ABSTRACTS FOR POSTER PRESENTATION

Embryo Transfer

168 FACTORS AFFECTING ON EMBRYO TRANSFER PREGNANCY RATES OF IN VITRO-PRODUCED BOVINE EMBRYOS

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The objective of this study was to analyze factors affecting the pregnancy rates after transfer of IVF-derived Japanese Black embryos. Holstein cows and heifers ($n = 7250$) were selected as recipients, and embryo transfers were performed for 3 yr (between 1998 and 2000). The IVM-IVF procedure was performed according to a method previously described (Hamano S and Kuwayama M 1993 Theriogenology 39, 703–712). IVF-derived embryos that developed into expanded blastocysts (grade 1, manual of IETS) after 7 to 8 days (insemination = Day 0) were used for this study. Some of these embryos were frozen in TCM-199 supplemented with 1.4 M glycerol, 20% calf serum, and 0.25 M sucrose. The embryos were seeded at −6°C, held at −6°C for 10 min, and then cooled to −25°C at a rate of 0.33°C min^{-1}. Frozen embryos were thawed in a 30 to 35°C water bath after 10 s of air thawing. Fresh ($n = 3952$) or frozen-thawed ($n = 3298$) embryos were nonsurgically transferred to recipients on Days 6 to 9 of the estrous cycle. Data collected at the time of embryo transfer included recipient parity (cow or heifer), whether recipient estrus was natural or synchronized with $PGF_{2\alpha}$, cloprostenol or CIDR, methods of estrous confirmation (showing standing heat, rectal palpation of ovary without standing heat, or showing only mucous vulvular discharge), number of examinations of the CL by palpation per rectum (twice on the day before embryo transfer and the day of embryo transfer, or once on the day of embryo transfer), type of embryos (fresh or frozen), and day of the estrous cycle at the time of embryo transfer. CATMOD procedures of SAS were used to determine the factors affecting the pregnancy rate. Overall pregnancy rates were 37.3% ($n = 2704$). Whether recipient estrus was natural or synchronized and the type of embryos did not influence the pregnancy rates. Heifers had significantly higher pregnancy rates than cows (44.0% v. 33.0%, respectively, $P < 0.05$). Pregnancy rates among the subset of heifers and cows showing standing heat were significantly higher than those showing only mucous vulvular discharge (39.5% v. 33.5%, respectively, $P < 0.05$). Examining the CL twive had a significantly higher pregnancy rate than did a single examination of the CL (41.1% v. 35.6%, respectively, $P < 0.05$). Pregnancy rate on Day 8 (38.4%, 1358/3533) of the estrous cycle at the time of embryo transfer was significantly higher than on Days 6 (27.7%, 23/83) and 7 (36.2%, 1235/3408) ($P < 0.05$), and the pregnancy rate on Day 6 of the estrous cycle at the time of embryo transfer tended to be lower than on Day 9 (38.9%, 88/226) ($P < 0.08$). These results demonstrate that confirming standing heat, performing CL examination twice before embryo transfer, freezing high quality embryos, and performing embryo transfers on Day 8 resulted in an improved pregnancy rate for the transfer of IVF-derived embryos.

169 PREGNANCY RATES OF THREE DIFFERENT RECIPIENT POOLS IN A COMMERCIAL BOVINE OPU/IVP PILOT TRIAL-NEW ZEALAND

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Commercial bovine IVP is being extended into new markets and regions worldwide. This study presents pregnancy results from 457 OPU ($n = 2860$ oocytes) sessions performed on a range of commercial donors ($n = 43$) collected twice weekly during a 6-month period in 2002 at a central donor facility. A SOF-culture system was utilized to produce blastocysts ($n = 750$, 1.6/session) for either fresh or frozen-thaw transfer into a wide range

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of recipient cattle on three sites. Cattle were synchronised with prostaglandin, were observed for behavioural estrus and were eligible to receive an embryo 7 days later. All OPU and embryo transfer were done by a single technician, and pregnancies were confirmed by ultrasound examination at 35 days of gestation. The following table summarizes early pregnancy results from 556 transferred embryos. Recipient selection remains a critical variable as shown in this pilot trial data set. New Zealand's seasonal calving meant the lactating beef cattle were approximately 60 days postpartum at the start of the embryo transfer programs and the lactating dairy cows were 80 days postpartum. Postpartum interval and body condition score could be interesting variables to elucidate. Bulls were run with the beef cows shortly after embryo transfer resulting in 68% pregnancy rate of those cows whose embryos failed and then were naturally mated. Despite the disappointing results with the lactating beef cows, the overall results have led to OPU/IVP being commercially offered in this new market. The authors would like to acknowledge the ArTech IVP team and the enthusiastic cattle breeders of New Zealand.

	Transferred embryos (% pregnant)	Fresh transfers (% pregnant)	Frozen transfers (% pregnant)
Pool A—dairy heifers	56 (55)	18 (77)	38 (45)
– dry dairy cows	81 (45)	70 (51)	11 (9)
– lactating dairy	56 (41)	22 (56)	34 (32)
Subtotal	193 (46)	110 (56)	83 (35)
Pool B—crossbred heifers	251 (53)	185 (55)	66 (45)
Pool C—lactating beef	112 (29)*	112 (29)**	
TOTAL	556 (46)	407 (48)	149 (40)

Within columns,* $P < 0.05$, ** $P < 0.001$, by pairwise comparisons using a 2-by-2 exact test.

170 INFLUENCE OF FACTORS ASSOCIATED WITH EMBRYO RECOVERY AND POLICY OF TRANSFER ON PREGNANCY RATES AFTER TRANSFER OF BOVINE FRESH EMBRYOS

