Embryo Transfer

144 HEAT TOLERANCE OF DEMI-EMBRYOS FROM A TROPICAL-ADAPTED BOS TAURUS BREED
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Long-time selection of autochthonous Bos taurus breeds in a tropical environment is known to result in some physiological changes, including gamete thermotolerance. The aim of this study was to evaluate the resistance to heat shock (HS) of semi-embryos from tropical-adapted B. taurus cattle. The experiment was carried out in a tropical wet climate, at the experimental station of Instituto de Zootecnia (latitude 22°46'S; longitude 47°17'W) from November to February (average maximum temperature = 30.0 ± 0.8°C, and average absolute precipitation = 153.1 ± 78.8 mm). Embryos (morulae) from 37 superovulated Caracu (a local B. taurus breed) cows were collected by uterine flushing on Day 6 after AI. Sixty-two grade I morulae were split with a metal microblade coupled to a micromanipulator, and each embryonic disc was placed separately in a 35-μL drop of culture medium [SOF supplemented with modified Eagle medium amino acids, sodium citrate, myo-inositol (SOFAci), and 10% FCS] under mineral oil in a humidified atmosphere of 5% CO₂ at 38.5°C for 24 h (control) or at 41°C for 12 h and a further 12 h at 38.5°C (HS). Halves of morulae that developed to the blastocyst stage (showing a central cavity) after a 24-h culture were transferred to recipient heifers previously synchronized with the donors. Pregnancy was monitored by ultrasonography scanning 40 days after transfer, and data were analysed by chi-square test. There was a significant difference in the development to the blastocyst stage between control (44/62, 71.0%) and HS (30/62, 48.4%) demi-embryos (P < 0.01). Pregnancy rate obtained from control (14/44, 31.8%) and HS (8/30, 26.7%) blastocysts was not different (P > 0.05). We conclude that HS affected embryonic development from the morulae to blastocyst stage of cultivated demi-embryos, although fertility of blastocysts that survived to HS was not further compromised. Further studies are necessary to elucidate whether Caracu embryos are more thermotolerant than other tropically sensitive B. taurus breeds.

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145 EFFECT OF TRANSFER OF CATTLE ELONGATING EMBRYO TO A REPEAT BREEDER COW ON PREGNANCY RATE AND INCIDENCE OF A RETURN TO ESTRUS
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In cattle, a low pregnancy rate directly affects calf and milk production and brings about economic loss to livestock producers. The number of repeat breeder cows in herds decreases the overall pregnancy rate. Therefore, many studies have focused on improving the cows’ reproductive performance. On the other hand, it has been demonstrated that the major embryonic loss occurs in the first or second week of pregnancy, after which mother cows recognize the presence of their embryos via a signal protein secreted by the embryos, interferon-τ. Therefore, to bypass the period with a high incidence of pregnancy loss in cattle, we used elongating embryos for embryo transfer (ET) to repeat breeder cows. Elongating embryos on Day 14 postoestrus were used for the present study because embryos of this stage have the ability to produce interferon-τ and embryos of later stages are easily damaged when they are collected from the uterus by flushing. In this study, Japanese Black cows were superovulated and inseminated when they showed standing oestrus. Elongating embryos were collected by nonsurgical uterine flushing. Collected embryos that had evident embryonic discs and intact trophectoderm were used for ET. These embryos were transferred, using plastic catheters or ET guns to ipsilateral uterine horns, to the corpus luteum of synchronized crossbred (Japanese Black × Holstein-Friesian) recipient cows. Recipient cows were divided into 2 groups according to the previous reproductive history of each cow: reproductively normal cows that were pregnant with fewer than 3 times of AI or regular ET (using Day 7 embryos) after every parturition (n = 33) and repeat breeder (RB) cows (n = 42). Elongating embryos were transfer on Day 14 after oestrus, and all cows were monitored for signs of standing oestrus twice a day until Day 80. Pregnancy was diagnosed with ultrasonography every 10 days from Day 30 to 80 postoestrus. The data were analysed by Fisher’s exact test. The pregnancy rate of reproductively normal cows was 54.5%. However, in repeat breeder cows, the pregnancy rate was significantly decreased (2.4%; P < 0.01). When the repeat breeder cows did not become pregnant, transfer of the elongating embryos was repeated another 5 times continuously and the incidence of a return of oestrus was monitored daily. At the first ET period, the average interestrus interval in nonpregnant repeat breeder cows was 59 ± 7.2 days. As the ET period proceeded, the interestrus intervals were gradually decreased, and at a sixth ET period, it approximated the normal oestrus cycle length (22.8 ± 0.8). These results indicated that although transfer of elongating embryos could bypass the critical period of high incidence of embryo loss, it did not improve the pregnancy rate of repeat breeder cows. Moreover, it suggests that the uterine environment of repeat breeder cows was gradually deteriorating in a time-dependent manner.

