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Corrigenda

131 CHROMOSOMAL ABNORMALITIES IN *IN VITRO*-PRODUCED 4-DAY-OLD CATTLE EMBRYOS: INFLUENCE OF THE OOCYTE MATURATION MEDIA

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Because of mistakes in the information supplied, the original list of authors was incorrect. The correct author list is:

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130 METABOLIC PROFILING OF MALE AND FEMALE BOVINE EMBRYOS USING NUCLEAR MAGNETIC RESONANCE (NMR) IMAGING

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It has been previously shown that during the pre-implantation phase of embryo development, the pentose phosphate energy pathway is 4 times more active in female embryos when compared with male embryos (Tiffin *et al.* 1991 J. Reprod. Fertil. **93**, 125–132). The different metabolic and growth rates can be attributed to the different expression of X-linked genes between the sexes during the early stages of pre-implantation development, in which both X chromosomes are still active in female embryos (Okamoto *et al.* 2004 Science **303**, 644–649). The aim of this study was to evaluate, by proton magnetic resonance (H1-NMR) imaging, the different behaviour of female and male embryos. In this study we evaluated only energetic substrates or Krebs cycle's intermediates. Matured bovine cumulus-oocyte complexes were fertilized *in vitro* according to our standard procedures (Rubessa *et al.* 2011 Theriogenology **76**, 1347–1355). Presumptive zygotes were placed in individual drops of 50 μL of SOF. Zygotes were incubated in a humidified mixture of 5% CO_2, 6% O_2, and 88% N_2 in air at 39°C. After 48 h, the zygotes were placed into WOW culture, and the drops collected in tubes. The embryos were scored for quality on the basis of morphological criteria. In this experiment, we evaluated 10 embryos for each sex, at stage of tight morula, early blastocyst, blastocyst, and expanded; data were obtained from 2 replicates. The embryos had their sex determined according to our standard protocol (Alomar *et al.* 2008 Anim. Reprod. Sci. **107**, 48–61). Samples of media (40 μL) were added to 660 μL of a stock solution prepared by dissolving 5.0 mg of sodium 3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionate (TSP) in 50 mL of deuterium oxide. The TSP acted both as a chemical shift reference and as an internal standard for the purposes of quantitation. The resulting diluted samples were transferred to a 5-mm NMR tube. Samples were analysed on a Varian VNS-750 NB (750 MHz) spectrometer (Agilent Technologies, Santa Clara, CA, USA). Data were statistically analysed with ANOVA using the Generalized Linear Model (GLM) procedure (SAS, version 9, 1999, SAS Institute Inc., Cary, NC, USA), where the independent variable was the sample (female, male embryos and control media without embryos). Tukey's post-hoc test was used to perform multiple comparisons. The *P*-level was set at 0.05. All data were expressed as quadratic means and with standard error of the means. The results, reported in Table 1, indicate that there are no statistical differences between the sexes after 48 h of embryo culture. In conclusion, these results confirm that in the first phase of development, the embryos derive their energetic substrates from substrates contained within the embryos themselves.

Table 1. Results, least squares mean (standard error)

Item	Myo-inositol	Citrate	Pyruvate	Lactate	Acetate
Female				2.710 (0.045)	0.548 (0.034)
Male	2.3366 (0.033)	0.222 (0.023)	0.448 (0.049)	2.778 (0.041)	0.565 (0.031)
Control	2.4150 (0.04)	0.195 (0.028)	0.355 (0.060)	2.830 (0.051)	0.590 (0.038)

