A125E OPU-IVP and ET

Pregnancy rates from different cattle breed embryos produced *in vitro* in a commercial program (part 1)

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Keywords: breed, embryo, pregnancy rate.

The objective of this study was to determine the pregnancy rate after transferring embryos from different cattle breeds in a commercial program of in vitro embryo production (IVP). Donors of different breeds, Angus, Nelore, Brangus, Holstein, Gyr and Girolando, were subjected to ovum pick up (OPU).Cumulus oocyte complexes were in vitro matured (22h, 38°C, 5% CO2 in humidified air) and then, fertilized in vitro with frozen-thawed semen, previously centrifuged for capacitation (18h, 38°C, 5% CO2 in humidified air). Embryos were cultured under the same conditions and procedures during 7 days (38°C, 5% CO2 in humidified air), when they were classified according to IETS criteria. Only grade 1 embryos were considered for transfer. Thus, 19195 fresh morula and blastocyst stage embryos (Angus=2576; Nelore=9715; Brangus=652; Hostein=647; Gyr=4231 and Girolando=1374) were transported up to 12 h in portable incubator and transferred to crossbred heifers. From day 28 to 32 after fertilization, the pregnancy status of recipients was determined through transrectal ultrasonography by visualization of the embryonic vesicle. For statistical studies, comparisons of frequency and analysis by Chi-square were performed, considering a significance level $P < 0.05$. Pregnancy rates according to the different breeds were: 36.8% for Brangus (240), 36.7% for Nelore (3569), 32.9% for Angus (848), 32.2% for Girolando (443), 29.7% for Gyr (1256) and 22.7% for Holstein (147). Significant differences ($P < 0.05$) were observed between all breeds except Angus vs. Girolando ($P = 0.66$), Nelore vs. Brangus ($P = 0.97$), Angus vs. Brangus (trend toward significance, $P = 0.06$) and Gyr vs. Girolando (trend, $P = 0.07$). In conclusion, the breed of the embryo significantly influences pregnancy rate in crossbred recipients, which affects the production results in commercial OPU/IVP programs, and furthermore demonstrating an improved performance of beef breed embryos.

Embryo Rio Preto and Bionorte SA.

A126E OPU-IVP and ET

Embryo mortality from different cattle breed embryos produced *in vitro* in a commercial program (part 2)

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Keywords: pregnancy, embryo mortality, breeds.

Embryo mortality (EM) in cattle is considered the main cause of reproductive failure, increasing the calving interval and implicating a greater economic loss in programs of in vitro embryo production (IVP) from high genetic merit animals. The objective of this study was to determine the EM rate after transferring embryos from different cattle breeds in a commercial program of IVP. Donors of different breeds, Angus, Nelore, Brangus, Holstein, Gyr and Girolando, were subjected to ovum pick up (OPU). Cumulus oocyte complexes (COC) were in vitro matured (22h, 38°C, 5%CO2 in humidified air) and then, fertilized in vitro with frozen-thawed semen, previously centrifuged for capacitation (18h, 38°C, 5%CO2 in humidified air). Embryos were cultured under the same conditions and procedures during 7 days (38°C, CO2 in humidified air) and classified according to IETS criteria. Only grade 1 embryos were considered for transfer. Thus, 19195 fresh morula and blastocyst stage embryos were transported up to 12h in portable incubator and transferred to crossbred heifers. From day 28 to 32 after fertilization, the pregnancy status of recipients was determined through transrectal ultrasonography by visualization of the embryonic vesicle. A total of 6503 pregnancies were diagnosed, 240 for Brangus (36.8%), 3569 for Nelore (36.7%), 848 for Angus (32.9%), 443 for Girolando (32.2%), 1256 for Gyr (29.7%) and 147 for Holstein (22.7%). Pregnant recipients were reevaluated by ultrasound 35 to 40 days later to confirm embryo survival by finding the heartbeat and/or spontaneous movements. For statistical studies comparisons of frequency and analysis by Chi-square were performed, considering a significance level $P < 0.05$. EM rate according to breeds were: 0.0% for Brangus, 4.9% for Gyr (61), 5.1% for Nelore (181), 6.3% for Girolando (28), 6.5% for Angus (55) and 12.2% for Holstein (18). Significant differences ($P < 0.05$) were observed between all breeds except Angus vs. Girolando, Angus vs. Nelore, Nelore vs.Gyr, Nelore vs. Girolando and Gyr vs. Girolando.In conclusion, the breed of the embryo significantly affects EM, demonstrating a better performance of embryos originated from Bos indicus and their crossbreeds than those obtained from pure Bos taurus breeds.

Embryo Rio Preto Ltda, Bionorte SA.

A127E OPU-IVP and ET

A comparison of Luprostiol and Dinoprost tromethamine for induction of oestrus in donkey embryo transfer

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Keywords: luprostiol, dinoprost acetate, oestrus.

Prostaglandin F2alpha (PGF2a) and its analogues have been routinely used after the embryo flush to induce the onset of oestrus in female donors (mares and jennies) and thus to obtain more embryos in a given season. The aim of this preliminary study was to compare the efficacy of Luprostiol (Prosolvin, Virbac, Spain) and Dinoprost tromethamine (Lutalyse, Pfizer Animal Health, Brazil) for induction of oestrus in jennies. Fourteen Andalusian jennies, 3-12 years of age, were used as embryo donors. Ovarian activity was evaluated daily during oestrus by transrectal ultrasound. When a ≥ *35 mm follicle was detected,* donor jennies were naturally mated every other day until ovulation (Day 0). Uterine flushing was done on Days 6 to 9. Each recovered embryo was measured and evaluated for morphology. In a total of 38 collections, donors were randomly treated with either 5.25 mg Luprostiol i.m. (n = 27) or 3.5 mg Dinoprost tromethamine i.m. (n = 11) immediately after the embryo recovery. Data were assessed by the chi-square test and the Kruskal-Wallis ANOVA. All donors responded well to prostaglandins (Luprostiol and Dinoprost tromethamine) given right after embryo collection. There were no significant ($P > 0.05$) differences between treatments for the interval from prostaglandin to ovulation (9.85 ± 0.72 days *vs.* 10.18 ± 0.84 days). In addition, the interovulatory interval was similar (16.56 ± 0.63 days *vs.* 17.27 ± 1.23 days; $P > 0.05$) between prostaglandins. Furthermore, there were no significant ($P > 0.05$) differences between these treatments for the embryo recovery rate (average, 74.3%) and embryo quality. Eighteen embryos were recovered from 27 flushes in Prosolvin group (66.7%), of which 14 were classified as Grade 1 (77.8%), 3 as Grade 2 (16.7%), and 1 as Grade 3 (5.6%). Meanwhile, nine embryos were recovered from 11 flushes in Lutalyse group (81.8%), of which 7 were classified as Grade 1 (77.8%), 1 as Grade 2 (11.1%), and 1 as Grade 3 (11.1%). Based in our results, we can conclude that both drugs used in the present study are adequate to consistently induce the onset of oestrus in jennies, with no deleterious effects on embryo recovery rate or embryo quality.