146 CONSEQUENCES OF EMBRYONIC EXPOSURE TO COLONY-STIMULATING FACTOR 2 ON TROPHOBLAST ELONGATION, INTERFERON TAU SECRETION, AND GENE EXPRESSION IN THE EMBRYONIC DISC AND TROPHECTODERM
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This study was designed to evaluate possible mechanisms by which colony-stimulating factor 2 (CSF2) acts during Day 5 to 7 of development to improve embryonic and fetal survival and decrease fetal losses. One hypothesis was that CSF2 causes increased secretion of interferon-τ (IFNT) by
the trophoblast of elongated conceptuses. Colony-stimulating factor 2 might also affect elongation of the trophoblast and improve survival of the embryonic disc (ED) because of evidence that treatment of embryo with CSF2 caused differential regulation of a large number of genes involved in developmental processes and a decrease in apoptosis. Bovine embryos were produced in vitro in the presence or absence of 10 ng mL\(^{-1}\) of CSF2 beginning at Day 5 after fertilization. Embryos were transferred at Day 7 to lactating dairy cows using a timed embryo transfer protocol \((n = 20\) and 15 for control and CSF2, respectively). At Day 15, embryos were recovered and assessed for length, stage, and presence of an ED. The flushings recovered from the first 60 mL were stored for antiviral activity analysis. A total of 4 filamentous conceptuses were divided into 1) the ED and a small amount of adjacent trophoblast and 2) tissue containing trophoblast only. These tissues were analysed for gene expression using the \textit{Bos taurus} Two Colour Microarray Chip from Agilent (Palo Alto, CA). Quantitative real time PCR analysis of 11 differentially expressed genes and 1 housekeeping gene (GAPDH) was performed to confirm the microarray results. In addition, quantitative real-time PCR of IFNT was performed in all recovered embryos (7 and 10 for control and CSF2, respectively). Results suggest that higher pregnancy rates at Day 30 represents increased embryonic survival at Day 15 (35% for controls vs. 66% for CSF2; \(P < 0.07\)), a greater capacity of the embryo to elongate (39 mm for controls vs. 62 mm for CSF2) and secrete IFNT (mean IFNT IU = 349.074 for control and 1883.060 for CSF2; \(P < 0.07\)) at Day 15. In addition, the amount of IFNT mRNA was 22 times higher (\(P = 0.06\)) in the CSF2-treated embryos. There was no difference in the presence of an ED between groups. Analysis of gene expression between the ED and trophoblast in filamentous embryos indicated no difference in transcription among this subset of embryos. However, more than 500 genes were identified as being preferentially expressed in the ED. These genes represent candidate markers for ED that should prove useful for studying the differentiation of the bovine conceptus through the periattachment period. In conclusion, results support the idea that the increased survival of embryos exposed to CSF2 from Day 5 to 7 of development is the result of increased embryonic survival before Day 15 and a greater capacity of the embryo to elongate and secrete IFNT at Day 15. The reduction in embryonic and fetal loss after Day 30 caused by CSF2 is probably not a direct reflection of altered gene expression at Day 15.

