Table 1. Comparison of cumulus cell removal techniques on bovine blastocyst rates

Treatment	Total embryos	Day 8 blastocysts	% Development
Vortexing	521	44[a]	8.4
Handstripping	644	99[b]	15.4
Microfluidics	529	135[c]	25.5

[a–c] Values with different symbols are significantly different; $P = 0.0004$.

157 HOLSTEIN-CHINESE YELLOW HYBRID RECIPIENT OOCYTES RECOVERED BY OVUM PICKUP CAN IMPROVE THE DEVELOPMENT OF CLONED BOVINE EMBRYOS

X.Y. Yang, *J.G. Zhao*, *H.W. Li*, *H. Li*, *H.F. Liu*, *Y.T. Zeng*, *and S.Z. Huang*

Institute of Medical Genetics, Shanghai Jiao Tong University, Shanghai, China. Email: ytzeng@stn.sh.cn

We used the subspecies hybrid F1 oocytes (Holstein/Chinese Yellow cattle) recovered by ovum pickup (OPU) as recipient cytoplasts to improve the development of bovine cloned embryos. Ten Holstein cattle, four Chinese Yellow cattle, and four hybrid F1 bovines were subjected to OPU once a week. In total, 44, 110, and 42 OPU sessions were respectively performed for hybrid cattle, Holstein cattle and Chinese Yellow cattle. The mean numbers of punctured follicles for hybrid and Yellow cattle were higher than for Holstein cattle (11.4 ± 0.5 and 11.7 ± 0.5 vs. 10.1 ± 0.7, mean $\pm$ SE), but the recovery rate for Holstein cattle was higher than that for hybrid and Yellow cattle (76.2% vs. 70.3% and 66.6%); therefore, recovered oocytes per session were similar in hybrid, Holstein, and Yellow cattle (8.0 ± 0.5, 7.7 ± 0.4 and 7.8 ± 0.5, respectively). No difference was shown in the quality of the recovered oocytes among the three breeds. The three kinds of recipient oocytes had a maturation rate of 72–73% (256/353, 614/847, and 238/327, respectively). Matured oocytes were used as recipients without selection, and same batch cumulus cells collected from Holstein cow were used as donor cells. The nuclear transfer procedure was essentially as described by Park *et al*. (2004 Mol. Reprod. Dev. 69, 365–374). Cleavage rate of reconstructed embryos was similar in the hybrid, Holstein, and Yellow groups (66%, 66%, and 75%, respectively). However, the blastocyst rate from the cleavage embryos (51% vs. 37% and 27%), cell number of each blastocyst on Day 8 (135 ± 4.1 vs. 116 ± 3.6, and 101 ± 4.2), and the percentage of Grade A blastocysts (54% vs. 42% and 29%) in the hybrid group were higher than in the Holstein and Yellow groups. The proportion of blastocyst production on Day 7 was greater in the hybrid group than in the Holstein and Yellow groups (89% vs. 71% and 63%). The blastocyst rate from morula in the hybrid group (84%, 37/44) was significantly higher than in the Holstein group (63%, 52/83) and the Yellow group (59%, 24/43). Taken together, these results strongly show that hybrid recipient oocytes can improve the development of cloned bovine embryos. It is suggested that the genetic heterogeneity of the hybrid recipient cytoplasm would lead to more possibilities of reprogramming and embryo development.

Table 1. Effect of different recipient oocytes on development of cloned embryos

Parameters	Hybrid	Holstein	Yellow	Total
No. of matured oocytes	256	614	238	1108
Fused reconstructed embryos (%)	110 (43)[a]	216 (35)[b]	120 (50)[a]	446 (40)
Cleavage (%)	73 (66)	142 (66)	90 (75)	305 (68)
Blastocysts (%)	37 (51)[a]	52 (37)[b]	24 (27)[b]	113 (37)
Cell number of blastocysts (Day 8)	135 ± 4.1[a]	116 ± 3.6[b]	101 ± 4.2[c]	118 ± 3.1

[a–c] Values with different superscripts within the same row are significantly different ($P < 0.05$).
Data were analyzed by ANOVA and chi-square using SAS (SAS Institute, Inc., Cary, NC, USA).

This work was supported by the Chinese "863" High-Tech Plan Program (Grant No. 2002AA206201).

