the DNA-binding fluorochrome Hoechst-33342 to identify the ICM cells that displayed only blue fluorescence. Stained blastocysts were examined under fluorescence microscopy. Results are expressed as means ± SD of six replicates, and differences between groups were analyzed by an unpaired Student's t-test corrected for inequality of variances (Levene's test). The VW+ group showed a higher ($P < 0.02$) survival rate (51.1 ± 20.9%) than the control group (34.8 ± 21.4%). However, there were no differences between groups in hatching rates (10.7±12.0% vs. 6.0±8.1%). There were also no differences between VW+ and control blastocysts in terms of ICM (14.5 ± 6.5% vs. 16.4 ± 7.5%) or TE (44.2 ± 18.2 vs. 46.3 ±12.1%) cells. In conclusion, the addition of 50 µg/mL of AsA to vitrification-warming media considerably enhances the cryotolerance of IVP porcine blastocysts but does not affect the quality of embryos in terms of number of cells in the ICM or TE. Supported by Séneca Foundation (19892/GERM/15).

A151E OPU - IVF and ET

Haematological and blood biochemical parameters in piglets derived from embryo transfer

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Keywords: porcine, *in vivo*, embryos.

Porcine embryo transfer (ET) has an important role in pig industry because it allows the transport of genetic material, avoiding the risk of diseases dissemination. However, there are not many studies that assess the characteristics of piglets born by ET and information on the haematological and biochemical parameters in the newborns is limited. Since those parameters can be indicators of metabolic disorders and other pathologies, we aimed to compare haematological and blood biochemical parameters from piglets obtained by ET (ETp, n=22, 2 litters) vs. piglets obtained by artificial insemination (AI) (AIp, n=27, 2 litters). For this study, sows were used with the same genetics, feeding and housing conditions and they were inseminated with the same Large White boar semen doses. *In vivo* produced embryos (7 days after AI) were transferred to recipient sows by non-surgical methodology (DeepBlue® Porcine ET catheter, Minitübe, Tiefenbanch, Germany). Piglets were weighed and blood samples were collected on days 3 and 15 after birth. Blood samples were analyzed by haematology analyzer (Siemens ADVIA® 120, Tarrytown NY, USA) and clinical chemistry analyzer (Olympus AU400, Tokyo, Japan). Statistical analysis was performed using Systat Software (v. 13, San Jose CA, USA) by ANOVA considering day of birth and group (ETp and IAp) as factors, and litter as covariable. Differences were considered to be statistically significant when $P \leq 0.05$. Sex of the piglets and weight were not different between ETp and AIp after birth. An increase in the number of white blood (WBCB), red blood cells (RBC), RBC distribution width (RDW), mean platelet volume (MPV), platelet component distribution width (PCDW) and platelet mass distribution width (PMDW) was detected in ETp in comparison to AIp. On the other hand, a reduction in platelets counts (PLT), plateletcrit (PCT), mean PLT component (MPC) and corpuscular haemoglobin concentration mean (CHCM) were observed in ETp. Furthermore, higher alkaline phosphatase (ALP) values were observed on day 3 in ETp, while gamma-glutamyl transferase (GGT) values increased on day 3 and 15. No significant differences were observed on the other parameters measured. In conclusion, these preliminary results (derived from 4 litters) suggest that changes in the haematological and biochemical parameters are associated to the ET, although there are not differences from the reference values in piglets (Ventrella, BMC Veterinary Research, 13: 23; 2017). The alterations in platelets related parameters could be explained by an immunological platelet injury, probably associated to presence of maternal antibodies incompatible with platelet antigens from the piglets (Forster, Can Vet J. 48:855-7; 2007). This hypothesis must be confirmed with further studies. Currently, we are evaluating the clinical significance of the data, as well as the gene expression and DNA methylation changes in blood cells and placental tissue from these animals. Supported by MINECO-FEDER (AGL 2015-66341-R) and Fundación Séneca, 20040/GERM/16.