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### 147 USE OF EQUINE CHORIONIC GONADOTROPIN AFTER EMBRYO TRANSFER IN NELORE AND CROSSBRED RECIPIENT CATTLE


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Recent studies that have used eCG administration either 14 or 22 days after fixed-time AI in dairy and beef cattle have indicated a positive effect on conception rates. The aim of this study was to evaluate conception rates in embryo recipients treated with eCG 7 days after fixed-time embryo transfer (ET). A protocol for synchronization of ovulation was used in 470 Nelore and crossbred embryo recipient heifers \((n = 179)\), dry cows \((n = 152)\), and lactating cows \((n = 139)\;\text{body condition score} = 3.0\) to 3.5; > 40 days in milk). All heifers and dry cows, but only 27.3% of lactating cows, were cycling at the beginning of the synchronization protocol. On Day 0, each female received IM injections of 2 mg of oestradiol benzoate (Gonadiol, Intervet Schering-Plough Animal Health), 0.075 mg of PGF\(_{2\alpha}\) (Prelolan, Intervet Schering-Plough Animal Health). An intravaginal progesterone-releasing device (DIB, Intervet Schering-Plough Animal Health) was also inserted on Day 0. On Day 8, the DIB device was removed and cows or heifers received another IM administration of 0.075 mg of PGF\(_{2\alpha}\) (Prelolan), 300 IU of eCG (Novormon, Syntex S.A., Buenos Aires, Argentina) and 0.6 mg of oestradiol cipionate (Pfizer, Brazil). Transrectal ultrasonography was performed in all recipients on the day of ET (Day 16, 17, or 18 of the protocol) and only those with a corpus luteum greater than 15 mm in diameter were selected \((n = 113)\) heifers, 114 dry cows, and 70 lactating cows. Transfers of fresh, frozen, and vitrified \(\text{in vivo}\) produced embryos from Nelore donors were balanced between experimental groups. Seven days after ET, 127 animals received an IM injection of 400 IU of eCG (eCG group), and the 170 remaining cows or heifers were not treated with eCG, and were therefore considered the control group. Pregnancy diagnosis was performed 30 and 60 days after ovulation by transrectal ultrason. Data were analysed using generalised linear models and results are presented as least squares means ± standard error. Conception rates were similar in the eCG and control groups at Day 30 (35.9 ± 5.5 and 33.5 ± 4.4, respectively; \(P > 0.10\)) and at Day 60 (27.6 ± 4.9 and 26.7 ± 3.9, respectively; \(P > 0.10\)). Contrary to other results in the literature, especially in dairy cows bred by fixed-time AI, the use of eCG 7 days after ET did not improve conception rates in \textit{Bos indicus} cattle used as embryo recipients.

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### 148 PIGLET GROWTH AND BLOOD COMPONENTS DURING LACTATION FOLLOWING RECIPROCAL EMBRYO TRANSFER BETWEEN MEISHAN AND WHITE CROSSBRED GILTS


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Sow productivity has a significant economic impact on the swine industry and is influenced by several factors, including preweaning piglet mortality. In Western breeds, low birth weight piglets exhibit the greatest susceptibility to preweaning mortality. In contrast, Meishan (MS) piglets have decreased birth weights but lower preweaning mortality rates, suggesting that birth weight is not the sole component of preweaning survival. The objective of the current study was to determine the contributions of the maternal and piglet breed and their interactions on piglet growth and blood components pertaining to survivability during lactation following reciprocal embryo transfer between MS and White crossbred (WC) gilts. Twenty-five successful pregnancies were produced by embryo transfer in 2 farrowing seasons that represented all maternal and piglet breed combinations;
Embryo Transfer