Embryo Transfer

158 PREGNANCY RATES IN RECIPIENT COWS TREATED WITH PROGESTERONE VAGINAL DEVICES AND INDUCED TO OVULATE WITH ESTRADIOL BENZOATE GIVEN AT THE TIME OF DEVICE REMOVAL OR 24 h LATER

G.A. Bo[A,B], *E. Balla*[A,B], *L. Cutaia*[A,C], *L. Peres*[A], *P.S. Baruselli*[D], *and R. Tribulo*[A,B]

[A]Instituto de Reproduccion Animal Cordoba, Cordoba, Argentina; [B]Universidad Catolica de Cordoba, Cordoba, Argentina; [C]Agencia Cordoba Ciencia, Cordoba, Argentina; [D]Departamento de Reproducao Animal, FMVZ-USP, Sao Paulo, Brazil.
Email: gabrielbo@iracbiogen.com.ar

Although treatments with progesterone (P4) releasing devices, estradiol benzoate (EB) and eCG have been shown to result in acceptable pregnancy rates after embryo transfer, the treatment requires that the cows be run through the chute at least four times for treatments. An experiment was

designed to compare pregnancy rates in cows treated with P4 releasing devices plus EB and eCG, and induced to ovulate with EB given at device removal or 24 h later. Non-lactating *Bos taurus* × *Bos indicus* crossbred beef cows ($n = 165$), with a body condition score between 2.5 to 3.5 (1 to 5 scale), were treated with a P4-device (DIB, Syntex, Argentina) and 2 mg EB i.m. (Syntex), on Day 0 and 400 IU of eCG i.m. (Novormon, Syntex) plus 150 μg D(+)cloprostenol i.m. (Ciclase, Syntex) on Day 5. On Day 8, DIB devices were removed and cows were randomly divided into two groups to receive either 1 mg EB i.m. at the time of DIB removal (EB0) or 24 h later (Day 9; EB24). Recipients were observed for signs of estrus for 48 h after DIB removal. On Day 16, all recipients observed in estrus and with >1 CL or a single CL with an area >256 mm^2 were selected to receive fresh embryos on Day 16 (EB0) or Day 17 (EB24). Furthermore, 20 recipients not observed in estrus but with a CL >256 mm^2 were randomly selected and transferred. The embryos used were 28 Grade 1, 40 Grade 2, and 24 Grade 3. Ovarian ultrasonography was performed on Day 0, to determine ovarian status (only cows with a CL or a follicle >10 mm and uterine tone were used), on Day 16 to measure CL area, and 60 days after embryo transfer to determine pregnancy status. Quantitative data were analyzed by Student's *t*-test and qualitative data were analyzed by logistic regression. There were no differences between groups in the mean (± SEM) CL area on Day 16 (EB0: 300.3 ± 12.0 mm^2 and EB24: 324.9 ± 11.7 mm^2; $P = 0.14$), the proportion of recipients in estrus (EB0: 38/82, 46.3%, and EB24: 34/83, 41.0%; $P = 0.49$), the number of recipients with >1 CL or a CL >256 mm^2 (EB0: 68/82, 82.9%, and EB24: 72/83, 86.7%; $P = 0.49$) and the number of recipients pregnant/transferred (EB0: 31/49, 63.4%, and 20/43, 46.5%; $P = 0.23$). Furthermore, there were no significant effects of embryo quality ($P = 0.31$) or technician ($P = 0.12$) on pregnancy rates. The mean interval from DIB removal to estrus was shorter ($P = 0.001$) for recipients in the EB0 group (22.7 ± 1.0 h) than for those in the EB24 group (37.4 ± 1.2 h). Nevertheless, pregnancy rates did not differ ($P = 0.14$) between recipients seen in estrus (EB0: 21/38, 55.3%, and EB 24: 16/34, 47.1%) and those not seen in estrus but with a CL >256 mm^2 on Day 16 (EB0: 10/11, 90.9%, and EB24 4/9, 44.4%). It was concluded that the two treatments evaluated are equally efficacious for synchronizing *Bos taurus* × *Bos indicus* recipients. Furthermore, the use of EB at device removal could reduce the number of trips through the chute without affecting pregnancy rates.

Research was supported by Syntex S.A., Estancia El Mangrullo S.A., and Agencia Cordoba Ciencia S.E.

159 TWIN vs. SINGLE TRANSFER OF IVP HOLSTEIN HEIFER EMBRYOS TO BEEF RECIPIENTS

A. Fischer-Brown[A], *G. Barquero*[B], *S. Clark*[B], *C. Ferguson*[B], *F. Ireland*[B], *N. Jensen*[A], *S. Lane*[B], *B. Lindsey*[A,C], *P. Lopes*[B], *R. Monson*[A], *D. Northey*[A], *A. Reeder*[A], *J. Rutledge*[A], *M. Wheeler*[B], *and D. Kesler*[B]

[A]University of Wisconsin-Madison, Madison, WI 53706, USA; [B]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [C]Minitube of America, Verona, WI 53593, USA. Email: afischer@ansci.wisc.edu