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A128E OPU-IVP and ET

Effects of ghrelin on activation of Akt and Erk1/2 pathways during *in vitro* maturation of bovine oocyte

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Keywords: ghrelin, oocyte maturation, Akt.

Ghrelin –a stomach derived peptide- acts, in general, as a negative feedback signal for reproductive functions affecting gonadotropin secretion, oocyte maturation and early embryo development. In a previous study we have shown that ghrelin at 800pg/ml accelerates *in vitro* maturation of bovine oocytes, reducing embryo yield (Dovolou et al 2014; RDA 49,665-72). Here we studied the role and possible mode of action of ghrelin on *in vitro* maturation of bovine oocytes. In exp. 1, in 3 replicates, COCs (n = 632) collected from abattoir material were matured *in vitro* for 18 or 24 hours in the presence of 0 or 800pg/ml of acylated bovine ghrelin (groups G18, G24, C18, C24). Matured oocytes were co-cultured for 24h with thawed bull semen, and presumptive zygotes were cultured for 9 days at 39^0C, 5%CO_2, and 5% O_2 . Cleavage and blastocyst formation rate were assessed on days 2 and 7 to 9, respectively and statistical analysis was carried out by one-way repeated measures ANOVA, with arcsine transformation. Exp. 1 served as internal control for the ensuing study on proteins. In exp. 2, after 0,6,10,18 and 24h in maturation, subsets of maturing COCs (each n = 10 in three replicates, totally n = 270) were mechanically denuded from the surrounding cells and cumulus cells and oocytes were separately stored in liquid N_2 for the assessment of activation of Akt and Erk1/2 pathways. Whole-cell extracts were prepared by lysis on ice using a buffer containing Tris-HCl NaCl, EDTA, Triton X-100, sodium deoxycholate containing protease inhibitors cocktail and sodium orthovanadate and assays were performed as recommended by the manufacturer (EMD Millipore, Darmstadt, Germany). Phospho-Akt1/PKBalpha (Ser473) and phospho-Erk/MAP Kinase 1/2 (Thr185/Tyr187) beadmates were used together. Protein expressions were measured on a Luminex 100 instrument (Luminex Corporation). The data output gives the mean fluorescence intensity (mFI) as a measure of protein abundance. We calculated the ratio of phosphoprotein mFI to number of treated or un-treated oocytes and cumulus cells and results were analyzed by ttest. In exp. 1 no difference existed in cleavage rate between groups. G24 yielded significantly less ($P < 0.03$) day 7 blastocyst in comparison to those of C18, C24 and G18 (16.3% , 29.3%, 26.9% and 30.0%, respectively).

In exp. 2, it was found that at 18 and 24 hours ghrelin caused significant increased phosphorylation of Akt in cumulus cells in comparison to the respective controls (pAkt/total Akt : 18h, 1.12 ± 0.1 and 0.79 ± 0.15; 24h, 0.99 ± 0.04 and 0.72 ± 0.08, for ghrelin and control groups). Oocytes matured in the presence of ghrelin at 10 h expressed lower Akt phosphorylation rate compared to controls (0.77 ± 0.13 and 1.21 ± 0.09). At 6 hours Erk1/2 phosphorylation was increased in ghrelin treated oocytes compared to the respective controls (11.48 ± 3.9 vs 6.08 ± 1.6).

We infer that ghrelin exerts antiapoptotic and antioxidant actions through the cumulus cells, and accelerates maturation via early phosphorylation of Erk1/2 that possibly brings about early activation of the maturation promoting factor.

A129E OPU-IVP and ET

Cryopreservation and intrauterine transfer of canine embryos

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UPSP 2011-03-101 ICE 'Interactions Cellules Environnement' & Centre de Ressources Biologiques – Infrastructure CRB-ANIM (ANR11-INBS-0003), VetAgro Sup, Marcy l'Etoile, Rhone France.

Keywords: embryo transfer, canine, cryopreservation.

The objective of the present study was to obtain live birth after transfer of cryopreserved canine embryos. To this end, seven bitches (Labrador retriever, 6.2 ± 1.8 years old) were used as donors according to ethical committee agreement (Vetagro Sup ethical committee number 1070) and ovulation timing was confirmed using blood sample monitoring (progesterone and LH assays). All donors were mated with fertile males 48 hours post ovulation and repeated 24-48 hours later to ensure fertilization. Females underwent ovariohysterectomies 9.7 ± 0.6 days post ovulation. Then, the embryos were collected *ex vivo* as previously described (Commin. L, Reproduction of Domestic Animals; Suppl 6:144-6; 2012), and classified under stereomicroscope. Among the 43 embryos collected (2 cells to blastocyst stage), 35 were slow freezed in a Vigro ® solution (Bioniche, Belleville Canada) containing ethylene glycol (EG, 1,5M) supplemented with BSA (0.4% *v/v*) and stored in 0.3 mL straws in liquid nitrogen until transfer to recipients. Six beagle bitches (16.5 ± 6.6 months old) were used as recipients and monitored by blood progesterone analysis and ultrasound examination to determine the time of ovulation. After thawing, embryos were observed under stereomicroscope to check for normal embryo morphology. Recipients were transferred at 9.6 ± 0.8 days post ovulation to allow less than 1 day of interval between the recipients and donors estrus cycles. Thawed embryos were surgically transferred to the recipients under gas anesthesia. The transfer were performed bilaterally on the top of the uterine horn, next to the oviduct junction, or close to the uterine bifurcation. Three to nine thawed embryos (undamaged, good quality) were transferred per recipient. After the surgery, the females were allowed to recover during at least 15 days before performing abdominal ultrasound for pregnancy diagnosis. None of the 6 bitches that were transferred became pregnant and no luteal deficiency was observed after blood progesterone analysis, suggesting more the inefficiency of the uterine transfer technique than the negative impact of the pregnancy maintenance. Even if the morphological assessment of thawed embryos did not allow to identify any significant damage after such a cryopreservation protocol, we were unable to confirm the viability of the embryos before transfer. Further *in vitro* studies (ie. *in vitro* culture or embryo differential staining) should be performed to confirm the intrinsic quality of slow frozen/thawed embryos. Also, improvement of the transfer technique (for example, using a transcervical endoscopic approach) should be evaluated with respect of a reduction the traumatic effects of the surgical technique (ie. capillary blood contamination).