MS × MS (n = 4 litters), MS × WC (n = 7 litters), WC × MS (n = 7 litters), and WC × WC (n = 7 litters). At Day 1, 10, and weaning (average weaning age = 18 days), piglets (n = 147, 97, and 94, respectively at Day 1, 10, and weaning) were weighed and blood samples were taken. Hematocrit, hemoglobin, glucose, nitrogen, nonesterified fatty acids, albumin, and cortisol were measured in all blood samples. All data were analysed for ANOVA using mixed model procedures. Piglet weights were greater (P < 0.001) throughout lactation in piglets from WC dams regardless of piglet breed. As a result, average daily gains from Days 1 to 10 and weaning were greater (P < 0.05) in piglets from WC dams. There were significant (P < 0.001) maternal × piglet × day interactions for hematocrit and hemoglobin levels in which levels were greatest at Day 1 in MS piglets from WC dams and at Day 10 in MS piglets from MS dams but decreased in WC piglets from WC dams at Day 1. Glucose was greater (P < 0.05) at Day 1 in piglets from WC dams regardless of piglet breed but was greater (P < 0.05) at weaning in WC piglets regardless of maternal breed. Nitrogen was similar at Day 1 for all maternal and piglet breed combinations, but at Day 10 and weaning, nitrogen levels were greater (P < 0.001) in MS piglets regardless of maternal breed. Nonesterified fatty acid was greater throughout lactation in piglets from MS dams irrespective of piglet breed. Albumin was greater (P < 0.05) in MS piglets throughout lactation regardless of maternal breed. Cortisol was not different between the maternal and piglet breed combinations throughout lactation, but cortisol was greater (P < 0.001) at Day 1 compared with Day 10 and weaning. This study demonstrated that piglet growth during lactation was influenced more by maternal breed in favor of WC dams, which supports previous crossbreeding studies. However, blood components pertaining to survivability displayed complex interactions between the piglet and maternal breed, which may signify possible mechanisms for improved preweaning survivability of MS pigs.

149 AN INEXPENSIVE LABORATORY PRACTICE TO TEACH EMBRYO COLLECTION AND TRANSFER

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The objective of this work is to describe a hands-on laboratory session for surgical embryo collection and transfer using rabbits as a model. Rabbits are characterised for their low purchase price, easy handling, and low maintenance, thus allowing their use as a tool for embryo transfer teaching and for its potential application in other species, such as the bovine, ovine, and caprine. These are important reasons in countries where the use of low-cost techniques and animal models is a must. Two females of the New Zealand breed were used as donors and one female of the Chinchilla breed was used as recipient. All females were healthy and multiparous. Three days after receiving natural service by a male, the donors were anesthetized (by injection of xylazine hydrochloride, 1.1 mg kg⁻¹) and ovariohysterectomy surgery was performed on each one. Each uterine horn was flushed 3 times with Ringer’s lactate (Hartmann) solution. Using a stereoscopic microscope, we searched for embryos and then placed them in a Petri dish for evaluation and selection. Once selected, embryos were moved to a 6-well dish containing 0.5 mL of a commercially available holding solution and washed 3 times. Twenty-one grades 1, 2, and 3 embryos were recovered and evaluated, showing developmental stages 1 to 6, according to IETS. The recipient female was anesthetized, and by laparotomy, her uterine horns were exteriorized to transfer a total of 15 grade 1 embryos with development stages 4 to 6. Pregnancy diagnosis was performed by real-time ultrasound on Days 10 and 20 post-transfer, with positive results. The transferred embryos induced a 34-day pregnancy, including 3 days of embryo development within the donors. Parturition was normal and without problems; 2 live offspring were born, with normal weight and size. The technique, used for teaching purposes, was successfully performed by 3 graduate students directed by 2 professors. The use of rabbits as a teaching model was efficient and the total cost of the laboratory practice was low, as compared with the use of other domestic species. The handling of the females and their offspring was easy, the feeding costs were low, and the practical laboratory session was fulfilled. It is proposed that the rabbit species be used for teaching surgical embryo collection and transfer to graduate and undergraduate students in underdeveloped and developing countries, where low-cost teaching models are of the utmost importance.