Use of sexed semen in conjunction with *in vitro* embryo production is a potentially efficient means of obtaining offspring of predetermined sex. Here we evaluate a production scheme involving single and bilateral twin transfer of Holstein female embryos to beef cattle recipients. Holstein oocytes were fertilized with the X-bearing fraction of gender-sorted Holstein semen. Cumulus cells were removed with aid of a vortex or microfluidic device (μFD). Half of the vortexed embryos were cultured in KSOMaaBSA (control), as were all μFD embryos. The remaining vortexed embryos were cultured in control medium with 6% avian white yolk (WY). Embryo production and transfer occurred across five replicates. Cows ($n = 475$) were synchronized using an Ovsynch protocol. They were administered GnRH on Day −9, PGF on Day −2, and GnRH on Day 0. Half of the cows received a CIDR (1.38 g progesterone) with the 1st GnRH injection. The CIDR was removed at the time of PGF treatment. Day 7 Grade 1 blastocysts were transferred fresh 7 days after the 2nd GnRH injection. Control and WY embryos were transferred as ipsilateral singles and bilateral twins; μFD embryos were transferred singly. Pregnancy was diagnosed with ultrasound between 41–46 days and confirmed between 60–90 days; fetal sexing confirmed that 95% of fetuses were female. Effects on embryo survival were analyzed by logistic regression. Chi-square analysis was applied to survival rates. Replication affected embryo survival ($P < 0.05$). There was no effect of cumulus removal, medium, or CIDR use. Fetal loss between ultrasounds was greater for twin vs. single transfers (30% vs. 15%, respectively; $P < 0.01$). Probability of embryo survival was estimated to increase ~0.006 with each increasing day postpartum. Five cases of hydrallantois were detected during the 5th month of gestation for 1 control twin, 1 WY single, and 3 WY twin transfers, originating from 3 replicates. On a production per transfer basis, the proportion of fetuses obtained for single and twin transfers was 30% and 55%, respectively ($P < 0.001$). Although there was greater embryonic loss for twin compared to single transfers, a higher percentage of cows receiving twins established and maintained pregnancy. Large-scale transfer of IVP Holstein heifer embryos to beef recipients is a feasible production scheme.

Table 1. Embryo survival and pregnancy rates

	No. embryos transferred	No. fetuses, 1st ultrasound (%)	No. fetuses, 2nd ultrasound (%)	No. recipients	No. pregnant, 1st ultrasound (%)	No. pregnant, 2nd ultrasound (%)
Single ET	291	104 (36)[a]	88 (30)[a]	291	104 (36)[a]	88 (30)[a]
Twin ET	368	146 (40)[a*]	102 (28)[a†]	184	84 (46)[b*]	73 (40)[b†]
Total	659	250 (38)	190 (29)	475	188 (40)	161 (34)

[ab] Values within columns with different superscripts differ significantly ($P < 0.05$); *62 twins, 22 singles;
† 29 twins, 44 singles; loss after 1st ultrasound included 5 singles, 1 fetus of 27 pairs, and 6 pairs.

160 *IN VIVO*-CULTURE OF BOVINE EMBRYOS: TRANSFER OF SEMEN PRE-INCUBATED OOCYTES, ZYGOTES AND 4 TO 8 CELL STAGE EMBRYOS INTO THE BOVINE OVIDUCT

V. Havlicek[A], *F. Wetscher*[A], *T. Huber*[A], *M. Gilles*[B], *D. Tesfaye*[B], *J. Griese*[B], *F. Rings*[B], *H. Müller*[B], *K. Schellander*[B], *G. Brem*[A], *and U. Besenfelder*[A]

[A]Institute of Animal Breeding and Genetics, VUW, Vienna, Austria; [B]Institute of Animal Breeding Science, University of Bonn, Bonn, Germany. Email: vitezslav.havlicek@boku.ac.at