This work was supported by a grant from the French Federation of Guide Dogs Associations (FFAC).

A130E OPU-IVP and ET

Ovulation induction for embryo transfer in Andalusian donkeys: human Chorionic Gonadotrophin *versus* Deslorelin acetate

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Keywords: hCG, Deslorelin acetate, ovulation.

The use of ovulation-inducing agents can improve the effectiveness of embryo transfer by synchronizing ovulation and artificial insemination (AI) or mating. Moreover, the duration of oestrus and the number of AIs or matings can be reduced with these treatments. This preliminary study was conducted to compare the efficacy of two drugs, human Chorionic Gonadotrophin (hCG; Veterin Corion, Divasa Farmavic, Spain) and Deslorelin acetate (Sincrorrelin, Ourofino Saúde Animal, Brazil), for induction of ovulation in jennies. Data on embryo recovery rates were recorded from 16 Andalusian jennies aged 3-12 years old. A total of 50 oestrus cycles were used and randomly divided into three groups: (i) no treated as control (n = 26), (ii) treated with 1500 IU hCG i.m. (n = 11), and (iii) treated with 0.75 mg Deslorelin acetate i.m. (n = 13). Donors were treated when they had a follicle larger than 35 mm of diameter and uterine oedema. Ovulation was diagnosed by daily ultrasound observations. Six to nine days after ovulation, donors were flushed three times for embryo recovery and each recovered embryo was measured and evaluated for morphology. Data were assessed by the chi-square test and the Kruskal-Wallis ANOVA. All donors (n = 24) responded well to the administration of both ovulation inducing agents. No differences ($P > 0.05$) were observed between treatments for the time to ovulation from treatment (hCG: 52.00 ± 4.00 h; Deslorelin acetate: 49.60 ± 4.95 h). The range of response was also similar ($P > 0.05$) for hCG and Deslorelin acetate (24 h: 9.1% *vs.* 15.4%; 48 h: 72.7% *vs.* 46.2%; 72 h: 18.2% *vs.* 38.5%). None of the variables studied (embryo recovery and mean diameter and age of embryos) were affected ($P > 0.05$) either by the ovulation treatment or the time to ovulation from treatment, but embryo quality ($P = 0.019$ for hCG *vs.* Deslorelin acetate). Nineteen embryos were recovered from 26 flushes (73.08%) in control jennies, of which 17 (89.5%) were classified as Grade 1 and 2 (10.5%) as Grade 2, whereas 8 Grade 1 embryos were collected from 11 flushes (72.73%) in jennies treated with hCG. Nine embryos were recovered from 13 flushes (69.23%) in jennies treated with Deslorelin acetate, of which 4 were classified as Grade 1 (44.4%), 3 as Grade 2 (33.3%), and 2 as Grade 3 (22.2%), showing a significantly lower percentage of Grade 1 embryos ($P < 0.05$) compared to hCG group. In conclusion, our results suggest that both drugs used in this study are adequate to consistently induce ovulation in jennies, but we noted that embryos recovered in jennies treated with hCG had better quality than those of the Deslorelin acetate ones.

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A131E OPU-IVP and ET

Cytological evaluation of PMN distribution in the genital tract of superovulated embryo donor cows

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Keywords: PMN, estrus cycle, donor cows.

Polymorphonuclear neutrophils (PMN) aid in the protection of the mucosae of the reproductive tract and comprise the first line of defense against infections. Since the mucosae of donor cows are challenged during inseminations (AI) and embryo collection (EC), the distribution of PMN in the genital tract of superovulated cows [n = 26; 1st–10th lactation; average milk yield: 33.2 ± 8 kg; 222 ± 192 days in milk (range: 72-1018d); healthy and gynecologically sound at pre-examination] has been examined. Superovulation followed the Georgsheil-Pluset protocol [PlusetÒ, 4 d, twice daily with decreasing dosage (in total 10 or 12.5 ml); PGF2α (PG) twice (am: PG analogue; pm: PG) on day 4 of FSH treatment]. Twelve to 16 h prior to EC animals were treated with a PG to induce corpora lutea regression and enhance embryo collection. Cytological samples were examined on 5 occasions: D9 ± 1.6 of the cycle before EC (E1); at 1st (E2) and second AI (E3) after superovulation; at EC (E4; D7 of the cycle); 16 ± 3 d after the last PG treatment (E5). Samples were taken with a modified cytobrush from vagina and cervix (E1, E2, E3, E4, E5) as well as corpus uteri and both uterine horns (E1, E4, E5), and from the tip of the insemination gun after AI into the uterine body (E2, E3). Following diff-quick® staining 300 cells/slide were differentiated into epithelial cells and PMN.
The mean percentage of PMN (PMN%) at all locations of the genital tract varied during the investigation period ($P < 0.05$ to $P < 0.001$; Dunn's method), except intrauterine samples E2 vs. E3. Values in the vagina at E1 (4.1 ± 5.0%) increased to a maximum of 17.7 ± 12.1% at E3. Thereafter, a decrease below E1-values was observed (E4: 3.7 ± 5.2%), whereas a second rise was obvious at E5 (10.4 ± 10.5%). The PMN-profile in the cervical canal ran parallel to that in the vagina, reaching a lower maximum level. Intrauterine cytobrush samples never exceeded 2.7 ± 1.8 PMN%, although cyclical variations were obvious (E1: 2.5 ± 1.8%; E4: 1.1 ± 0.9%; E5: 1.7 ± 2.7%). Samples taken from the tip of the insemination gun varied largely in relation to PMN% (E2: 13.1 ± 19.0%; E3: 15.6 ± 16.2%) which might be due to some cell contamination from the cervix, although a disposable plastic cover had been used for AI.
This study is the first to outline the PMN-distribution in different areas of the genital tract of superovulated embryo donor cows throughout the estrus cycle. The results show that PMN%-profiles in the different parts of the genital tract of superovulated embryo donor cows are subject to cyclic changes. This is most obvious in vagina and cervix which might be linked to the type of natural ejaculate deposition in bovines. The uterine PMN% (cytobrush samples) indicate that superovulation might have a positive effect on the endometrial health status, since values at EC are significantly lower than in the preceding interestrus. Samples from the tip of the insemination gun are not directly comparable to endometrial cytobrush samples. Yet they fit the PMN% fluctuations of the other localizations.