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Success of embryo transfer (ET) is related to many well identified factors such as quality and stage of embryos, or parity of recipients. However, there are no data on the effects of factors associated with embryo recovery and ET policy (proportion of embryos transferred as fresh or frozen) on pregnancy rates (PR) after ET. A retrospective study was designed to study how these factors affect PR achieved with fresh embryos (FE). A total of 3380 embryo recovery sessions (RS) realized by Embryotop (France) between 1995 and 2002 were studied, corresponding to 14,755 transfers with FE. Forty-four RS were made with only frozen embryos and removed from data set. A total of 1716 RS involved transfer from both frozen and FE, whereas in 1620 RS all transfers were made with FE. The organization of RS was assessed by the number of operators (OP) per RS and per donor cow, the number of embryos collected per RS and per OP. The operator at RS and ET was also noted. Embryo characteristics such as stage and quality (according to IETS criteria), paternal origin, and breed of donor cow were recorded together with the breed, date of ET and parity of recipients. The association between each variable and PR was analyzed by Chi-square. Only the significant factors were considered for further analysis ($P < 0.05$) and introduced with a stepwise procedure in a multivariate model of logistic regression to calculate adjusted odds ratio (OR). The number of RS was stable among years (463 to 493), whereas the number of embryos collected increased by 10% from 1995 to 2002. For 681 RS (20.4%), more than one donor cow were used for collection. Mean number of donor cows per RS and OP increased from 1.16 in 1995 to 1.34 in 2002. The mean number of transfers of FE per RS and OP was quite stable (3.82 in 1995 to 4.16 in 2002). This was explained by the increase in the number of frozen embryos per OP, averaging 1.99 in 1995 and 4.14 in 2002. The proportion of RS with only transfers of FE decreased by 20 points from 1995 (57%) to 2002 (36%). The number of transfers of FE per RS and OP was highly variable, ranging from 0 to 26. PR with FE averaged 52.1% and were significantly influenced by the number of donor cows per OP, operator of RS, embryo quality and paternal origin as well as recipient parity. Transfer of FE collected in RS with one donor cow resulted in higher PR than when more than one cow (53.0% v. 49.9%, OR = 1.12, $P = 0.003$). This could be partly explained by a higher mean number of transfers per RS and OP (1 donor cow = 3.7 ± 2.0 v. > 1 = 5.3 ± 3.1 ; $P < 0.05$) as well as a higher proportion of RS with only FE transferred (1 donor cow = 51.2% v. > 1 = 38.5% ; $P < 0.05$). Although the embryo quality was lower in RS combining ET of both frozen and FE than when only FE were transferred, these effects were not related to the lower quality of FE used in RS involving more than 1 donor cow (1 donor cow = 1.74 ± 0.50 v. > 1 = 1.75 ± 0.44; $P > 0.05$), but may be explained by a longer time between embryo recovery and ET. After adjustment for the usually well identified sources of variation of PR, the conditions of RS as well as ET policy (leading to the selection of the best embryos for transfer as frozen and to influence on the quantity of work per OP) can significantly influence PR. These sources of variation should be taken into account when analyzing PR results.

171 IMPROVEMENT IN EMBRYO RECOVERY USING UTERINE DOUBLE FLUSHING

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The aim of the present study was to evaluate the effects of uterine double flushing on embryo recovery rates (total structures and viable embryos), after a resting period with the uterus filled with PBS. There were 210 embryo recovery procedures conducted using the uterine double flushing method,

and the results were compaired with 432 conventional single flushing procedures. All procedures were conducted with Limousin ($n = 403$) and Guzerá ($n = 239$) cows, following the same superovulation protocol. Cyclic donors received a progestagen implant (CIDR) and 1 mL of oestradiol benzoate (Estrogin®) at Day 0. Between Day 5 and Day 9 animals received doses ranging from 200 to 300 UI (zebu cows) and 300 to 500 UI (taurine cows) of FSH (Pluset® – Serono) in decreasing doses. Between Day 6 and Day 8 PGF2a (Ciosin®) was administered, followed by withdrawal of the progestagen after 24 h. Artificial insemination was performed between 14 and 26 h after the beginning of treatment. For the double flushing procedure, after the first uterine flushing of both horns with 1 L DPBS (Nutricell), a Foley catheter was positioned in the uterine body in order to fill the uterus with the same solution (80 to 150 mL). After this procedure the catheter was closed with a disposable 5-mL syringe gasket, and the animals were allowed to rest in the surroundings of the work place for 30 min. After this period, a second flush was performed in order to recover the remaning liquid used during manipulation of the uterus. Animals from the control group (group A) were subjected to a single uterine flushing procedure. From 210 double flushing procedures (group B – test), 1409 viable embryos were produced, classified as grades I, II, III and IV (IETS), (average of 6.7 embryos per procedure), whereas, in the 432 single flushing procedure (group A – control), 1993 embryos were produced (average of 4.6). Statistical analysis showed the increase of viable embryo recovery rate. When consecutive double flushing was performed, the average of recovered embryos increased from 8.3 to 12.7 ($P < 0.05$) in Limousin cows, and from 7.9 to 11.5 ($P < 0.05$) in Guzerá cows. Comparing recovery after single flushing with that after double flushing, the mean number of viable embryos increased from 4.7 to 6.9 ($P < 0.05$) in Limousin cows and from 4.5 to 6.4 ($P < 0.05$) in Guzerá cows. In order to assure the nonexistence of negligence effects or operator influence on results, the mean values of total embryo recovery rate after single flushing (control group) was compared to the mean values of the same rate after the first uterine flushing on test group. Results indicated no difference between recovery rates. The present work showed the viability of using the uterine double flushing procedure for improvement of embryo recovery rates in cattle.

172 ELECTIVE SINGLE EMBRYO TRANSFER (eSET) IN HUMANS DOES NOT AFFECT THE CHANCES OF A SUCCESSFUL PREGNANCY

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Improvements in human IVF have led to increased pregnancy rates but at the expense of increasing twinning rates. Twins are a bad outcome for the offspring, parents and the healthcare system. An obvious solution to this is to transfer only one embryo and freeze the rest for potential further treatment. This study looked at the effect of doing this on the cumulative live birth rate (when the cryopreserved embryos were thawed and transferred). Patients less than 38 years of age presenting for IVF treatment and with more than two embryos suitable for transfer were offered the chance of transferring only one embryo (elective single embryo transfer, eSET) and freezing the rest. Those patients declining a single embryo transfer had two transferred and served as the controls. Patients not achieving a pregnancy returned for a frozen embryo transfer but were not restricted on the number transferred (to a maximum of two). Cumulative live birth rates were recorded over the ensuing two years. Statistical comparisons were made using paired chi-square tests. The live birth rates from the initial fresh transfer was 41% for eSET (41/111) and significantly higher (53%, $P < 0.05$) for the two-embryo transfer group. These differences were eliminated when the frozen embryos were factored in, both groups rising to 61% of patients treated (68 and 172 live births, respectively). The twinning rate was significantly reduced ($P < 0.01$) from 33% in the two-embryo transfer group to 6% (arising from 4 sets of twins in the frozen embryo transfers) in the eSET group. eSET in the fresh embryo transfer cycle does not affect the chances of a live birth and reduces the twinning rate at least fivefold. Currently, 70% of patients under the age of 38 are electing to have eSET.