Oviduct as well as oocyte and embryo development are subject to developmental changes which have crucial effects on the application of *in vivo* culture. The present study aimed at optimizing *in vivo* culture of IVP bovine embryos at different developmental stages in the bovine oviduct. Cumulus oocyte complexes (COC) were collected from slaughterhouse ovaries, matured *in vitro* for 22 h and assigned to four groups. In groups I and II, oocytes were pre-incubated for 3 to 4 h with 5×10^6 sperm/mL, and then immediately transferred to recipients, which had just completed ovulation (group I), or kept *in vitro* for a further 12 to 18 h and transferred to Day 1 synchronized recipients (group II). In groups III and IV, COC were subjected to standard IVF/IVC; then embryos were either transferred at the 4- to 8-cell stage on Day 3 into the oviducts of Day 3-synchronized recipients (group III) or kept *in vitro* for a further 4 to 5 days (group IV). Thirty-four 18- to 30-month-old temporary recipients were synchronized using a standard Ovsynch protocol. COC and embryos were transferred and re-collected by transvaginal endoscopy. COC or embryos were loaded into a 180° curved glass capillary, which was inserted via the infundibulum 5 to 8 cm deep into the ampulla ipsilateral to the CL. On recipient Day 7, a 90° curved metal canula served for tubal flushing prior to conventional uterine embryo flushing. Sixty mL of PBS containing 1% fetal calf serum were rinsed through the oviduct into the uterus and a further 400 mL of medium were finally used for flushing of the uterine horn and collected via an embryo filter. Embryo development was evaluated directly after flushing (Day 7) and on Day 8. For statistical analysis (ANOVA), the blastocyst rates (Days 7 and 8) in group III were related to COC corrected by the collection rate. In group I, 575 COC were transferred to 11 recipients and 420 (73%) were re-collected as oocytes or embryos. The blastocyst yields on Day 7 and Day 8 were 23% (97) and 25% (104), respectively. In group II, the transfer of 489 presumptive zygotes into 13 heifers resulted in only 175 re-collected (36%), of which 15% developed into blastocysts (Day 7: 26; Day 8: 27). Ten heifers (group III) served for *in vivo* culture of 643 embryos at the 4- to 8-cell stage. On Day 7, 568 (88%) embryos were flushed and 171 (30%) reached the blastocyst stage. A further 24 h culture *in vitro* finally resulted in 244 (42%) blastocysts. The complete *in vitro* production system delivered 13% (63/477) blastocysts on Day 7 and 34% (161/477) blastocysts on Day 8. The collection rates ($P < 0.001$) and the blastocyst rates on Day 7 ($P < 0.05$) and Day 8 ($P < 0.001$) differed significantly in all groups. The present data demonstrate that the developmental stage of transferred complexes has an influence on embryo recovery as well as an embryo development.

This work was supported by Austrian BMBWK and BMLFUW (#1227).

161 PREGNANCY RATES OBTAINED AFTER EMBRYO TRANSFER AT FIXED TIME OF *IN VIVO*-, IVF- AND CLONED-DERIVED EMBRYOS

J. Lagioia, *M. Panarace*, *M. Marfil*, *M. Basualdo*, *J. Gutierrez*, *M. Révora*, *and M. Medina*

Centro de Investigaciones Reproductivas Perez Companc, Goyaike, Ea. San Joaquín, Argentina. Email: jlagioia@goyaike.com.ar

The most important factor in bovine embryo transfer programs is the low efficiency in the utilization of the recipients; this low efficiency is associated with low response to synchronization protocols and failures in estrus detection. It has been shown that cows transferred at fixed time with *in vivo*-derived embryos resulted in high rates of recipients selected for transfer and high overall pregnancy rates (recipients pregnant/recipients treated) (Tribulo *et al*. 2002 Theriogenology 57, 563). An experiment was designed to evaluate the pregnancy rate in recipients transferred with *in vivo* (fresh and frozen), IVF, and cloned-derived embryos without estrus detection. A total of 1555 non-lactating *Bos Taurus* crossbred beef cows was divided into two groups. Cows from group 1 ($n = 421$) were synchronized with a progesterone intravaginal releasing device (1 g P4; DIB, Syntex®, Buenos Aires, Argentina) plus 2 mg of estradiol benzoate (EB) i.m. (Syntex®) on Day 0. On Day 5, they received 400 IU of eCG (Novormon 5000, Syntex®) i.m. and 150 μg of D-Cloprostenol ($PGF_{2\alpha}$) (Bioprost-D, Biotay®, Buenos Aires, Argentina). The DIB devices were removed on Day 8 and on Day 9, 1 mg of EB was injected. Day 10 was arbitrarily considered as the day of estrus. Cows from group 2 ($n = 1134$) received 2 doses of $PGF_{2\alpha}$ 14 days apart and were checked for heat during 5 days after the second $PGF_{2\alpha}$ dose. Cows of both groups were examined 7 days after estrus by ultrasonography (Pie Medical Scanner 200®) and those with a corpus luteum >10 mm of diameter were transferred nonsurgically with *in vivo* (fresh and frozen), IVF, and cloned-derived embryos. In group 1, 360 cows were transferred, and in group 2, 726 cows were transferred (Table 1). Pregnancy was diagnosed 23 days later by ultrasonography (Pie Medical Scanner 200®). The pregnancy rates were compared statistically between groups 1 and 2 by analysis of variance (Infostat, LSD Fisher). There was no significant statistic difference ($P > 0.05$) between pregnancy rate in group 1 and 2 with *in vivo* (fresh), IVF, and cloned-derived embryos. However, pregnancy rate of frozen *in vivo*-derived embryos was lower in group 1 than in group 2 ($P < 0.05$). Results showed that treatment using DIB combined with EB, $PGF_{2\alpha}$, and eCG associated with embryo transfer without estrus detection (group 1) had no difference in pregnancy rate when compared with the treatment where synchronization with $PGF_{2\alpha}$ and heat detection were used (group 2). Another important advantage is the use the group 1 treatment for increasing the flexibility and efficiency in the management of the recipients of *in vivo*, IVF, and cloned-derived embryo transfer programs.