150 CONCEPTION RATES OF FRESH AND FROZEN IN VIVO-PRODUCED MORULAE AND BLASTOCYSTS IN LACTATING DAIRY RECIPIENT COWS


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The aim of this study was to evaluate conception rates of fresh or frozen embryos in the morula and blastocyst stages of development when transferred to lactating Holstein cows. During a 365-day period, 213 Day-7 embryos were produced by superovulation in lactating Holstein cows or nonparous Holstein heifers and transferred to lactating Holstein cows synchronized with the Ovsynch protocol at the University of Wisconsin–Madison. After collection, embryos were evaluated and classified according to IETS standards. Subsequently, grades 1 and 2 embryos were conventionally frozen in ethylene glycol or transferred fresh to recipients on Day 7 of the cycle in the uterine horn ipsilateral to the corpus luteum. When there were more recipients than fresh embryos available, frozen embryos were thawed and transferred. Pregnancy diagnosis was performed on Days 30, 60, and 120 by ultrasound evaluations. Moreover, calving data were recorded. Data were analysed using generalized linear models and results are presented as least squares means ± standard errors. The average conception rates between 30 and 280 days of fresh (n = 45) and frozen (n = 168) embryos were similar (38.7 ± 7.3 and 29.4 ± 3.3%, respectively; P > 0.10). There was an interaction between treatment and embryo developmental stage (P < 0.10). The average conception rate was higher in fresh blastocysts (54.2 ± 12.8%) than fresh morulae (25.2 ± 5.1%) and cryopreserved embryos (P < 0.05). Moreover, there was no difference between conception rates of cryopreserved morulae and blastocysts (26.8 ± 3.6 and 32.1 ± 4.8%, respectively; P > 0.10). Embryo quality seems not to have influenced the conception rates of Holstein embryos (34.9 ± 3.3 and 32.9 ± 6.9% of grades 1 and 2, respectively).
respectively; \( P > 0.10 \). We concluded, based on the results of this study, that cryopreservation of in vivo-produced embryos in Holstein donors did not substantially affect embryo viability and that the developmental stage of frozen embryos did not influence conception rates in lactating dairy recipients.

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**151 PREGNANCY RATES AFTER A NONSTEROIDAL ANTI-INFLAMMATORY (FLUNIXIN MEGLUMINE) AND PROGESTERONE \((P_4)\) ADMINISTRATION TO MANGALARGA MARCHADOR RECIPIENT MARES**


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The aim was to evaluate whether pregnancy rates could be improved by randomly injecting recipient mares with flunixin meglumine (FM; Banamine®, Schering-Plough, São Paulo, Brazil) on the day of embryo transfer combined or not with long-acting progesterone (\( P_4 \)) in commercial embryo transfer programs. One hundred sixty-five Mangalarga Marchador recipient mares from the 2006–2007 breeding season of 4 different commercial programs were used. Ages ranged from 4 to 15 years old. In 86 mares, 1.1 mg kg\(^{-1}\) of FM was given intravenously immediately after embryo transfer, and in 79 mares, only saline was injected. Additionally, 59 mares receiving saline were injected with 1 800.0 mg of progesterone at embryo transfer and every week thereafter until 110 days of gestation. Of the 86 receiving FM, 17 received the same progesterone protocol. Embryos were transferred nonsurgically in Ham’s F-10 or Encare medium. Pregnancy was checked by ultrasonography at 15, 30, and 60 days after transfer, and only a positive diagnosis on the latter was considered pregnancy in the statistical analysis. In a 2 \times 2 factorial design (2 levels of FM and \( P_4 \)), pregnancy rates were analysed using the GENMOD procedure considering a binomial distribution (SAS®, Cary NC), and means were compared by orthogonal contrasts. Pregnancy rates were lower \((P < 0.0001)\) in FM-treated (50.2 ± 5.3%) compared with control (88.2 ± 5.1%) mares and in \( P_4 \)-treated (85.9 ± 5.0%) compared with control (52.4 ± 5.5%) mares. An interaction effect between FM and \( P_4 \) was not observed \((P < 0.49)\). For mares receiving or not receiving FM, the addition of the \( P_4 \) protocol lowered pregnancy rates. In mares not treated with FM, pregnancy rates were 81.4 ± 5.1% and 95.0 ± 8.8% for \( P_4 \)-treated and nontreated mares, respectively \((P < 0.05)\). In FM-treated mares that did not receive \( P_4 \), pregnancy rates were 76.8 ± 4.8% compared with 23.5 ± 9.6% of those treated with \( P_4 \) \((P < 0.0001)\). In conclusion, the indiscriminate use of progesterone and nonsteroidal anti-inflammatory drugs may actually decrease reproductive efficacy and even act synergistically to potentiate their effect. These results must be interpreted with caution, considering that the data are derived from field observations, although sources are from very reliable and well-controlled operations. Nevertheless, the authors consider it imperative to have a proper diagnosis before generalizing the use of medical tools to improve pregnancy rates in horses.