A132E OPU-IVP and ET

Route of Oxytocin administration and nonsurgical embryo recovery in Santa Inês ewes after induction synchronous estrus

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Keywords: oxytocin, transcervical embryo recovery, sheep.

Oxytocin has been used to promote cervical dilation with the objective to access uterus both in artificial insemination and transcervical embryo recovery in sheep and goats. The objective of this study was to test the effect of two routes of oxytocin administration on nonsurgical embryo recovery efficiency in Santa Inês ewes after induction of synchronous estrus. A total of 46 pluriparous ewes randomly chosen after weaning received intravaginal sponges (60 mg MAP; Progespon®,Syntex, Buenos Aires, Argentina) for six days plus 200 IU eCG (Novormon 5000®, Syntex, Buenos Aires, Argentina) i.m. and 37.5 µg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina) latero-vulvar, 24 h before sponge removal. Estrus was monitored twice daily after sponge removal and ewes were naturally mated by fertile rams while in estrus. Embryo recovery was attempted by cervical route (Theriogenology, 86:144-151, 2016) at day 7 after estrus onset. A total of 46 ewes that showed estrus received 37.5 µg d-cloprostenol latero-vulvar and 1 mg estradiol benzoate (Estrogin®, Biofarm, São Paulo, Brazil) i.m. 16 h before embryo recovery and 50 IU oxytocin (5 mL; Ocitocina Forte UCB®, São Paulo, Brazil) 20 min before embryo recovery by i.v. (T1; n = 21) or intravaginal route (T2; n = 21). T2 ewes were kept in anterior bipedal position when sponge applicator was gently introduced into the vulva and vagina and oxytocin was instilled in the vaginal fornix. Applicator was then immediately removed and ewes came back to quadrupedal position. Qualitative data were analyzed by chi-square test, while quantitative data were evaluated by one way ANOVA, both at 5% significance. Overall estrus response was 95.6% (44/46). Two ewes showed estrus later than the others and were not used. Interval to estrus and estrus duration were similar ($P > 0.05$) for T1 (46.4 ± 11.9 h and 33.8 ± 12.6 h) and T2 (48.0 ± 9.8 h and 31.6 ± 10.8 h) ewes, respectively. Successful uterine flushing was equal to T1 (12/21) and T2 ewes (12/21). Fluid recovery rate was 96.4 and 96.5% for T1 and T2 ewes, respectively ($P > 0.05$). The duration of the embryo recovery was similar ($P > 0.05$) for T1 (24.1 ± 5.7 min) and T2 ewes (23.2 ± 5.3 min). The average total structures recovered was 0.9 ± 0.4 for T1 and 0.5 ± 0.5 for T2 ewes ($P > 0.05$). Results of this study showed that nonsurgical transcervical embryo recovery can be efficiently done in some ewes; a higher number of individuals is needed to conclude that transcervical embryo recovery can be efficiently done in ewes and surgery embryo collections can be avoided in near to 60% of pluriparous Santa Inês ewes; and that the route of oxytocin administration did not affect the parameters evaluated.

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A133E OPU-IVP and ET

Effect of oocyte transport between two European countries on the bovine blastocyst production

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Keywords: oocyte transportation, bovine blastocyst production, European countries.

Commercial OPU is often performed on sites that are quite distant from the IVP labs (Ward et al., Theriogenology 54, 433-446, 2000). Maturing oocytes during shipment is a practical solution to overcome the problem of distance and help to apply the technique in many regions without IVF facilities. Despite free circulation agreements for trade and people through European countries, specific limitations are set for biological specimens and specifically for bovine gametes. We hypothesized that trans-border cooperation for IVP provided that sanitary status of donor females is in agreement with EEC requirements. The aim of this study was to evaluate the effect of oocyte transport during 6 hours between Spain and France on the bovine blastocyst production.

In the transport group, Holstein Cows (n = 6) from Ponderosa Holstein farm in Lleida-Spain were stimulated with decreasing doses pFSH (Folltropin, Vetoquinol, France) twice daily during 3 days (total dose: 350 µg). Sanitary tests were performed on donors one week prior to FSH treatment investigating for Tuberculosis, Brucellosis, Leucosis, infectious bovine rhinotracheitis, Bovine viral diarrhea, and Paratuberculosis. COCs were collected by OPU after 36 hours coasting period. COCs were recovered, evaluated immediately after OPU and placed into 2.0 mL tubes (Corning, USA) in 500 µL maturation medium. Gas mix (5% CO_2 in air) was injected into each tube tightly sealed and placed at 38.0°C in portable incubator (Minitub, Tiefenbach, Germany) during 6 hours. On arrival in Auriva IVP lab, tubes were opened and placed into the incubator with 5% CO_2 at 38.5°C with maximum humidity to complete the 24 hours maturation period. They were fertilized with frozen–thawed X-sorted sperm in TALP medium. Presumptive zygotes were cultured in SOF medium (Minitub) up to day 7 at 38,5°C in 5% CO_2, 5% O_2 and 90% N_2 atmosphere with maximum humidity. Grade 1 blastocysts and expanded blastocysts according to IETS classification were recorded on days 6.5 and 7. The same procedure was used in control Holstein cows (n=8) from Auriva IVP Laboratory in Denguin France, in the same period, except COCs were placed directly into CO_2 incubator, after retrieval and inseminated with X-sorted semen of different bulls. Oocyte recovery and embryo production were analyzed by student T-test and blastocyst yield by Chi-Square. The variation among X-sorted semen on the blastocyst rate in the control group was analyzed by ANOVA.