Table 1. Comparison of pregnancy rates between group 1 (embryo transfer at fixed time) and group 2 (embryo transfer 7 days after estrus detection)

Embryo	Group	Embryos transferred	Pregnant recipients	(%)
Cloned	1	55	30	54.5[a]
Cloned	2	67	31	46.3[a]
IVF	1	13	9	69.2[a]
IVF	2	76	32	42.1[a]
Fresh	1	47	28	59.6[a]
Fresh	2	304	184	60.5[a]
Frozen	1	245	120	49.0[a]
Frozen	2	279	165	59.1[b]

[a,b] Values with different superscripts are significantly different ($P < 0.05$).

162 EMBRYO QUALITY AND COLOR IN HOLSTEIN FRIESIAN HEIFERS AND COWS IN RELATION TO SERUM PARAMETERS

J.L.M.R. Leroy[A], *L. Goossens*[B], *A. Geldhof*[B], *T. Vanholder*[A], *S. De Vliegher*[A], *G. Opsomer*[A], *and A. Van Soom*[A]

[A]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, University of Ghent, Ghent, 9820, Belgium; [B]Flemish Cattle Breeding Association, Oosterzele, Belgium. Email: jo.leroy@ugent.be

Bovine embryo freezability is closely linked with quality and differs between breeds (Visintin JA *et al*. 2002 Theriogenology 57, 345–359). Dark embryos have a poor cryotolerance resulting in low pregnancy rates (Hill BR and Kuehner LF 1998 Theriogenology 49, 168), and are characterized by excessive accumulation of lipid droplets which in turn may be influenced by the biochemical composition of the embryonic environment. Earlier work revealed that Holstein Friesian (HF) cows yield significantly darker embryos compared to Belgian Blue beef cows (Leroy JLMR *et al*. 2004 Reprod. Fert. Dev. 16, 211). In this field examine we aimed to study the differences in embryo quality and color between HF cows (producing milk) and heifers (non-producing) in relation to four serum parameters that have previously been linked with embryo quality and fertility: urea, total protein (TP), total cholesterol (TC), and triglycerides (TG). Blood samples were collected from HF cows ($n = 54$) and HF heifers ($n = 33$) prior to embryo flushing on Day 7 after superovulation and subsequent insemination. Serum was stored frozen until assay with commercial photometric assays. Embryos were scored individually by the same operator for quality (excellent to poor: score 1 to 4) and color: light (L), medium (M), or dark (D) using a binocular stereomicroscope (40×). Independent Student's *t*-test and chi-square test were used when appropriate. Heifers and cows yielded on average (± SEM) 5.1 ± 0.82 and 6.1 ± 0.71 embryos per flushing, respectively. Significantly more cow embryos were classified as dark compared to heifer embryos (L, M, D: 20.4%, 55.5% and 24.1% vs. 70.8%, 27.4%, and 1.8%, for cow and heifer embryos, respectively) ($P < 0.05$). Significantly more heifer embryos showed an excellent morphological quality (62.5% of heifer embryos compared to 13.1% of cow embryos) ($P < 0.05$). Serum concentrations (± SEM) of urea (4.5 ± 0.2 vs. 2.8 ± 0.2 mM), TP (7.59 ± 0.07 vs. 6.57 ± 0.09 g/dL) and TC (183 ± 5.3 vs. 105 ± 3.8 mg/dL) were significantly higher and serum concentrations of TG (17.2 ± 0.6 vs. 23.8 ± 0.9 mg/dL) significantly lower in cows compared to heifers ($P < 0.05$). In this study, embryo color (i.e. lipid content) and quality as well as urea, TP, TC, and TG serum concentrations were clearly influenced by the production parameter (cow = producing vs. heifer = non-producing). These findings imply that producing milk (parity = 0 or >0) may be an important confounder. Therefore, this variable should be taken into account when studying multiple variables influencing embryo quality or color simultaneously. Further analyses are ongoing to reveal which factors at the cow level are affecting the embryo quality and color.

This research was funded by IWT-Flanders (Grant no. 13236).

163 DOES LH PLUS hCG, USED TO INDUCE OVULATION IN SUPERSTIMULATED COWS, IMPROVE PREGNANCY RATES AFTER TRANSFER OF EMBRYOS THAT ARE NOT EXCELLENT?