173 TRANSFER OF VITRIFIED BLASTOCYSTS FROM ONE OR TWO SUPEROVULATED LARGE WHITE HYPERPROLIFIC DONORS TO MEISHAN RECIPIENTS: REPRODUCTIVE PARAMETERS AT DAY 30 OF PREGNANCY

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The present study was designed to determine the effect of pooling embryos from two donors on the reproductive success of transfer of vitrified/warmed porcine blastocysts. Superovulated Large White hyperprolific gilts ($n = 24$) were used as embryo donors. Gilts were artificially inseminated 12 and 24 h after initial detection of estrus using fresh semen, and slaughtered on Days 5.5 to 6 of the estrous cycle (Day 0 = Onset of estrus). Embryos were recovered by flushing the uterine horns, and unhatched blastocysts were selected. Vitrification and warming were performed as reported previously (Berthelot *et al.*, 2000 Cryobiology 41, 116–124). Embryo transfers were conducted in asynchronous (−24 h) Meishan gilts ($n = 20$). Twenty vitrified/warmed blastocysts were surgically transferred into one uterine horn. Ten recipients received embryos from one donor (group 1) and the other ten transfers were performed with mixed embryos from two donors (group 2). Pregnancy was assessed ultrasonographically at Day 25 after estrus and recipients were slaughtered five days later. The pregnancy rate from the different groups was compared using Fisher exact test. The GLM procedure of SAS was used to determine the effect of the origin of embryos (one or two donors) on the number of developed fetuses and viable fetuses at Day 30 of pregnancy. The ovulation rate was 32.5 ± 11.8 (mean ± SD). The total number of embryos collected was 634, of which 57 (9.0%), 36 (5.7%), 513 (80.9%) and 28 (4.4%), were unfertilized oocytes and degenerated embryos, morulae, unhatched blastocysts and hatched blastocysts, respectively. The ratio of collected embryos to the number of corpora lutea was 81.3%. The pregnancy rate for group 1 (70%) was not different ($P > 0.05$) than that for group 2 (90%). No significant differences were detected between group 1 and group 2 for in vivo embryo development (number fetuses/transferred embryos in pregnant recipients; 33.3% v. 40%) or in vivo embryo survival (number viable fetuses/transferred embryos in pregnant recipients; 27.9% v. 33.9%). However, the in vivo efficiency (number viable fetuses/total transferred embryos) was higher ($P < 0.05$) when transfers were performed with embryos from two donors (19.5% v. 30.5%). These results indicate that pooling embryos from two donors increases the in vivo efficiency after transfer of vitrified/warmed porcine blastocysts. This study was supported by grant from SENECA (FPI/99, Spain).

174 EMBRYO TRANSFER OF VITRIFIED IVF EMBRYOS IN CATTLE: PREGNANCY COMPARISON AFTER SINGLE AND DOUBLE TRANSFER

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Advancement in vitrification of in vitro-produced bovine embryos will benifit the cattle breeding and production industry. The objective was to evaluate whether bilateral (double) embryo transfers (ET) can improve pregnancy rate compared to ipsilateral (single) transfers. Bovine cumulus-oocyte complexes collected from slaughterhouse ovaries were matured for 20–22 h, and subsequently subjected to a standard Brackett and Oliphant in vitro fertilization (IVF). Six hours after IVF, embryos denuded of cumulus were cultured in defined CR1 medium supplemented with essential and non-essential amino acids (CR1aa), plus 6 mg mL^{-1} BSA for 2 days at 39°C under 5% CO_2, 5% O_2 and 90% N_2, and then cultured in CR1aa medium supplemented with 7.5% FBS for a further 5 days on bovine cumulus monolayers. Expanded blastocysts with tighter compaction of the inner cell mass (quality 1) were selected on Day 7 for cryopreservation via modified solid surface vitrification (Dinnyes *et al.*, 2000 Biol. Reprod. 513–8). Vitrification solution contained HEPES-buffered TCM199 supplemented with 20% FBS, ethylene glycol and dimethylsulphoxide. A droplet of 1–2 μL vitrification solution containing 4–5 blastocysts was dropped directly onto a cooled surface within 30 s after 3-min incubation in equilibration solution. Prior to ET, embryos were warmed and subsequently washed several times in 0.25 M sucrose rehydration solution and M199 + 7.5% FBS medium. The warmed embryos from initial trials were cultured for 2 to 72 h to evaluate their viability after vitrification. During ET trails, vitrified embryos were loaded into transfer straws (one embryo per straw) after warming. The treatments were as following, (1) single transfers, one embryo was transferred into the horn ipslateral to CL; (2) double transfers, one embryo was transferred by non-surgical means into each uterine horn of a synchronous recipient on Day 7. ET trails were conducted in both the USA (double transfers) and China (single v. double transfers). Pregnancy was determined by palpation per rectum around Day 70 after transfer. The data were compared by Student's *t*-test. The survival rate of vitrified IVF embryos reached as high as 91.4% ($n = 256$) 2 h post-warming, and hatching rate was 70.7% ($n = 154$) 72 h after culture in vitro, respectively. The data (Table 1) show that double transfers resulted in a significantly higher pregnancy rate than did single transfers ($P < 0.05$). With double transfers, a higher pregnancy rate was achieved in the USA than in China (76.2% v. 45.6%, $P = 0.079$). This study confirms that double embryo transfers can improve the pregnancy outcome after ET, perhaps because bilateral placement of embryos may increase embryonic signals to the maternal environment. Further evaluation of gestation length, single/twin conception and calving difficulty is under investigation.

Table 1. Pregnancy rate (Day 70) of vitrified bovine IVF embryos following single and double transfer

Treatment	No. Recipients	No. Pregnancies (%)
Double ET-USA	21	16 (76.2)[a]
Double ET-China	127	58 (45.6)[a]
Single ET-China	151	51 (33.8)[b]

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

175 SAVING PREGNANCIES IN BEEF CATTLE AFTER A LUTEOLYTIC DOSE OF PROSTAGLANDIN

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Three experiments were conducted to determine if pregnancy (PREG) could be maintained in cattle after receiving a luteolytic dose of PGF2α (PGF) (25 mg, Lutalyse) i.m. during 30 to 40 d or 80 to 90 d of gestation (GEST). The objectives were to determine whether PREG could be maintained in the absence of a functional CL with administration (ADM) of progesterone (P4) or altrenogest (ALT) following PGF injection and to determine if induced luteal tissue (LT) could support the PREG to term. In Exp I, crossbred cows (BCS = 5 to 7) 30 to 40 d of (GEST) were randomly assigned to receive 100 mg of P4 in 3 mL of vehicle s.c. at 2 h post-PGF (p-PGF) (Trt A; $n = 2$), 6 h p-PGF (Trt B; $n = 3$), 10 h p-PGF (Trt C; $n = 3$), 14 h p-PGF (Trt D; $n = 2$) or 18 h p-PGF (Trt E; $n = 2$). After PGF, all cows were given 100 mg P4 daily i.m. for ≥ 7 d. PREG was maintained in 100%, 100%, 67%, 50% and 0% of the females in Trts A, B, C, D and E, respectively. Following the 7-d P4 Trt, 3 cows were selected from the remaining PREG females for induction of LT. These cows were evaluated via ultrasound 2 to 3 times weekly until a follicle ≥ 10 mm developed on the ovary ipsilateral to the gravid horn. At that time, 2500 IU of hCG were given i.m. to induce LT development. LT developed successfully in 2 of 3 (67%) females at which time the P4 was decreased over 10 d. One cow lost her PREG prior to LT formation, 13 d p-PGF. Of the 2 remaining females with induced LT, 1 female lost the PREG at 60 d post-P4 Trt and the remaining female gave birth to a healthy calf at 286 d of PREG, without dystocia. In Exp II, similar beef cows (BCS = 5 to 7) at 80 to 90 d of GEST were randomly assigned to receive a dose of 100 mg ALT (drenching) starting at 2 h p-PGF (Trt A; $n = 3$), 6 h p-PGF (Trt B; $n = 2$) or 12 h p-PGF (Trt C; $n = 2$). At 24 h after ALT Trt, all cows received two 15-mg norgestomet implants to maintain PREG. One female, in Trt B, did not respond to PGF and was removed from the study. PREG was maintained in 67%, 100% and 0% of the females in Trt A, B and C, respectively. From the remaining 3 PREG cows, 2 were selected for induction of LT, as described above. Both cows formed induced LT and 14 d later both implants were removed. To date, both females have maintained their PREG and are expected to calve in 30 d. In Exp III, crossbred heifers (BCS = 5 to 6) at 30 to 40 d of gestation were randomly assigned to receive 100 mg ALT 6 h p-PGF