In the transport group and the control group, respectively 22.2 ± 10.0 COCs (6 OPU sessions) and 8.1 ± 3.7 COCs (21 OPU sessions) were collected ($P < 0.05$) and were processed for in vitro maturation. 7.5 ± 5.4 and 4.7 ± 2.0 G1 embryos were produced ($P > 0.05$). The mean embryo development rate (grade 1 embryos / number of oocytes entering maturation process) was 33.8% in the transport group and 57.7% in the control group ($P < 0.05$). No difference was found between bulls.

Although it is still necessary to increase embryo development rate, these results demonstrate that successful inter-border cooperation for bovine IVF/IVP is possible in neighbour areas.

A134E OPU-IVP and ET

Effect of X-sorted sperm on development grade 1 in vitro-produced embryos derived from bovine ovum pick up oocytes under commercial conditions

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Keywords: embryo developmental kinetic, OPU, FIV.

Sex-sorted sperm has been used for IVF to produce in vitro sexed bovine embryos. Following IVF, developmental kinetics, specifically the timing of blastocyst formation, has been related to embryo developmental competence (Lonergan et al, Journal of Reproduction and Fertility 117, 159-167, 1999). The objective of this work was to analyze developmental kinetics of OPU-IVP bovine embryos produced with unsorted and X-sorted sperm under commercial conditions. The work was performed at the Biotechnology AURIVA Station located in Denguin, South West, France.

Four to fifteen years old Holstein cows (n = 31) and 16-22 months old heifers (n = 18) were used in an OPU-IVP program. Donor animals were stimulated with decreasing pFSH doses (Stimufol; Reprobiol, Liège, Belgium) twice daily during 3 days, (total dose: 350 µg for cows and 250 µg for heifers). Cumulus oocyte complexes (COCs) were collected by OPU 12 to 24 h after the last FSH injection and matured *in vitro* using a standard IVM protocol. Oocytes were fertilized with frozen–thawed unsorted or X-sorted sperm in modified Tyrode's bicarbonate buffered medium using different non pre-tested bulls (n = 58). Presumptive zygotes were cultured in SOF medium (Minitub, Tiefenbach, Germany) up to Day 8 at 38.5°C under an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 with maximum humidity. OPU/IVP was repeated one to 16 times (2.3 ± 3.0) for each donor cow or heifer. Grade 1 (G1) expanded blastocysts, according to IETS classification, were recorded on days 6.5, 7.0, 7.5 and 8. Oocyte recovery and embryo production were analyzed by Student T-test and the rate of expanded blastocyst by Chi-Square. Sixty one OPU sessions were performed with unsorted semen and 66 with X-sorted semen, with a mean number of oocytes processed for in vitro maturation 10.9 ± 6.4 and 9.1 ± 4.8 ($P > 0.05$) and a mean number of G1 produced embryos 7.4 ± 4.5 and 4.7 ± 2.9 ($P < 0.05$) per session, respectively. The mean embryo developmental rate (G1 embryos/number of oocytes entering maturation process) was 67.8% (unsorted group) and 52.1% (X-sorted group; $P < 0.05$). On day 6.5, 52.8% of total blastocysts had already expanded after IVF with unsorted sperm, compared with 37.0% with X-sorted sperm ($P < 0.05$); while 41.3% were developed with unsorted and 53.1% with X-sorted sperm ($P < 0.05$) on day 7.0. Expansion was observed in 4.6% of embryos produced with unsorted and 9.7% with X-sorted sperm ($P < 0.05$) on day 7.5. No difference was observed on day 8 in proportion of expanded blastocysts produced with unsorted and X-sorted sperm. Unlike previous studies in which sexed embryos develop on days 8 and 9, in our study, although the number of expanded blastocysts was high with unsorted semen on day 6.5, the majority of expanded blastocysts developed on day 7 with unsorted (94%) and X-sorted sperm (90%).

A135E OPU-IVP and ET

Intrafollicular Oocyte Transfer (IFOT) of immature oocytes improves developmental rates and results in healthy calves

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Keywords: IVP, IFOT.

Although the in vitro production (IVP) of bovine embryos is a well-established technique, there are still major differences of IVP-derived blastocysts compared to their in vivo derived counterparts. To circumvent the negative impacts of in vitro culture on bovine embryos, we have recently established the so called intrafollicular oocyte transfer (IFOT). This technique allows injection of oocytes into the dominant follicle without breaking it, so they move to the oviduct and regular IA can be performed and blastocysts can be recovered from the uterus on day 7. However, it remained an open question whether IFOT could be applied to immature slaughterhouse derived bovine oocytes allowing in vivo fertilization and development, too. Therefore, recipient heifers were synchronized by two injections of PGF2α (2 ml Estrumate ®) 11 days apart followed by a final injection of GnRH (2.5 ml Receptal ®) 42 hours after the second PGF2α administration. Groups of immature oocytes (n = 50) derived from slaughterhouse ovaries were injected into the presumed dominant follicle of synchronized Simmental heifers 37-42 hours after the second PGF2α injection using a modified Ovum-Pick-Up (OPU) equipment. All in all, a total of 800 immature bovine oocytes were transferred to 16 heifers. Subsequently, 307 embryos (38.6 ± 27.2%) were recovered after flushing the uteri at day 7. Among all recipients, 13 of 16 delivered extra embryos above the recipient's native one presumed to be derived from the dominant follicle's own oocyte. Of these recollected embryos, 83.2 ± 11.5% had cleaved and 48.2 ± 11.2% had developed to the blastocyst stage. However, when excluding one blastocyst per recipient (considered to be derived from its native oocyte) IFOT embryos still reached a blastocyst rate of 45.5% being significantly higher (45.5% vs. 29.3%, ANOVA, $P < 0.05$) compared to fully in vitro produced embryos (SOFaa + 0,4% BSA in 5 % O_2 & 5 % CO_2) although derived from the same charge of slaughterhouse ovaries. In contrast, cleavage rates did not differ (83.2% vs. 83.7%). Hence, our results indicate that the maturational environment profoundly affects the ability of bovine oocytes to develop to the blastocyst stage. Finally, transfer of 13 cryopreserved IFOT-derived blastocysts resulted in 2 pregnancies (15.4%) and birth of 2 healthy calves. To the best of our knowledge, for the first time our study reports full in vivo development of immature slaughterhouse derived oocytes to the blastocyst stage and these are the first calves reported after transfer of immature slaughterhouse derived oocytes into preovulatory follicles. Thus, our study proofed that IFOT offers a new method to circumvent harmful effects of in vitro production conditions for immature bovine oocytes derived from slaughterhouse ovaries also enabling attractive and innovative new experimental setups.