M.F.G. Nogueira[A], *J. Buratini Jr*[B], *and C.M. Barros*[A]

[A]Department of Pharmacology, Instituto de Biociências, UNESP; [B]Department of Phisiology, Instituto de Biociências, UNESP, Sao Paulo, Brazil. Email: marcelo:femanet.com.br

The LH receptor (LHr) present in ovarian follicle cells is essential for the biological response due to its binding to the ligand (LH). The events following the LH surge are related to the presence of LHr and its affinity to LH. Studies on LHr gene expression in theca and granulosa cells from bovine follicles have demonstrated the presence of at least four isoforms of LHr mRNA by alternative splicing. Two of the four detected isoforms have an open reading frame (ORF) and can be translated to functional proteins (receptors coupled with G protein) with different affinities to their ligands (Robert C *et al.* 2003 Reproduction 125, 437–446). From those two isoforms with ORF, one (full) has affinity to both LH and hCG molecules, whereas the second isoform (with deletion of exon 10) has affinity to hCG only. Recently we have detected the same four isoforms described by Robert *et al*. in both granulosa and theca cells from abattoir. Based on this information, the present study tested the hypothesis that, in cows superstimulated with FSH, administration of both LH and hCG, as an attempt to stimulate any variety of LH receptor present in the follicles, would result in improvement of oocyte quality and/or increase in ovulation rate. Nelore cows (*Bos taurus indicus*) were superstimulated according to the protocol termed P-36 (Barros CM *et al.* 2003 Theriogenology 59, 524 abst), and embryos were flushed 7 to 8 days after inducing ovulation. Ovulation was induced with LH

(pLH, 12.5 mg, i.m.; Lutropin, Vetrepharm, London, Ontario, Canada; Group 1) or both LH (12.5 mg) and hCG (1500 IU, i.m.; Choragon, Ferring GmbH, Kiel, Germany; Group 2). Superstimulation protocol and embryo transfer were performed simultaneously on both groups. Mean (± SEM) of total structures, viable embryos, and viability rate were: 12.4 ± 2.36, 10.0 ± 2.38 and 80.8% (Group 1, $n = 8$ flushings) and 12.2 ± 2.03, 8.9 ± 1.66, and 73.1% (Group 2, $n = 14$); there was no significant difference between groups ($P = 0.96$; $P = 0.71$ (ANOVA), and $P = 0.18$ (Fisher's Exact Test), respectively). In a subset of embryos (excellent, good, fair, and poor qualities) transferred without freezing, the pregnancy rates for groups 1 and 2 were 41.7% (25/60) and 56.9% (37/65), respectively ($P = 0.11$, Fisher's Exact Test). The overall pregnancy rates from embryos of excellent, good, and fair qualities were 46.3% (25/54) and 58.9% (33/56), respectively for Groups 1 and 2 ($P = 0.25$). When only embryos of good, fair, and poor qualities were pooled, the pregnancy rates were 18.8% (06/32) and 60.0% (30/50), respectively for Groups 1 and 2 ($P = 0.0003$). It is concluded that simultaneous administration of LH and hCG to induce ovulation in superstimulated animals did not alter production of viable embryos or viability rate. However, the use of hCG in addition to LH administration could be beneficial for embryos of good, fair, and poor qualities.

Nogueira has a fellowship from FAPESP (São Paulo).

164 EFFECT OF ACCUMULATION OF LIPIDS DURING *IN VITRO* CULTURE ON BOVINE BLASTOCYST YIELD AND FETAL DEVELOPMENT

J.A. Rooke, M. Ewen, G.J. McCallum, R.G. Watt, and T.G. McEvoy

Scottish Agricultural College, Aberdeen, AB21 9YA, UK. Email: j.rooke@ab.sac.ac.uk

Previous research has shown that increased embryo lipid content, through culture in the presence of serum, is associated with reduced blastocyst yield and quality; provision of antioxidants ameliorates the effects of lipid accumulation (McEvoy *et al*. Reprod. Fertil. Dev. 16, 200). The present study extended these observations to assessment of fetal development from blastocysts which had accumulated lipid *in vitro*. Bovine oocytes, aspirated from abattoir-derived ovaries, were matured and fertilized using standard procedures. Cleaved zygotes were assigned to culture (5% CO_2; 5% O_2; 90% N_2; 38.5°C) in four treatments: (1) synthetic oviductal fluid containing 0.3% bovine serum albumin and amino acids (SOF); (2) SOF plus supplementary bovine lipoproteins (2%, SOFLP); (3) SOF plus the antioxidant Trolox® (100 μM; 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, Sigma-Aldrich Co., Inc., Irvine, UK; SOFT); and (4) SOF plus lipoproteins and Trolox (SOFLPT). Blastocysts were synchronously transferred to recipient cattle (day 7; 23/treatment) together with a control group ($n = 21$) of artificially inseminated (AI) cattle. Reproductive tracts were recovered on Day 70 and fetal total and organ weights recorded. Data were analyzed by ANOVA (with fetal sex as covariate) and chi-square analyses. Culture in the presence of lipoproteins increased blastocyst total fatty acids (mean ± SD) from 98 ± 9.7 to 124 ± 7.3 ng/blastocyst. Blastocyst yields (%; 23 batches of ovaries) were reduced ($P = 0.002$) by inclusion of lipoproteins in culture (SOF, 22.0 ± 8.20; SOFLP, 16.4 ± 8.57; SOFT, 22.8 ± 9.03; SOFLPT, 24.2 ± 7.30) unless Trolox was present. Blastocyst grade was poorer ($P < 0.001$) after culture in the presence of lipoproteins irrespective of the presence of Trolox (SOF, 2.4 ± 0.43; SOFLP, 2.6 ± 0.45; SOFT, 2.4 ± 0.47; SOFLPT, 2.6 ± 0.40). Pregnancy rates (Day 70) were greater ($P < 0.001$) for AI (91%) than culture (26%) but were not affected by culture treatment. Although there were no differences in fetal weights between AI and IVC fetuses, SOFLP fetuses (Table 1) were lighter, had smaller relative liver weights, and had greater crown-rump length-to-weight ratios than other IVC fetuses. Therefore, IVC in conditions that increased blastocyst lipid content without adequate antioxidant protection reduced blastocyst yield and influenced fetal development.