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**152 EFFECT OF EMBRYO TRANSFER AFTER ARTIFICIAL INSEMINATION ON THE CONCEPTION RATE IN DAIRY COWS UNDER HEAT STRESS IN SOUTHERN JAPAN**

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A serious decline in the reproductive performance of dairy cows occurs in southern Japan in the summer period, when the total number of hot days \(\geq 35^\circ\)C numbers more than 20 days annually. Previous reports have mentioned the effectiveness of embryo transfer (ET) at 7 days after AI (AI/ET) under heat-stressed conditions. In the present study, we investigated the effect of AI/ET on conception rate (CR) under heat-stressed conditions in the summer period. Artificial insemination was performed at 13 commercial dairies in this study from August through September in 2007 and 2008. Seven days after AI, a single embryo was transferred into the uterine horn contralateral to the ovary with a corpus luteum (AI/ET, \( n = 82 \)). Artificial insemination at oestrus without further treatment was assigned as the control group (AI, \( n = 367 \)). In 2007, frozen–thawed embryos of Japanese Black cattle were transferred, and the same cattle were used for ET of fresh embryos in 2008. The temperature-humidity index \([0.8 \times \text{temperature} + 0.01 \times \text{relative humidity (temperature} - 14.4) + 46.4], \) rectal temperature, and diurnal highest or lowest and average ambient temperatures were measured at the time of AI and ET. Cows were diagnosed for pregnancy at 42 days after AI by palpation per rectum and were reexamined by transrectal ultrasonography at 60 days after AI. The CR was calculated as the number of cows diagnosed as pregnant 60 days after AI divided by the number of cows inseminated. Fetal loss was calculated as the number of cows that did not deliver calves after term divided by the number of cows diagnosed as pregnant. The CR, number of AI, fetal loss, and type of newborn (Holsteins, AI origin; Japanese Black, ET origin) were confirmed retrospectively. For statistical analysis, Fisher’s exact test and Student’s \( t \)-test were used for comparison of the CR, fetal loss, and body temperature by using a statistical software program for PC (Excel Statistics 2006). The CR for AI/ET was 30.4% and for AI was 13.8% in 2007 \((P < 0.01)\), and the CR for AI/ET was 30.8% and for AI was 21.5% in 2008 \((P < 0.05)\). The average diurnal temperature was 31.1°C in 2007 and 30.1°C in 2008, and the temperature-humidity index was 81.8 and 80.8, respectively. On Day 8, the pregnant cows had a lower rectal temperature than the open cows in 2007, but not in 2008 \((38.9 \pm 39.4^\circ\)C in 2007; \( P < 0.05 \); and 39.1 ± 38.9°C in 2008; \( P > 0.05 \)). The fetal loss was 38.1% in AI/ET v. 7.4% in AI in 2007 \((P < 0.05)\) and 12.5% v. 0% in 2008 \((P < 0.05)\), respectively. The AI/ET procedure could improve CR in dairy cows during the summer period in southern Japan. However, other problems may accompany AI/ET, such as higher fetal losses.
153 EFFECT OF DIFFERENT HOLDING AND TRANSPORT MEDIA ON CONCEPTION RATES FOLLOWING TRANSFER OF IN VIVO AND IN VITRO FERTILIZATION-DERIVED BOVINE EMBRYOS
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Two experiments were conducted to compare conception rates following embryo transfer (ET) of bovine embryos held and transported in Synpro® holding medium (Bioniche, Belleville, Ontario, Canada) with other 2 holding media: Emcare® (ICPbio, Auckland, New Zealand) for in vivo-derived embryos and HEPES-buffered synthetic oviduct fluid (H-SOF) for IVF-derived embryos. The first trial was performed in the period from October through December 2006 at the Curitiba farm in Poços de Caldas, Minas Gerais, Brazil. A total of 140 in vivo-derived embryos were produced from 20 Nelore donor cows and transferred fresh at the same farm. After each donor recovery, embryos were equally separated per stage (morula or blastocyst) and classification (grades 1, 2, and 3) into 2 Petri dishes, each containing either Synpro or Emcare. The embryos were held for an average of 3 h after recovery, loaded into 0.25-mL straws, and transferred fresh into recipients heifers, which were all previously synchronized with the same hormonal protocol treatment and presented a corpus luteum on the day of transference. Conception rate was checked at approximately 60 days of conception by pregnancy diagnosis. Transport time ranged from 1 to 9 h, and the recipient farms ranged from 100 to 1200 km in distance from the Embriza Laboratory. Crossbred Nelore donor cows and transferred fresh at the same farm. After each donor recovery, embryos were equally separated per stage (morula or blastocyst) and classification (grades 1, 2, and 3) into 2 Petri dishes, each containing either Synpro or Emcare. The embryos were held for an average of 3 h after recovery, loaded into 0.25-mL straws, and transferred fresh into recipients heifers, which were all previously synchronized with the same hormonal protocol treatment and presented a corpus luteum on the day of transference. Conception rate was checked at approximately 60 days of conception by pregnancy diagnosis. The chi-square test was used for statistical analysis. The conception rate of embryos maintained in Synpro was significantly higher than those in Emcare: 64.2% (43/67) vs. 47.9% (35/73; P < 0.05). A second experiment was performed between September and December 2008 at Embriza Biotechnology Laboratory, Campo Grande, Mato Grosso do Sul, Brazil. A total of 1689 IVF-derived embryos (stage 1, quality 1), produced from Nelore donor cows, were randomly assigned to be held and transported in either Synpro (769) or H-SOF transport medium (920). Transportation time ranged from 1 to 9 h, and the recipient farms ranged from 100 to 1200 km in distance from the Embriza Laboratory. Crossbred recipient heifers (Bos taurus × Bos indicus) were synchronized with prostaglandin or vaginal progesterone device protocols. Pregnancy diagnosis was performed by ultrasonography approximately 60 days after ET. Statistical comparisons were performed using the chi-square test. Conception rates resulting from embryos transported in Synpro (45.1%, 347/769) and in H-SOF (42.0%, 386/920) were not different (P = 0.19).