A136E OPU-IVP and ET

In vino veritas? – How resveratrol attenuates oxidative stress in bovine oocytes of prepubertal and adult donors

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Keywords: reproductive aging, cattle, OPU.

The oocyte aging phenotype has been well characterized, but the adaptive response machinery to oxidative stress has been poorly investigated. Recently, a growing body of evidence supports the hypothesis that the aging process is regulated by a continuous crosstalk between reactive oxygen species (ROS) and SIRT1. The SIRT1 protein belongs to the family of NAD^+-dependent deacetylases, and has been shown to regulate several key processes, including fertility and ageing. Resveratrol (3,4',5-trihydroxystilbene) is an antioxidant identified in various plant species and red wine which stimulates SIRT1 activity. Based on these observations, the goal of the present study was to examine, if resveratrol protects bovine oocytes and blastocysts from oxidative stress.We hypothesize that SIRT1 signalling activated by resveratrol could play a role in reproductive aging. Cumulus-oocyte-complexes (COCs) of prepubertal (5-6 months old) and adult (2.-6. lactation) cows were collected by Ovum Pick-up. The medium for *in vitro* maturation and *in vitro* fertilization was supplemented with 2µM Resveratrol® (Sigma-Aldrich, Buchs, Switzerland). Our standard lab protocol was used as control. Cleavage rates at day 4 and blastocyst formation at day 7 were evaluated. ROS levels in oocytes and embryos were assessed by using BODIPY C11 (Thermo Fisher, Bonn, Germany). Oocytes were incubated for 30min at 37°C in TCM culture supplemented with 10 mM BODIPY, blastocysts respectively were incubated in SOF. As positive control, oocytes and blastocysts were treated with 10mM H_2O_2 for 10 min. Finally, oocytes and blastocysts were analyzed under a fluorescent confocal microscope (LSM510, Zeiss, Germany) and relative fluorescent intensity was calculated. For each treatment group 12 independent replicates were performed.One-way ANOVA from JMP software was implemented to evaluate differences of fluorescence intensity units (FIU). Data were expressed in mean ± SEM. In total 456 COCs of cows and 482 of claves were used for the IVP. The cleavage rates of adult and prepubertal donors did not differ significantly among the treatments (standard protocol: 56.5 ± 5.4% for cows and 53.0 ± 4.7% for calves, resveratrol supplemented protocol: 62.1 ± 4.3% for cows and 63.6 ± 3.9%). The blastocyst rates were slightly enhanced in the resveratrol supplemented groups (cows: 34.2 ± 3.8% and calves: 33.1 ± 4.2%) compared to those of standard protocol (cows: 27.5 ± 4.8% and calves: 26.4 ± 3.3%). The relative fluorescence levels of ROS were significantly lower (121 ± 34 FIU) in the resveratrol treated samples than in that of the control group (865 ± 45 FIU, $P \leq 0.05$). Additionally, the ROS levels in the untreated groups were significantly higher in MII-oocytes (1255 ± 56 FIU) and blastocysts (984 ± 26 FIU) derived from cows compared to their younger counterparts (442 ± 37 FIU and 310 ± 23 FIU, respectively, $P \leq 0.05$). In conclusion, these preliminary results indicate that resveratrol is able to influence the cellular redox status in oocytes and blastocysts of donors in different age.

A137E OPU-IVP and ET

The application of bovine *in vitro* embryo production technology to the rescue of Valdostana Castana breed

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Keywords: genetic rescue, bovine, embryo.

The Aosta Valley breed brown (Valdostana Castana) is a native rustic breed of alpine zone with a good attitude to the production of milk and meat. Excellent grazer, easily exploits even the high-altitude pastures, a characteristic that has led her to develop a defensive behaviour of its territory and an instinct for pugnacity. This propensity has been selected over the centuries and has turned the breed into "sporting animals" to be presented in local competitions and increasing the interest toward the most performing individuals. Moreover the Valdostana Castana counts a limited number of about 5000 animals giving further incentive to apply modern biotechnologies to preserve the breed. Sporadic attempts to apply superovulation have met with inconsistent results most likely due to the lack of a reliable stimulation protocol for the breed.
Therefore over the last three years the ovaries of 15 donor cows have been referred to our laboratory for embryo production by in vitro technologies. The oocytes were aspirated from ovarian follicles larger than 2mm and those with a non atretic cumulus cells were matured in medium TCM199 supplemented with 10% FCS, ITS (insulin, transferrin, sodium selenite, Sigma), FSH and LH (1:1, Menopur, Ferring) for 24h. Frozen semen was used for IVF following separation on a Redigrad gradient composed of two fractions: 45% and 90%. The motile fraction was resuspended in medium SOF-heparin (1µg/ml) at a concentration ranging from 0.3 to 1 million sperm/ml depending on the bull. The day after IVF (day 0) the presumptive zygotes were transferred in 500 microl of medium SOF1 plus aminoacids. On Day 4 and Day 6 half of the medium was changed with SOF2 plus aminoacids. On day 7 and 8 of culture the G1 embryos (IETS grading) that had reached the full/expanded blastocyst stage were frozen in 1.5M ethylene glycol. The freezing curve was seeding at -6°C and cooling at 0.5°C/min down to -32°C and plunging in liquid nitrogen.
The total number of oocytes recovered was 694 and the average number per donor was 46.27, ranging from 15 to 88. In total 8 different bulls were used for IVF and cleavage rate was between 25% and 89% with an average of 66.28%. The number of transferrable embryos was 141 ranging from 0 (1 cow) to 30 with an average of 9.4 per cow. The number of freezable embryos was 112 ranging from 0 (1 cow) to 26 with an average of 7.47 per cow. The percentage of transferable and freezable embryos was 30.65% and 24.35% of cleaved respectively. All the grade 1 embryos (112) were frozen in ethylene glycol. None of the non freezable embryos (29) was transferred as fresh.
Sixty-three frozen-thawed embryos were transferred into recipient heifers obtaining 21 pregnancies (33% pregnancy rate). At present 14 calves have been born from 17 pregnancies gone to term and 3 pregnancies were lost.
These results confirm that the in vitro embryo production technology can contribute to the preservation of special cattle breeds by generating valuable calves from donors destined to the abattoir.