(Trt A; $n = 3$), 12 h p-PGF (Trt B; $n = 2$) or 18 h p-PGF (Trt C; $n = 2$). PREG was maintained in 100% of females. Subsequently, induction of LT was attempted in all 7 females. To date, 5 females have induced LT. Of the remaining 2 females, 1 lost her PREG at 80 d of GEST, while the other female is currently in the process of developing fresh LT. These results demonstrate that PREG can be maintained with P4 or ALT administration following a PGF injection, and that induced luteal tissue can support these PREG to term, thus eliminating frequent ADM of progestin in an attempt to maintain a viable PREG. To our knowledge, this is the first report of a pregnancy developing to term, resulting in normal parturition and a viable calf following induced luteal tissue in the absence of the initial CL of PREG.

176 PRODUCTION OF TRANSFERABLE EMBRYOS IN BRAHMAN COWS TREATED WITH BOVINE SOMATOTROPIN

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It is the goal of numerous research laboratories around the world to increase the number of transferable bovine embryos and to improve the viability of these embryos after ET into recipients. Treatment of donor cows with bovine somatotropin (bST) has given satisfactory results that have increased the hopes for an improved commercial production of embryos (Moreira *et al.* 2002 Theriogenology 57, 1371–1387). In order to demonstrate the effect of bST on the production of transferable embryos, degenerated embryos and non-fertilized oocites, 16 high-value genetic Brahman cows were synchronized and later subjected to a superovulatory (SO) treatment. The SO treatment consisted of decreasing doses of FSH (Folltropin-V®, Vetrepharm Inc, Ontario, Canada) every 12 h for 4 days (2.5, 2.0, 1.5, 0.5 × 2/day). Forty-eight hours after the beginning of the SO treatment, a dose of $PGF_{2\alpha}$ (Lutalyse®, Upjohn, Kalamazoo, MI, USA.) was given to all donors (i.m.). Estrus was detected by careful observation. At the time of first AI (6 hours after estrus detection) all cows were given (i.m. a GnRH (Buserelin, Conceptal®, Intervet, Boxmeer, The Netherlands) analogue and randomly assigned to one of the following groups: bST ($n = 8$), which were treated with 500 mg (s.c.) of bST (Boostin-S®, Schering-Plough Caracas, Venezuela) or control group ($n = 8$) not treated. The second AI was done 10 hours after the first AI. Immediately after the uterine recovery, oocytes and embryos were evaluated and classified, according to their morphologic characteristics, into transferable embryos (TE) of good (TE1) or medium (TE2) quality, degenerated embryos (DE) and non-fertilized oocytes (NF). CL were evaluated by rectal palpation to estimate the ovulatory response. The statistical comparison between experimental groups (percentage of each category) was carried out using a chi-square test of SAS. Results are presented in Table 1. Results show that bST donor treatment increases the number and quality of transferable embryos. Key words: bST, ET, donor, embryo, superovulation.

Table 1. Number and percentages of transferrable and degenerated embryos and non-fertilized oocytes in superovulated Brahman cows after treatment with bST

Reproductive response	Treatments	
	Control (%)	bST (%)
Total ova/embryos	131 (56.4)[a]	101 (43.5)[b]
Transferable embryos	66 (50.4)[c]	79 (78.2)[d]
Good quality	48 (36.6)[c]	67 (66.3)[d]
Medium quality	18 (13.7)[c]	12 (11.9)[d]
Degenerated embryos	38 (29.0)[a]	17 (16.8)[b]
Non-fertilized oocytes	27 (20.6)[c]	5 (4.9)[d]

Values within the same row without a common letter differ ([a,b] $P < 0.05$; [c,d] $P < 0.01$)

177 HYSTEROSCOPIC BLASTOCYST IMPLANTATION—A NOVEL EMBRYO TRANSFER PROCEDURE

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Various techniques using different types of catheters have been advocated to increase pregnancy rates while reducing side effects from the embryo transfer procedure. However, all of these techniques are 'blind' procedures of catheter introduction into the uterus, and the problems of 'lost embryos' and the occurrence of ectopic pregnancies persist. A novel hysteroscopic-guided direct embryo transfer procedure with visually directed embryo implantation was developed to improve the current 'blind' embryo transfer procedures by increasing chances of success while eliminating tubal pregnancies and decreasing high-order multiple pregnancies from IVF related techniques. At West Coast Infertility Medical Clinic, 57 patients with average age of 28.43 ± 4.54 were analyzed. Stimulation method: controlled ovarian hyperstimulation was initiated with Follitropin β; (Follistim®, Organon Pharmaceuticals, Inc.). Premature endogenous gonadotropin surge (i.e. the prevention of an LH surge) was controlled with ganirelix acitate (AntagonTM, Organon Pharmaceuticals, Inc., West Orange, NJ, USA.). Oocyte retrieval was performed in an office setting under local anesthesia and mild sedation, followed by routine IVF/ICSI, IVC. By Day 5–6, up to 2 best quality blastocyst stage embryos were transferred to patient's

uterus by 'hysteroscopic embryo implantation' procedure: a lightweight hybrid (rigid/flexible) mini hysteroscope (Napoli, Inc., Los Angeles, CA, USA) was used for visualization of the endometrial cavity. The scope incorporates a flexible distal end of 3 mm in diameter with a straight-through operating channel. In addition, the optic filter is directly connected to a light source, decreasing the weight of the scope and giving a better feel for the scope. The transfer catheter (Napoli, Inc.) is polycarbon based with a tapered tip (to 500 μm), beveled to 60°. During embryo transfer procedure, the catheter tip was inserted into a depth of 1 mm from the surface of the endometrium under direct hysteroscopic visualization. The loaded embryos with 10 μL medium was released underneath the endometrium to produce a 'bubble' cushion. Luteal phase support was provided (3000 IU of hCG at Day 3 and Day 6 post-retrieval, separately). Pregnancies were determined by serum hCG concentration of 5 IU mL^{-1} or more at Day 16 post-retrieval. Thirty out of 57 (52.6%) women became pregnant. Multiple pregnancy rate was 4 out of 30 (13.3%) and comprised only of twins, and no ectopic pregnancy was found. In conclusion, a newly developed instrument and embryo transfer procedure by mechanical implantation of the embryo was achieved. By implanting the embryos, we have reduced the number of embryos that are transfered, minimized the chances of 'losing' embryos, and eliminated ectopic pregnancies.