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Supplementation of α-tocopherol in two sheep breeds: effect on *in vivo* embryo production

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Keywords: ewes, α-tocopherol, superovulation.

The efficiency of a multiple ovulation and embryo transfer (MOET) program depends on the number of good quality embryos obtained, some studies suggest that supplementation with antioxidants can help to improve embryo quality. The objective of the study was to evaluate the effect of α-tocopherol supplementation on embryo quality of a MOET program in two sheep breeds. In total 43 females were superovulated, from which 12 Charollais and 12 Dorper were treated with 500 IU of α-tocopherol given 60 h before sponge removal, while 11 Charollais and 8 Dorper were not treated (0 IU). The ewes were synchronized with intravaginal sponges containing 20 mg FGA for 12 days and on day 10th were superovulated with a purified source of follicle stimulating hormone. Estrus was detected with teaser rams and ewes in estrus were inseminated by laparoscopy 18 h after estrus onset with 4 doses of fresh semen containing $100x10^6$ spermatozoa each. Embryo recovery was attempted 7 d after estrus by laparotomy. Ovulation rate, recovery rate, fertilization rate, and embryo quality were measured. The results were analyzed using ANOVA and t-test for means comparison or Chi-square tests as it was required. There was no effect ($p>0.05$) of α-tocopherol application, breed or their interaction on ovulation and recovery rates. Fertilization rate was similar ($p>0.05$) among ewes treated or not with α-tocopherol, but was higher ($p<0.05$) in Dorper than Charollais ewes (45.10 vs. 36.42%). Embryo quality was similar ($p>0.05$) among breeds, but lower ($p<0.05$) in treated (53.91%) than non-treated (70.33%) α-tocopherol ewes. The same trend occurred in the two breeds. In conclusión, the application of α-tocopherol did not improve fertilization rate and embryo quality of superovulated ewes under the conditions of the study.

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The STEINER OPU System: A new autoclavable device for flushing follicles in equine oocyte collection

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Keywords: eqine OPU, flushing follicles, new autoclavable device.

In equine IVF, oocyte collection is particularly challenging. Because equine IVF veterinarians must perform in vitro maturation (IVM), collecting oocytes from unstimulated follicles, it is essential to optimize the follicular flushing technique. The STEINER-TAN Needle (available in 17-, 19- and 21-gauge sizes) System was developed as a result of many years of research (Rose and Laky 2013. J Assist Reprod Genet. 30: 855-860) and practical work conducted in the field of human in vitro fertilization (IVF) (Schenk et al. 2016. J Assist Reprod Genet. 34:283-290.). It is manufactured by IVFETFLEX.com Handelsgmbh & Co KG (Graz, Austria). This needle combines the advantages of both single lumen and double lumen needles in that the option of flushing follicles is retained. Double lumen needles (12-gauge) are currently used for equine IVF in combination with epidural anesthesia and sedation. The STEINER (ovum pick-up) OPU System could also potentially be useful to veterinarians due to the fact that it is autoclavable and has the same functional properties as the low-cost STEINER-TAN Needle System currently used in human IVF. In this study, an autoclavable device (Steiner OPU System) was adapted to meet the needs of the veterinarian and demands of the IVF market. A 15-, 16-, or 17-gauge disposable needle or EchoTip® autoclavable needle, approximately 10-15 cm in length, is attached via a male luer lock to distal end of a piece of metal tubing. The aspiration tubing is inserted into this tubing with open end a few millimeters (proximal) from the male luer lock, enabling the free flow of fluid between outer (flushing) and inner (aspirating) tubing and facilitating follicle flushing. Female luer locks are attached to the aspiration and flushing tubing at the proximal end. The complete length of the tubing is 45 cm. This system can be used in combination with an autoclavable needle guide and elongated with a vaginal probe (available from IVFETFLEX.COM and manufactured to fit any US probe on the market). This tubing may be flushed manually. For optimal temperature control, a STEINER flush/valve, which is a flushing pump with a syringe warmer fitted for 50-cc syringes, may be used (also available from IVFETFLEX.COM), two models (mechanical or electrical) of which are available. Based on our research findings in humans, we hypothesize that the newly-designed OPU system could potentially be used by veterinarians to facilitate IVF in horses and openly welcome opportunities for scientific collaboration. Our results using this needle for oocyte retrieval in humans (Rose BI and Laky DJ. 2013. J Assist Reprod Genet. 30: 855-860) have led us to the conclusion that this new system could have significant advantages for IVF in horses over existing systems because it reduces pain (use of a much smaller OPU needle), obliterates the need for epidural anesthesia, allows for better temperature control (use of the syringe warmer), and helps keep the costs of the OPU procedure low.