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A138E OPU-IVP and ET

Melatonin accelerates the timing of *in vitro* porcine embryo development

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Keywords: Melatonin, embryo culture, porcine.

Melatonin (MEL) has been used to promote in vitro oocyte maturation and embryo development in different species, including the pig, due to its potential as antioxidant, anti-apoptotic and free radical scavenger. However, the effects of MEL on the timing of embryo development are still unknown. This study aimed to determine the influence of MEL on the developmental kinetics of in vitro produced porcine embryos. Immature oocytes (N = 868) collected from abattoir-derived ovaries of prepubertal gilts were cultured in 500 µL drops of 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 then inseminated with thawed sperm (1000 spermatozoa per oocyte) in 100 µL drops of fertilization medium for 5 h. Presumed zygotes were cultured in 500 µL drops of glucose-free embryo culture medium that was supplemented with pyruvate and lactate for 2 days and then changed to fresh embryo culture medium containing glucose for an additional 5 days. MEL (1 nM) was added to both the maturation and culture media (IVM+IVC group), or only to the culture medium (IVC group). Media without MEL supplement were used as control. Embryo developmental stages were evaluated after 24, 48, 120 and 144 h of culture to assess the timing of embryonic development. Data are presented as means ± SEM of 4 replicates, and differences among groups were analyzed by ANOVA. Our results show that MEL increased ($P < 0.001$) the rate of cleavage (59.1 ± 2.0% and 61.6 ± 3.5% for IVM+IVC and IVC groups, respectively) at 48 h of culture compared to controls (44.0 ± 5.6%). In addition, a higher ($P < 0.002$) proportion of embryos that cleaved within 48 h of fertilization reached the 3-4-cell stage at 24 h of culture in IVM+IVC (16.9 ± 1.9%) and IVC (17.8 ± 1.5%) groups compared to the control (10.1 ± 3.4%). MEL also increased ($P < 0.04$) the blastocyst formation rates at 120 and 144 h of culture (30.5 ± 2.5% and 40.2 ± 2.7%, and 29.9 ± 2.3% and 40.6 ± 2.2%, for IVM+IVC and IVC groups, respectively) in comparison with the controls (19.5 ± 1.4% and 29.3 ± 3.3%, respectively). At 120 h of culture, blastocysts formed in the IVM+IVC and IVC groups consisted of a population with less ($P < 0.002$) early blastocysts (47.2 ± 3.9% and 45.1 ± 2.6%, respectively) and more ($P < 0.002$) full-expanded blastocysts (52.8 ±3.9% and 54.9 ± 2.6%, respectively) compared with the controls (68.3 ± 3.9% and 31.7 ± 3.9%, respectively). Similarly, from the total number of blastocysts formed at 144 h of culture, the percentages of expanded-hatching-hatched blastocysts were higher ($P < 0.05$) in the IVM+IVC (58.7 ± 5.7%) and IVC (59.7 ±3 .8%) groups than in the controls (38.7 ± 8.8%). In conclusion, the addition of 1 nM MEL to embryo culture medium accelerated the timing of embryo development regardless of whether MEL was present or not in the maturation medium.

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A139E OPU-IVP and ET

Reproductive efficiency at a commercial farm comparing AI *versus* ET at first insemination

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Keywords: Reproductive efficiency, ET, first AI.

To evaluate reproductive efficiency using ET versus AI at first breeding post-calving procedures were performed on a commercial dairy farm in Spain. Eleven heifers were superovulated (Bo et al. Reprod Fertil Dev 2010 22:106-12). The same commercial frozen bull semen (Gerard, ABS) was used. Flushings were performed by the same ET-team (Nr. ES11ET05B). Quality 1 embryos were frozen in Ethylen-glycol and used. 170 primiparous (DIM=158 ± 17.9), were selected to be first-AI with the same semen (Group AI; n = 82; DIM=159 ± 16.3) or, ET with collected embryos (Group ET; n = 88; DIM=157 ± 19.4); DIM did not differ between groups (P = 0.402). Estrus of recipient/inseminated cows was detected (Westfalia Rescounter II pedometers). ET-cows were scanned (7.5-MHz linear, Ibex® Pro) and subjected to ET if at least one CL ≥18 mm diameter present. Luteal Surface (LS) was calculated. Early pregnancy diagnosis was performed at 28-34d (ED) and pregnant cows confirmed at 56-63d (CD). Data were analyzed with Chi squared test and logistic regression. Quality-1-embryos average/donor was 5.36; 32.9% of recipient cows were rejected. LS was 460 ± 223.5 mm2 and did not affect pregnancy. Two donors produced more fertile transferred embryos [88.9% (8/9) and =75% (6/8)], and two gave less fertile embryos [0% (0/4) and 14.3% (1/7) P=0.009]. These individual differences would not introduce bias into the study; the distribution of transferred embryos was 28% (17/59) of high fertility embryos, 19% (11/59) low fertility embryos and 52% (31/59) of average fertility embryos. Pregnancy loss did not differ among donors (P = 0.114). Fertility was higher for ET-cows [45.8% (27/59 P = 0.011)] than for AI-cows [24.4%; (20/82); OR of ED-pregnancy=2.616; P = 0.009]. DIM did not affect fertility (P = 0.7) with similar DIM (158.8 ± vs. 157.5 ± 18.1) for non-pregnant and pregnant cows. If rejected cows were included as non-pregnant, fertility did not differ [24.4% (20/82) and 30.7% (27/88) for AI and ET-groups; P = 0.39]. This highlights the relevance of the non-physiological ovarian activity resumption. Discarding recipients means to reject those with altered ovarian functionality. Therefore, one mean factor affecting fertility that can be overcome with ET-programs is to diagnose altered ovarian cycle-cows. 75.6% of non-pregnant cows after AI followed pregnancy diagnosis at day 28, with this date being the earliest date to treat. Rejected recipients (32.9%) could be treated at day 7. This accelerated reproductive rhythm has a positive impact on the farm pregnancy rate, and implies benefits of this first-AI ET-program. Early fetal loss did not differ among groups [5% (1/20) vs. 11% (3/27); P = 0.63], according with previous works showing similar pregnancy loss after day 42 of pregnancy. In conclusion, the ET-program resulted in a higher farm reproductive efficiency not through a higher fertility of the transferred embryos but through a better-expected global pregnancy rate. Economic implications should be further evaluated.