178 EMBRYO QUALITY AND COLOR IN HOLSTEIN FRIESIAN AND BELGIAN BLUE CATTLE IN RELATION TO DONOR BLOOD CHOLESTEROL AND TRIGLYCERIDES

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Bovine embryo freezability is closely linked with quality and differs between cow breeds (Visintin JA *et al.*, 2002 Theriogenology 57, 345–359). Dark embryos have a bad freezability, resulting in low pregnancy rates (Hill BR and Kuehner LF, 1998 Theriogenology 49, 168). Excessive accumulation of lipid droplets in dark embryos is suggested to be the cause of this inferior embryo quality and in turn may be influenced by the biochemical composition of the embryonic environment. In a field trial we studied the relationship between donor breed (Holstein Friesian dairy cows v. Belgian Blue beef cows), donor serum total cholesterol and triglyceride concentration, embryo quality and embryo color. The preliminary results are presented. Blood was drawn from Holstein Friesian (HF) ($n = 74$) and Belgian Blue cows (BB) ($n = 55$) prior to embryo flushing on Day 7 after superovulation and subsequent insemination. Serum was analyzed for total cholesterol and triglycerides using commercial photometric assays. Embryos were scored individually by the same operator for quality (excellent to bad: score 1 to 4) and for color: light (L), medium (M) or dark (D) using a binocular stereomicroscope (40X). Student's *t*-test, chi-square test and Spearman correlations were used when appropriate. Holstein Friesian cows yielded significantly more embryos (± SEM) per flushing than did BB (6.0 ± 0.59 v. 4.2 ± 0.44) ($P < 0.05$). However, significantly more HF embryos were classified as dark compared to the BB embryos (L, M, D: 39.4%, 43.8% and 16.8% v. 80.5%, 19.5% and 0% of the HF and BB embryos, respectively) ($P < 0.05$). Significantly more BB embryos showed an excellent morphological quality (55.0% of BB embryos compared to 28.4% of HF embryos) ($P < 0.05$). The serum concentrations (± SEM) of total cholesterol (148 ± 5.9 mg dL^{-1} v. 106 ± 4.1 mg dL^{-1}) were significantly higher and the serum concentrations of triglycerides (19.6 ± 0.71 mg dL^{-1} v. 28.4 ± 3.34 mg dL^{-1}) were significantly lower in HF compared to BB ($P < 0.05$). Within the HF breed, there was a significant positive correlation between donor blood total cholesterol and the color of each embryo ($r = 0.545$) and a negative correlation between serum triglycerides and embryo color ($r = -0.484$) ($P < 0.05$). In our study, embryo quality and color as well as serum total cholesterol and triglyceride concentrations are clearly influenced by donor breed. Within the HF breed, darker embryos originate from donors with higher serum total cholesterol and lower serum triglyceride levels. These preliminary data suggest that factors influencing total cholesterol and triglyceride concentrations in the donor blood (e.g. breed, milk yield, nutrition) may influence embryo color and thus embryo lipid content, freezability and subsequent pregnancy rate.

179 IMPORTANCE OF EMBRYO TRANSFERS IN TRANSGENIC MOUSE FACILITIES

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With the increasing demand for and production of transgenic and mutant mice for biomedical research, embryo transfer plays a paramount role. The purpose of performing embryo transfer in this species is to generate transgenic mice via blastocyst injection of embryonic stem cells or pronuclear injection of DNA constructs, to revitalize cryopreserved sperm and embryos, and to generate mouse lines that meet specific pathogen-free health standards for breeding in barrier areas (rederivation). We present results from two years of carrying out embryo transfers for rederivation purposes in the large mouse breeding facility of the GSF—National Research Center for Environment and Health, Neuherberg, Germany. Pathogens to be eradicated from inbred transgenic (C57BL/6 background) and mutant (C3H background) mouse lines included mouse hepatitis virus, mouse minute virus, and mouse parvovirus. In vitro- and in vivo-produced two-cell embryos were washed 3 times in M2 medium. A total of 20 embryos each were transferred to the oviduct of 8- to 12-week-old specific pathogen-free pseudopregnant (Day 0.5) Swiss recipients under aseptic conditions. Mice were then kept singly in individually ventilated cages and manipulated in a Class II laminar flow hood. From each transfer to one to five recipients with embryos originating from the same mouse line, one recipient was tested for the presence of microorganisms 6 to 12 weeks after embryo transfer, i.e. at 0 to 6 weeks after weaning, according to the FELASA (Federation of European Laboratory Animal Science Associations) Guidelines. A total of 290 embryo transfers were performed for revitalization of cryopreserved sperm from 52 mouse lines, cryopreserved two-cell embryos from 18 mouse lines and rederivation of 12 mouse lines using freshly collected two-cell embryos. From these 290 embryo transfers, 59 mouse lines were re-established (40 from cryopreserved sperm, 7 from cryopreserved embryos and 12 from in vivo-produced embryos). Health monitoring of 54 recipients showed that all mouse lines generated were free of all pathogens stated in the FELASA list. The results presented here show that all 12 (100%) mouse lines were re-established after transfer of freshly collected two-cell embryos whereas 77% and 39% success rates were observed for revitalization of cryopreserved sperm and embryos, respectively. The success of embryo transfer in eradicating pathogens depends

on the inability of these pathogens to transverse the zona pellucida and enter and/or infect embryonic cells. In our mouse facility, embryo transfer provided an efficient method to successfully revitalize cells of the mouse germ line as well as to eradicate prevalent murine pathogens. Furthermore, the results demonstrate the efficiency of transferring embryos of different origins and thereby obtaining and maintaining specific pathogen-free health standards in our mouse colonies.