Table 1. Fetal weights (g) and relative liver weights (g/kg) and crown-rump lengths (CRL, mm/g) at day 70

	SOF	SOFLP	SOFT	SOFLPT	AI
Total wt	48 ± 1.6^{b}	39 ± 1.5^{a}	43 ± 1.8^{ab}	45 ± 0.7^{b}	42 ± 0.9
Liver wt	37 ± 1.5^{ab}	35 ± 2.3^{a}	38 ± 0.9^{ab}	46 ± 3.5^{b}	41 ± 1.0
CRL	1.9 ± 0.09^{a}	2.4 ± 0.11^{b}	2.1 ± 0.08^{ab}	2.0 ± 0.05^{a}	2.2 ± 0.07

Row means with different superscripts differ ($P < 0.05$).

SAC receives financial support from SEERAD.

165 SYNCHRONIZATION TREATMENT WITH NEW AND REUSED CIDR-B DEVICES: ESTRUS AND PREGNANCY RATES IN AN EMBRYO TRANSFER PROGRAM

C.W. Solorzano[A], *J.H. Mendoza*[B], *A. Villa-Godoy*[A], *C.S. Galina*[A], *and S. Romo*[C]

[A]FMVZ, Universidad Nacional Autonoma de Mexico, Cuautitlan, Mexico; [B]Private Practice;
[C]FESC, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico. Email: romo@servidor.unam.mx

An earlier study demonstrated that in CIDRs used in adult cows on two previous occasions, there was still a sufficient amount of progesterone (P4) remaining that allowed estrus synchronization in heifers (Solorzano *et al*. 2004 Reprod. Fert. Dev. 16, 214). However, the diverse conditions existing in that study made a statistical comparison impossible. The objective of this study was to study the effect of new and used CIDRs, combined with estradiol benzoate (EB) and prostaglandin F2-α (PGF) treatments, on estrus synchronization rates (ESR) and pregnancy rates (PR) in an embryo

transfer program in Brangus cows in a tropical climate. We used the same set of CIDRs in the same location in cows of the same breed, age, and body condition during three consecutive weeks. Cows were randomly allocated to one of three treatment groups. In Group 1, all cows ($n = 44$) were treated with a new 1.9-g CIDR (CIDR-B, InterAg, Hamilton, New Zealand), combined with 2 mg EB on Day 0. In Group 2, all cows ($n = 43$) were treated with a reused (first reuse) 1.9-g CIDR and 2 mg EB on Day 0. In Group 3, all cows ($n = 42$) received a reused (second reuse) 1.9-g CIDR and 2 mg EB on Day 0. CIDR devices were removed on Day 7 and all cows received PGF (0.25 μg cloprostenol) at that time. Estrus was expected to occur 24 h later. Seven days after estrus all cows showing heat were examined by rectal palpation, and those with a CL 15 mm in diameter or larger were selected to receive a frozen/thawed embryo (in 1.5 M ethylene glycol) by nonsurgical direct transfer. PR were determined by rectal palpation 60 days after estrus. In Group 1, a total of 90.9% of the cows displayed signs of estrus (40/44), and 42% of those that received a frozen embryo were pregnant (16/38). In Group 2, a total of 88.4% of the treated cows showed signs of estrus (38/43), and 37% of those recipients became pregnant (13/35). In Group 3, 88% of treated cows showed signs of estrus (37/42), and 36% of cows receiving an embryo were pregnant (13/36). ESR and PR were compared by χ^2 and Fisher's tests, and no differences were found among the three groups studied. This confirms that, in 1.9-g CIDRs used on two previous and consecutive occasions, there is still a sufficient amount of P4 remaining that allows successful estrus synchronization and pregnancy rates in a third use.