A140E OPU-IVP and ET

Genomic breeding value for number of OPU derived oocytes in bovine

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Keywords: OPU-IVP, breeding value, genomics.

The success of embryo production by ovum pick-up (OPU) in vitro production (IVP), is determined to a large extent by the initial number of oocytes that can be collected per donor cow. This number of oocytes varies per cow and has a heritability of 0.3 (Merton et al 2009, Theriogenology. 72:885-893). A genomic (based on DNA markers) breeding value for the number of oocytes could be used to select female animals (at young age) that give a high number of oocytes. Since OPU-IVP within CRV is already carried out for more than 20 years and almost all animals are genotyped, the development of such genomic breeding values for OPU should be possible and more accurate than a pedigree-based breeding value. The aim of this study is to determine the reliability of genomic breeding values for OPU.

The total dataset contained 2,543 female Holstein Friesian animals with in total 40,734 OPU sessions. From these animals, 890 were genotyped with the Illumina 50K SNP chip (reference population). Classical (pedigree based) and genomic (pedigree + DNA marker based) breeding values were estimated using the singe-step BLUP method. Reliability was determined as the correlation between the pedigree-based or genomic breeding value before OPU records were obtained with the realized number of oocytes after OPU using cross-validation.

Based on this dataset the reliability was 0.21 for the pedigree-based breeding values and the reliability increased to 0.29 when genomic information was added. The reliability of genomic breeding values for OPU oocytes was significantly lower than reliabilities obtained for milk production traits (which are around 0.65), but in that case the reference population is much larger, i.e. ~40,000 animals. When selecting the best or the worst 10% based on the genomic breeding value for oocytes, the best animals give two times more oocytes than the worst, 5.0 and 10.8 oocytes respectively. This shows that there is substantial genetic variation in OPU oocytes.

This study shows that it is feasible to estimate genomic breeding values for the number of oocytes derived after OPU before OPU records are obtained (no phenotypic data yet). This breeding value has a reliability that is high enough to be of practical use. The breeding value can be used to select donor female animals for optimal use in an OPU-IVP embryo production program. Since all animals in the CRV OPU program are genotyped, the reference population will grow over the coming years resulting in more reliable genomic breeding values.

A141E OPU-IVP and ET

The ability of boar epididimal semen for in vitro fertilization

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Keywords: pig, IVF, epididimal semen.

Boar semen tends to have different abilities of *in vitro* capacitation and *in vitro* fertilization (IVF). It has been demonstrated that epididimal boar spermatozoa can reach *in vitro* capacitation and IVF of oocytes easier than spermatozoa after ejaculation (Matás et al., Theriogenology, 74, 1327- 1340, 2010). Therefore the main purpose of this study was to compare the ability of *in vitro* capacitation and IVF of boar epididimal spermatozoa and spermatozoa after ejaculation and as well as *in vitro* development of fertilized oocytes to the blastocyst stage.
The epididimal semen was obtained after slaughter from 4 out of 18 selected boars of various breeds. Then the collected sperm was diluted in Biosolwens Plus (Biochefa, Sosnowiec, Poland). Ejaculated semen (n = 4) was obtained from one cross-breed boar with the correct motility parameters, selected from among 10 boars. The semen motility was evaluated under a microscope and by using the System SCA. The semen capacitated in a medium based on TCM-199 (Sigma-Aldrich) and incubated for a period of 1 hour in 5% CO_2 in the air and at a temperature of 39ºC. Cumulus-oocytes complexes were aspirated from follicles obtained from slaughterhouse ovaries. After maturation, oocytes were fertilized *in vitro* with epididimal semen (experimental group, n = 107 of oocytes) and with ejaculated semen (control group, n = 70 of oocytes). Presumptive zygotes were cultured in NSCU-23 medium up to the blastocyst stage. Blastocyst development was evaluated after 6-8 days of culture, and then the total cell number and apoptosis of blastocysts was detected with TUNEL. For statistical data Chi-squared-tests were used. The total motility (mean) of epididimal spermatozoa before and after capacitation was 70-85% (78.75 ± 5.15) and 65-85% (70 ± 5), respectively, and of ejaculated spermatozoa: 75-90% (82.5 ± 3.23) and 60-80% (68.75 ± 5.15), respectively, before and after capacitation. A slightly higher (no significant difference) proportion of cleavage rates, morula and blastocysts was observed in the experimental group (55.1, 30.8 and 16.8%, respectively) compared with the control group (48.6, 17.1 and 12.8%, respectively). Mean number of total cell nuclei per blastocyst was also slightly higher (no significant difference) in the experimental group (27.4 ± 1.78) than in the control group (21.2 ± 2.65). However, the mean number of apoptotic nuclei for both the experimental and control groups was low (0.67 ± 0.29 and 0.38 ± 0,18, respectively).
Summing up the results it can be concluded that the ability of *in vitro* fertilization of both the epididimal and ejaculated spermatozoa is similar, however, the selection of ejaculated semen was higher than selection of epididimal boar semen. Moreover, these studies show that developmental competence of embryos obtained after IVF of boar epididimal and ejaculated semen is comparable.

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