180 EFFECT OF TIME OF eCG TREATMENT ON PREGNANCY RATES IN *BOS INDICUS* × *BOS TAURUS* RECIPIENTS SYNCHRONIZED WITH PROGESTERONE VAGINAL DEVICES AND TRANSFERRED WITHOUT ESTRUS DETECTION

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It has been shown recently that treatments with progesterone (P4)-releasing devices combined with estradiol benzoate (EB) plus P4 on Day 0, eCG and PGF on Day 5 and a second application of EB one day after device removal (Day 9) can be used successfully to transfer bovine embryos at a self-appointed time, without the necessity of estrus detection. Although the treatment solved one of the major problems in recipient management, estrus detection, it requires handling the recipients at least five times for treatments and embryo transfer. An experiment was designed to evaluate whether reducing one day of handling, by the administration of eCG and PGF at the time of removal of the P4 device (Day 8), results in comparable pregnancy rates than giving eCG on Day 5. A secondary objective was to determine the effect of injectable P4 at the time of device insertion plus EB treatment. Crossbred *Bos taurus* × *Bos indicus* beef heifers ($n = 301$) were randomly assigned to 4 treatment groups in a 2 by 2 factorial design. All Heifers received a P4 device (DIB, Syntex, Argentina) plus 2 mg EB i.m. (Syntex) at unknown stages of the estrous cycle (Day 0), with or without 50 mg of P4 given i.m. at the same time. Heifers were further subdivided to receive PGF (0.150 mg d-cloprostenol, Prolise, Tecnopec, Sao Paulo, Brazil) and 400 IU of eCG (Novormon, Syntex) i.m. on Days 5 or 8. In all heifers, DIB devices were removed on Day 8 and 1 mg EB was administered i.m. on Day 9. Day 10 was arbitrarily considered as the day of estrus. On Day 17, heifers were bled for plasma P4 concentrations and examined by ultrasonography to determine the number of CL and their diameter. Heifers that had >1 CL or a single CL with diameter ≥18 mm received an in vitro-produced (IVP) embryo by nonsurgical transfer performed by the same veterinarian. Pregnancy rates were determined by ultrasonography 30 days later. The effects of Day of eCG administration (Day 5 or Day 8), P4 of treatment (E2 or E2 + P4) and the day-by-P4 treatment interaction on the numbers of CL and plasma P4 were analyzed by ANOVA, and the proportion of recipients selected and pregnant were analyzed using non-parametric tests (NPAR1WAY, SAS). There was no significant effect of P4 treatment or the P4-by-day of eCG interaction in any of the parameters evaluated. However, there was a significant effect of day of eCG administration on plasma P4 concentrations (Day 5 = 2.4 ± 0.3 v. Day 8 = 1.7 ± 0.2; $P = 0.03$) and the number of CL (Day 5 = 1.4 ± 0.1 v. Day 8 = 1.1 ± 0.0; $P = 0.02$) on Day 17. Furthermore, the proportion of recipients pregnant/treated tended ($P = 0.1$) to be higher in heifers in the Day 5 Group (71/151, 47.0%) than in those in the Day 8 Group (61/150, 40.7%). Although delaying the eCG and PGF administration from Day 5 to Day 8 saves one trip through the chute for treatments, it resulted in lower plasma P4 concentrations and tended to decrease pregnancy rates in bovine embryo recipients synchronized with DIB devices and EB and transferred at a fixed time. Furthermore, the administration of injectable P4 at the time of DIB insertion did not affect pregnancy rates.

181 COMPARISON OF THE PREGNANCY RATES AFTER SYNCHRONIZATION OF OVULATION USING GnRH AND $PGF_{2\alpha}$ IN RECIPIENT DAIRY CATTLE

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Treatments with GnRH and $PGF_{2\alpha}$ for synchronization of ovulation has resulted in acceptable pregnancy rates after fixed-time artificial insemination in dairy cows without estrus detection. The objective of the present study was to evaluate the practicability of ovulation synchronization (Ovsynch, Pursley JR *et al*. 1995 Theriogenology 44, 915–923) in dairy cattle using GnRH and $PGF_{2\alpha}$ for the embryo transfer recipients. Dairy cattle (cows; $n = 100$, heifers; $n = 88$) were randomly allocated to one of two groups. The control group (cows; $n = 45$, heifers; $n = 37$) was composed of cows in natural estrus. The ovulation synchronization group (cows; $n = 55$, heifers; $n = 51$) was treated with an intramuscular injection of 100 μg of GnRH at a random stage of the estrous cycle. Seven days later, the cattle received $PGF_{2\alpha}$ (Cows; 25–30 mg) or $PGF_{2\alpha}$ analog (Heifers; 0.5 mg) in order to regress the corpora lutea (CL). Forty-eight hours later, cows and heifers received a second injection of 100 μg GnRH. Embryo transfer was carried out 7 days after the second injection of GnRH in the ovsynch group and 7 days after estrus in the control group. The cattle judged to have CL 17 mm were classified as acceptable recipients. The size of the follicles and the CL were determined to be of estrus stage and embryo transfer by means of ultrasonography. The mean numbers of follicles and CL were analyzed by ANOVA, while pregnancy rates were analyzed by chi-square test. The results are presented in the Table. The proportion of cows and heifers determined to be acceptable embryo transfers was not different between the control group and the ovsynch group. There were no differences in the proportion of acceptable embryo transfers between the control group and the ovsynch group. Follicle diameter at the time of estrus in the control group (cows; 20.7 ± 0.7 mm, heifers; 16.8 ± 0.5 mm) were significantly larger than that of the ovsynch group (cows; 18.0 ± 1.0 mm, heifers; 14.7 ± 0.2 mm) ($P < 0.05$). Although CL diameter at the time of embryo transfer in heifers showed no differences between the control group and the ovsynch group (25.0 ± 1.0 mm *v.* 22.8 ± 1.5 mm), The CL diameter of the control cow group was larger than that of the ovsynch group (29.8 ± 0.7 mm *v.* 26.1 ± 1.0 mm, $P < 0.05$). However, no differences in pregnancy rate were seen between the control group and the ovsynch group. These results suggest that ovsynch can be effectively applied in an embryo transfer program for cattle.

Table 1. Proportion of acceptable embryo transfer recipients and pregnancy rate in dairy cattle in the control ovsynch groups

Group	Parity	No. of recipients	Acceptable recipients (%)	No. of pregnancies (%)
Control	Cows	45	33 (73.3)	17 (51.5)
	Heifers	37	33 (89.1)	20 (60.6)
Ovsynch	Cows	55	40 (72.7)	20 (50.0)
	Heifers	51	46 (90.2)	27 (58.7)
Total		188	152 (80.9)	84 (55.2)

182 LAPAROSCOPIC EMBRYO TRANSFER IN KOREAN BLACK GOATS

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In this study, laparoscopic embryo transfer (ET) was conducted to overcome the disadvantages of laparotomic ET including invasiveness, adhesions and duration of surgery in Korean black goats (*Capra hircus aegagrus*). Transferred transgenic embryos were produced by DNA pronuclear microinjection of in vivo derived zygotes. Recipient goats were synchronized in estrus by using an intravaginal progesterone device CIDR® for 13 days and injection of 400 IU PMSG 48 hrs before CIDR® removal. Embryos were transferred on day 4 after CIDR® removal. Recipient goats were starved for 48 hrs and suspended head down in an operating table at an angle of 45° to the horizontal under general anesthesia. A Veress needle (Vomed, Tuttlingen, Germany) was inserted through the abdominal wall to make a pneumoperitoneum. After obtaining sufficient pneumoperitoneum, a 5-mm laparoscope and grasping forceps (MGB, Berlin, Germany) were inserted through the 5-mm trocar sleeves. After investigation of the ovaries, uterine horns and oviducts, embryos in a polyethylene tube (SP 65, Nastume, Tokyo, Japan) were transferred into the oviduct via the infundibulum in 76 recipients. To compare the pregnancy rates, laparotomic ET was also conducted in 21 recipients. In both groups, two microinjected embryos were transferred per recipient. Pregnancy of the recipient goats was examined by ultrasound on day 30 after embryo transfer. Pregnancy rates of laparoscopic ET were significantly higher than those of laparotomic ET (46.1% v. 28.6%; $P < 0.05$). Our results suggest that laparoscopic ET is a highly efficient method for the transfer of goat embryos.