166 EFFECT OF TREATMENT WITH hCG OR GnRH AT THE TIME OF EMBRYO TRANSFER ON PREGNANCY RATES IN COWS SYNCHRONIZED WITH PROGESTERONE VAGINAL DEVICES, ESTRADIOL BENZOATE, AND eCG

R. Tribulo[A,B], *E. Balla*[A,B], *L. Cutaia*[A,C], *G.A. Bo*[A,B], *P.S. Baruselli*[D], *and L. Peres*[A]

[A]Instituto de Reproduccion Animal Cordoba, Cordoba, Argentina; [B]Universidad Catolica de Cordoba, Cordoba, Argentina; [C]Agencia Cordoba Ciencia, Cordoba, Argentina; [D]Departamento de Reproducao Animal, FMVZ-USP, Sao Paulo, Brazil.
Email: gabrielbo@iracbiogen.com.ar

Although several studies have investigated the relationship between circulating progesterone and pregnancy rates in cattle, the beneficial effect of treatments that increase progesterone concentrations, by insertion of a progesterone (P4) releasing device or induction of an accessory CL with hCG, GnRH, or LH treatment, has resulted in inconsistent effects on pregnancy rates in embryo recipients. An experiment was designed to evaluate the effect of hCG or GnRH treatment, given at the time of embryo transfer without estrus detection, on pregnancy rates in recipients treated with intrauterine P4-releasing devices, estradiol benzoate (EB), and eCG. The experiment was performed in two replicates; non-lactating *Bos taurus* × *Bos indicus* crossbred beef cows with a body condition score between 2.5 to 3.5 (1-to-5 scale) were used (replicate 1, $n = 180$; replicate 2, $n = 140$). All cows received 1 g of P4 via a P4-releasing device (DIB, Syntex, Argentina) and 2 mg EB i.m. (Syntex) on Day 0, and 400 IU of eCG i.m. (Novormon 5000, Syntex) plus 150 μg D(+)cloprostenol i.m. (Ciclase, Syntex) on Day 5. DIBs were removed on Day 8 and all cows received 1 mg EB i.m. on Day 9. Recipients were not observed for signs of estrus, and those >1 CL, or a single CL with an area >256 mm^2, received 195 Grade 1 and 46 Grade 2 frozen/thawed "direct transfer" embryos on Day 17. At the time of embryo transfer, recipients were randomly allocated to 1 of 3 treatment groups to receive 1500 IU hCG (Ovusyn, Syntex), 50 μg Lecirelina (GnRH, Gonasyn, Syntex), or no treatment (control) at that time. Ovarian ultrasonography was performed on Day 0 to determine ovarian status (only cows with a CL or a follicle >10 mm and uterine tone were used), on Day 17 to measure CL area, and 40 days after embryo transfer to determine pregnancy status. Data were analyzed by logistic regression and the effects of replication, technician, treatment, and embryo quality were considered in the model. From the 320 recipients treated with a DIB plus EB and eCG, 241 (75.3%) were selected to receive an embryo. Nine (3.7%) and 1 (0.4%) of the selected recipients had 2 and 3 CL, respectively. Pregnancy rates did not differ between replicates (replicate 1: 80/140, 57.1%; and replicate 2: 57/101, 56.4%; $P = 0.84$), technicians (technician 1: 65/118, 55.1%; and technician 2: 72/123, 58.5%; $P = 0.64$), or treatments (hCG: 43/80, 53.8%; GnRH: 45/83, 54.2%; and control: 49/78, 62.8% $P = 0.99$). However, pregnancy rates were higher ($P = 0.001$) in recipients receiving Grade 1 embryos (121/195, 62.1%) than in those receiving Grade 2 embryos (16/46, 34.8%). GnRH or hCG treatment at the time of embryo transfer did not increase pregnancy rates in recipients synchronized with P4 releasing devices, EB, and eCG.

Research was supported by Syntex S.A., Estancia El Mangrullo S.A., and Agencia Cordoba Ciencia S.E.

Embryonic Stem Cells

167 ISOLATION AND COMPARATIVE PROFILING OF HUMAN ADIPOSE-DERIVED ADULT STEM CELLS

A. Boquest[A], *A. Shahdadfar*[B], *K. Fronsdal*[B], *J. Brinchmann*[B], *and P. Collas*[A]

[A]Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; [B]Department of Immunology, Rikshospitalet, Oslo, Norway.
Email: andrewcb@medisin.uio.no

The stromal compartment of mesenchymal tissues is thought to harbor stem cells that display extensive proliferative capacity and multilineage potential. However, despite their potential impact in the field of regenerative medicine, little is known about the biology of stromal stem cells prior to culture. After removing adipocytes and erythrocytes from collagenase digested human adipose tissue, we identified two cell populations using flow cytometry which shared expression of stem cell markers SH2 and CD34, but lacked the phenotypic characteristics of leukocytes (CD45−). However, they were found to be discernible based on CD31 expression, a marker for endothelial cells. Using CD31 conjugated magnetic beads, we separated these cells (CD45-CD31− and CD45-CD31+) from three patients and compared global gene expression profiles using an Affymetrix platform. The