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Effect of resveratrol-cyclodextrin complex supplementation during oocyte maturation or embryo culture *in vitro* in bovine

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Keywords: resveratrol, embryo, bovine.

The damaging effects of reactive oxygen species on *in vitro* embryo production have been widely studied on the past decade. Thus, many antioxidants such as resveratrol have been added to the *in vitro* production media mimicking endogenous antioxidants in an attempt to decrease their negative impact. Resveratrol has been reported to have a positive effect when added to *in vitro* maturation (0.1- 10 µM) or to culture media (0.25- 1.0 µM) in bovine and porcine *in vitro* embryo production. Higher concentrations of resveratrol in the embryo culture medium had been proved to have toxic effects on the developing embryos. Methyl ß-cyclodextrin, a group of cyclic oligosaccharides, has been used to improve the solubility of drugs. The present study evaluates the effect of the complex resveratrol-cyclodextrin during *in vitro* oocyte maturation (IVM) or *in vitro* embryo culture (IVC) on developmental competence and quantitative changes in gene expression of developmental important genes. In experiment 1, a concentration of 1 or 10 µM resveratrol (R1 or R10 respectively) diluted in 0.001% cyclodextrin was added to IVM media (TCM-199+10% FCS) and after 24 h a representative number of oocytes (n=330) were fixed to examine maturation level or snap frozen for gene expression analysis by RT-qPCR (n=120). The remaining were *in vitro* fertilized and cultured in SOF+3 mg/ml BSA to the blastocyst stage (n=1293). In experiment 2, 744 *in vitro* produced zygotes were cultured in SOF+3 mg/ml BSA supplemented with 0.5 or 1 µM resveratrol (R0.5 and R1 respectively) diluted in 0.0001% cyclodextrin. In both experiments, cleavage rate and blastocyst yield were recorded and blastocysts on Day 7 and 8 of the experiment 2 were snap frozen for gene expression analysis. A group without complex resveratrol-cyclodextrin (control$^-$) and a group with cyclodextrin (control$^+$) were included during IVM and IVC. A higher percentage of oocytes remained arrested in germinal vesicle when 10 µM resveratrol was added to the IVM medium (16.6 ± 2,6 %) compared to R1 (9.03 ± 0.6) and control groups (4.22 ± 0.6 and 10.3 ± 0.7 for control$^-$ and control$^+$ respectively ANOVA, $P<0.05$). No differences were found in cleavage rate or blastocysts yield between groups in both experiments. Regarding gene expression in oocytes, 10 µM of resveratrol during IVM decreased the expression of genes involved in competence of oocytes and subsequent embryo development (*NLRP2* and *BMP15* and *POU5F1,* ANOVA, $P<0.05$). Moreover, the expression of *BAX* was lower in oocytes treated with resveratrol compared to control$^-$group (ANOVA, $P<0.05$). Blastocysts produced with 0.5 µM f resveratrol showed a positive effect on the expression of genes related to lipid metabolism (*LIPE, CYP51, PNPLA2 and MTORC1)* compared to control groups (ANOVA, $P<0.05$) indicating that resveratrol could decrease lipid accumulation leading to a higher survival rate after vitrification. Further studies are needed to study the long-term effects of resveratrol supplementation on *in vitro* embryo production.

Funded by Spanish MINECO: AGL2015-66145-R & AGL2015-70140-R.