183 PREGNANCY RATE FOLLOWING TRANSFER OF IN VITRO- AND IN VIVO-PRODUCED BOVINE EMBRYOS TO LH-TREATED RECIPIENTS

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The objective was to evaluate the effect of pLH treatment on pregnancy rates in recipients receiving in vivo- or in vitro-produced bovine embryos. Heifers ($n = 37$) and lactating ($n = 28$) and non-lactating ($n = 150$) beef cows were treated at random stages of the cycle with 100 μg GnRH i.m. (Cystorelin, Merial Canada Inc., Victoriaville, Quebec, Canada) on Day −9, 500 μg cloprostenol i.m. (PGF; Estrumate, Schering Plough Animal Health, Pointe-Claire, Quebec, Canada) on Day −2 and GnRH on Day 0 (66 h post-PGF; without estrus detection). Cattle were placed at random, by class, into three groups: no further treatment (Control; $n = 71$), or 12.5 mg pLH (Lutropin-V, Bioniche Animal Health, Belleville, Ontario, Canada) on Day 5 ($n = 72$) or on Day 7 ($n = 72$) after the second GnRH. On Day 7, cattle with a CL > 10 mm in diameter (determined ultrasonically) received in vivo-produced, fresh (Simmental) or frozen (Holstein), or in vitro-produced frozen (Holstein) embryos (embryo type balanced among groups). Embryos were cryopreserved in 10% ethylene glycol; in vivo-produced frozen embryos were thawed 5 to 10 s in air, 15 s in a water-bath at 30°C and then "direct-transferred" nonsurgically. In vitro-produced frozen embryos (donated by IND Lifetech Inc., Delta, British Columbia, Canada) were thawed in a water-bath at 27°C for 10 s and placed in ViGro Holding Plus medium (AB Technology, Pullman, WA, USA) at room temperature, evaluated and then transferred nonsurgically. Pregnancy was determined by ultrasonography on Day 35. Data were analyzed with CATMOD, chi-square and GLM procedures (SAS Institute, Cary, NC, USA.). Twenty cattle (9.3%) did not receive embryos; five heifers had cervical problems, and five heifers and 10 cows did not have a CL > 10 mm. Overall, 7.1% of the recipients had two CL on the day of embryo transfer. There was no effect ($P > 0.05$) of treatment, embryo type (or interaction) or class of recipient on pregnancy rate (overall, 44.1%, 86/195; Table 1). Similarly, mean (± SD) CL diameter and luteal area did not differ ($P > 0.05$) among groups or between pregnant and open recipients (overall, 22.0 ± 3.4 mm and 352.0 ± 108.7 mm, respectively). However, recipients with a CL diameter ≥ 18 mm tended ($P < 0.1$) to have a higher pregnancy rate (45.8 vs 25.0%). In a subset of 40 recipients examined ultrasonically on Day 12, 50% of those treated on Day 5 and 70% of those treated with pLH on Day 7 had two CL. In summary, overall pregnancy rate in GnRH-synchronized recipients receiving in vitro- or in vivo-produced embryos by nonsurgical transfer was 44.1%. Embryo survival to Day 35 was not affected by type of embryo or treatment with pLH 5 or 7 days after ovulation.

Table 1. Pregnancy rate in recipients on Day 35 based on pLH treatment and embryo-type

	Control	pLH Day 5	pLH Day 7	Fresh in vivo	Frozen in vivo	Frozen in vitro
Recipients (n)	64	67	64	10	49	136
No. pregnant (%)	32 (50.0)	28 (41.8)	26 (40.6)	4 (40.0)	24 (48.9)	58 (42.6)

184 PREGNANCY RATES AFTER ESTRUS SYNCHRONIZATION TREATMENT WITH NEW AND REUSED CIDR-B DEVICES

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It is not well known whether used CIDR devices containing progesterone (P4) combined with estradiol benzoate (EB) and prostaglandin F2α (PGF) can provide acceptable estrus synchronization rates (ESR) and pregnancy rates (PR) in ET or AI programs. Three experiments were designed to study the effect of new and used CIDR-B, with different P4, EB and PGF treatments on ESR and PR in a reproductive program in beef cattle in a tropical climate. Experiment 1 was a control to evaluate ESR and PR in lactating recipient females. All cows ($n = 284$) were treated with a new 1.9-g CIDR (CIDR-B, InterAg, New Zealand), combined with 2 mg EB and 50 mg P4 on Day 0. CIDR devices were removed on Day 8 and all cows received 0.25 mg 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 (1.5 M ethylene glycol = EG) by nonsurgical direct transfer (DT). PR were determined by rectal palpation 60 d after estrus. Ninety percent of the cows displayed signs of estrus (256/284) and 40% of those that received a frozen embryo were pregnant (96/239). Experiment 2 was designed to evaluate ESR and PR in dry recipient cows treated with a used CIDR-B (first reuse). All cows ($n = 274$) were treated with a reused 1.9-g CIDR combined with 2 mg EB and 50 mg P4 on Day 0. CIDR devices were removed on Day 8 and all cows received 0.25 mg cloprostenol at that time. Estrus was expected to occur 24 h later. Seven days after estrus, all cows that showed estrus were rectally evaluated and those with a CL 15 mm in diameter or larger received a frozen/thawed embryo (1.5 M EG) by DT. A total of 93% of the treated cows showed signs of estrus (254/274) and 51% of those that received an embryo were pregnant (110/217). Experiment 3 was designed to evaluate ESR and PR in virgin heifers, treated with a used CIDR (second reuse). All heifers ($n = 414$) were treated with a reused 1.9-g CIDR combined with 1 mg EB on Day 0. CIDR devices were removed on Day 8 and all heifers were expected to show estrus 24 h later. Approximately 12 h after estrus, all heifers that showed signs of estrus were inseminated, using frozen/thawed semen from a single bull. Of the treated females, 78% showed signs of estrus (323/414) and 69% of the inseminated were pregnant (223/323). These results suggest that in a CIDR that was used in two previous occasions, there is still a remaining amount of P4 that allows estrus synchronization in heifers. Furthermore, the reutilization of CIDR-B devices can contribute to reduce the costs related to ET or AI programs in cattle. However, the diverse existing conditions among the 3 experimental groups in this study make a statistical comparison impossible. Therefore, further studies are needed, under controlled experimental conditions, to confirm the results obtained.