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154 EVALUATION OF THE EFFECTIVENESS OF TREATMENT WITH TRYSPIN IN MURINES EMBRYOS EXPERIMENTALLY EXPOSED TO BOVINE HERPES VIRUS TYPE-1 (BoHV-1) BY THE NESTED-PCR TECHNIQUE
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The cryopreservation of bovine embryos has allowed an increase in their commercialization causing concern about the transmission of pathogens through the production and transfer of in vitro embryos. The International Society of Embryo Transfer (IETS) suggests the treatment of embryos with trypsin or antibiotics in alternating washes with culture medium in order to remove and/or inactivate infectious agents that may interfere with the final product. The aim of this study was to evaluate the effectiveness of trypsin treatment in the elimination and/or removal of bovine herpes virus type-1 (BoHV-1), Colorado strain, in murine embryos. Viral detection was made by n-PCR and cytopathic effect in Madin Darby bovine kidney (MDBK) cells. Six- to 8-wk-old female mice (Swiss) were superovulated and mated with fertile males of the same strain. After 24 h, the zygotes (n = 262) were divided into 3 groups: control group submitted to sequential wash (CseqW), the group exposed to the virus (30 μL; 10^8.5 virus mL^-1) and submitted to sequential wash (EseqW), and the group exposed to the virus and submitted to the trypsin treatment (ET). All the groups of zygotes and the last sequential wash drops were tested by nested-PCR and inoculated in MDBK cells to allow observation of cytopathic effects. All groups except for CSw showed positive results for the nested-PCR for both zygotes and for the last wash drops. A cytopathic effect was observed in all groups except for CSw, demonstrating the viability of the virus after treatment. These results demonstrated that trypsin treatment was not effective in eliminating or removal of BoHV-1. The need for such studies is fundamentally important when considering the potential risk of pathogen transmission by reproductive biotechnologies.

155 EMBRYONIC APOPTOSIS AFTER BTV-8 INFECTION IN BOVINE HATCHED IN VITRO PRODUCED BLASTOCYSTS
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The recent bluetongue virus serotype-8 (BTV-8) epidemic in central Western Europe has been associated with field fertility problems. Previous research clearly showed that in vitro produced bovine hatched blastocysts are susceptible for BTV-8 infection (Vandaele et al. 2010 Reprod. Fertil. Dev. 22, 254 abst.). The aim of the present study was to investigate the effect of a BTV-8 infection on the occurrence of apoptosis in embryos in order to gain a clear insight into the role BTV-8 might play in early embryonic death. Immature