185 ASSESSMENT OF VIABILITY OF IN VITRO PRODUCED BOVINE EMBRYOS BY TRIPLE AND SINGLE TRANSFER

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Factors that affect the viability of in vitro-produced (IVP) embryos are usually evaluated by comparing pregnancy rates of a treatment and a control group. The 'er' model of embryo survival (McMillan WH *et al.*, 1998 Theriogenology 50, 1053–1070) utilizes twin embryo transfer to estimate embryo ('e') and recipient ('r') contributions to embryo survival, and allows the comparison of treatment effects without using a control group, when treatment is the only change in operations. Application of the model to data of contemporaneous single and twin transfer indicates that 'e' and 'r' are independent of the number of embryos transferred. Thus, twin transfers enable the efficient use of costly recipients while providing meaningful estimates of single embryo survival rates. The objective of this study was to assess the embryo survival rates of fresh IVP embryos of a newly established IVP lab by applying the model to triple transfers and comparing the expected embryo survival rates with those achieved for single transfers. Cumulus-oocyte complexes (COCs) were aspirated from abattoir-derived ovaries of cows of unknown breeds or by ovum pick-up (OPU) from Holstein-Friesian 2- or 3-yr-old donor cows. COCs were matured in 500 μL of TCM199 + 10% FCS (Life Technologies, Auckland, NZ), 10 $\mu g\,mL^{-1}$ FSH and LH (ICPBio, Auckland, NZ), 1 $\mu g\,mL^{-1}$ estradiol (Sigma, Auckland, NZ), 100 μM cysteamine (Sigma) for 24 h under 5% CO_2 and then fertilized with 1×10^6 percoll-separated sperm mL^{-1} from a single bull (Tervit HR and Pugh PA, 2000 14th ICAR 18, 37(abst)). Twenty-four h after insemination, presumptive zygotes were transferred into 500 μL mSOF (Pugh A *et al.*, 2001 Theriogenology 55, 314 (abst)) and cultured for 4 days under humidified 5% CO_2, 7% O_2 and 88% N_2. On Day 4, cleaved embryos were transferred into fresh culture medium and culture continued for a further 3 days under the same conditions. Embryo stage and grade were evaluated on Day 7 of culture. Grades 1, 2 and 3 (IETS manual, 2002) compact morulae and blastocysts produced from abattoir-derived COCs were transferred in triplets, while grades 1 and 2 compact morulae and blastocysts from OPU-derived COCs were transferred singly, in 0.25 mL insemination straws into synchronized Holstein-Friesian heifers. Recipients received a CIDR (CIDR Cattle Insert, Pharmacia, Auckland, NZ) at Day −12 followed by a prostaglandin (Estroplan, Parnell Laboratories, Auckland, NZ) injection at Day −6. CIDRs were removed at Day −2, followed by estrus at Day 0 (= day of IVF). Embryos were transferred on Day 7 and recipients received a CIDR after transfer (ET). CIDRs were removed at Day 19 to synchronize any returns. Two experienced practitioners performed all the transfers. Pregnancies (single transfers) and number of live fetuses (triple transfers) were confirmed at Days 60 and 42, respectively. Pregnancies were terminated between Days 62 and 65 by two prostaglandin injections 48 h apart. A total of 76 single transfers resulted in 36 pregnancies (47.4%, binomial SD 5.7%). A total of 75 triple transfers (225 embryos) resulted in 98 viable fetuses (44%) and 58 pregnant recipients (77.3%). For triple transfers, the estimates for 'e' and 'r' were 0.50 and 0.89, respectively, with the product yielding an expected triple embryo survival

rate of 44.1%. The actual distribution of 17, 23, 30 and 5 recipients carrying 0, 1, 2, or 3 fetuses, respectively, was not significantly different from the expected values of 16, 25, 25 and 8 estimated from the model (chi-square = 2.49, NS). Estimates for 'e' and 'r' were not significantly different when combined single and triple data were included in the model ('e' = 0.55 and 'r' = 0.90), indicating that embryo survival is independent of the number of embryos transferred. Results indicate that multiple transfers do increase pregnancy rate (from 47.4 to 77.3%), but not embryo survival posttransfer (44.1 v. 47.4%). Although single ET was done with OPU-derived embryos and triple with slaughterhouse-derived embryos and results are not strictly comparable, the similarity of estimates for 'e' suggests that using the same in vitro-embryo assessment criteria resulted in embryos of similar intrinsic viability from the two sources. In the near future, we will perform triple transfers of cryopreserved IVP embryos and use the model to estimate embryo and recipient contributions to embryo survival of frozen IVP embryos, without using a fresh control. We will continue to build a dataset based on triple and single transfers to further assess the effect on embryo survival rates of triple and single transfers.

186 SEROCONVERSION OF CALVES TO BOVINE VIRAL DIARRHEA VIRUS (BVDV) FOLLOWING INTRAVENOUS INOCULATION WITH ARTIFICIALLY EXPOSED IN VIVO-DERIVED EMBRYOS

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An early study demonstrated that washing embryos was effective for removal of virus after artificial exposure of in vivo-derived embryos to a cytopathic isolate of BVDV (Singh *et al.*, 1982 Theriogenology 17,437–444). However, a recent study using representative noncytopathic isolates of BVDV demonstrated that washing was less beneficial for removing some isolates of BVDV than for others (Waldrop *et al.*, 2000 Theriogenology 57, 575). Thus, the objective of this study was to determine if the quantity of a high affinity isolate of BVDV that remains associated with single-washed or trypsin-treated embryos is sufficient to cause infection in vivo. Twenty zona pellucida-intact Day 7 morulae and blastocysts (MB) were collected from superovulated cows. After collection, all MB were washed according to International Embryo Transfer Society (IETS) standards, and all but 4 (negative controls) were exposed for 2 h to approximately 106 cell culture infective doses (50% endpoint) per mL (CCID50/mL) of viral strain SD-1. Following exposure, one-half of the MB were washed and one-half were trypsin-treated according to IETS standards. All MB were then individually sonicated, and sonicate fluids were injected intravenously into seronegative calves. Blood was collected from each calf on Days 0, 3, 6, 9, 12, 15, 20, 25, and 30, and sera were assayed for BVDV and anti-BVDV antibodies. All cattle used in the study were determined to be virus- and antibody-negative 30 d prior to and the day of intravenous inoculation of sonicate fluids into calves. Viremia was not detected in any calf following injection, possibly due to intermittent sampling and/or small amount of embryo-associated virus present. However, seroconversion of 38 and 13% of the calves occurred following injection with sonicate fluids from washed and trypsin-treated embryos, respectively. Findings demonstrated that the quantity of a high affinity isolate of BVDV associated with single-washed or trypsin-treated embryos is sufficient to be infective in vivo as evidenced by seroconversion. These results emphasize the need for studies to determine if the virus associated with exposed individual embryos constitutes an infective dose when placed into the uterus.