131 CHROMOSOMAL ABNORMALITIES IN *IN VITRO*-PRODUCED 4-DAY-OLD CATTLE EMBRYOS: INFLUENCE OF THE OOCYTE MATURATION MEDIA

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Chromosomal abnormalities were related to embryo developmental failures in cattle, being highly increased in IVF-produced compared with multiple-ovulation embryo transfer-produced embryos. The origin of this difference remains unclear, but they were related to the suboptimal environmental culture laboratory conditions. We studied the influence of 3 different maturation media in the appearance of chromosomal abnormalities in early *in vitro*-produced embryos. A total of 562 oocytes classified as Class A were collected by follicular aspiration in slaughterhouse ovaries and matured by 20 h in 3 different culture media supplemented with 10% FCS and antibiotics as follows: TCM-199 (T, 193 oocytes); DME (D, 178 oocytes); and RPMI-1640 (R, 191 oocytes). Matured oocytes were fertilized by 16 h with 1×10^6 spz mL^{-1} in IVF-TALP media and cultured by 72 h in SOF media. Later, 48 h after fertilization, 562 presumptive embryos were evaluated showing a normal cleavage rate of 64.7, 55.2, and 54.9% in T, D, and R groups, respectively. After culture, only 181 early embryos (90 hpi) showing normal development were correctly karyotyped following our standard laboratory techniques. Individual blastomeres were stained with Giemsa and assessed by direct observation at ×1250 magnification in a brightfield microscope. The percentage of normal diploid embryos (D) and abnormal haploid (H), polyploid (P), or aneuploid (A) embryos were determined. Only embryos showing almost 2 different blastomeres correctly karyotyped were included in this study. Cleavage rates were statistically higher ($P < 0.05$) in the oocytes matured in T media in comparison with the oocytes matured in D or R media. The percentage of diploid embryos were almost equals in the 3 groups evaluated (Table 1). There was some variation when each kind of chromosomal abnormality was assessed individually, but no statistical differences were observed. These results are in consonance with our previous studies in which we demonstrated the maturation time and morphological quality are the 2 main oocyte-derived factors influencing the ploidy of early embryos. It was also demonstrated that chromosomal complements were affected, in a much lesser extent, by the maturation media supplementation. However, in the present study, the maturation media did not statistically affected embryo ploidy. However, the higher percentage of cleaved embryos using TCM-199 observed agree with fact that this

maturation media is one of the most widely used in IVF procedures in cattle. Based on our data, we suggest that oocyte maturation, a well established technique in cattle, could be performed using different maturation media without expecting major differences in the embryo ploidy, and therefore, the differences observed in cleavage rate must be originated in other physiological factors.

Table 1. Results

Oocyte maturation media	Number of embryos analysed	Chromosomal complements									
		Normal embryos		Abnormal embryos							
		Diploid		Total		Haploid		Polyploid		Aneuploid	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
TCM-199	63	50	79.37	13	20.63	6	9.52	5	7.94	2	3.17
DME	57	45	78.95	12	21.05	7	12.28	4	7.02	1	1.75
RPMI-1640	61	46	75.41	15	24.59	6	9.84	5	8.20	4	6.56

132 VITRIFICATION SYSTEM (OPEN AND CLOSED) IN NEW INCUBATOR WITH REDUCED OXYGEN

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Most IVF laboratories uses high oxygen tension (20%) during embryonic culture. However, it is known that under physiological conditions, oxygen tension in the female reproductive tract ranges between 2 and 8%. Therefore, the aim of this study was to evaluate survival and hatching rate after *in vitro* culture of vitrified/thawed bovine *in vitro*-produced blastocysts cultured under different oxygen concentrations. The experiment consisted of comparing 2 culture systems using different concentrations of oxygen: conventional incubator (20% O_2, Thermo Scientific, model 3130) and a new incubator (5% O_2, WTA Watanabe Tecnologia Aplicada, model Eve). Only Day 7 expanded blastocysts grade 1 were used. Embryos were produced according to conventional IVF protocols. Briefly, cumulus-oocyte complexes were aspirated from postmortem ovarian follicles, matured in TCM199 + 10% FCS + 0.5 mg FSH mL^{-1} + 50 mg LH mL^{-1} + 1 mg oestradiol mL^{-1}, for 24 h at 38.5°C, 5% CO_2 in air. Live spermatozoa from Nellore bull were obtained by centrifugation in Percoll gradients (45 and 90%) and cultured with cumulus-oocyte complexes at 1 million of sperm mL^{-1} in TALP medium + 10 mg of heparin mL^{-1}. After 20 h incubation, zygotes were transferred to CR2 + 2.5% FCS + 4 mg of BSA mL^{-1} and granulosa monolayer for 7 days. Expanded blastocysts were randomly allocated to 2 treatments for vitrification (open system – cryotop and closed system – HSV Kit, IMV-Technologies) using the same vitrification media and protocol (VS1: 10% ethylene glycol + 10% dimethyl sulfoxide and VS2: 20% ethylene glycol + 20% dimethyl sulfoxide for 8 min and 50 s, respectively). After exposure to the vitrification solutions, 2 embryos were loaded/straw, and the straws were plunged into LN. The warming procedure consisted of, immediately after removal from LN2, transferring the embryos in 2 successive warming solutions with decreased concentrations of sucrose (1 M and 0.50 M for 5 min each). The vitrified/rewarmed embryos were transferred to *in vitro* culture. There were no differences in survival rates ($P < 0.05$) between the open and closed vitrification system for blastocysts produced in reduced oxygen in the Eve incubator – 5% O_2 (96% – 109/114 and 98% – 158/161, respectively) compared with embryos produced in the high oxygen environment in the Thermo incubator – 20% O_2 (93% – 214/230 and 92% – 94/102, respectively). Hatching rates were increased for blastocysts cultured in the lower oxygen environment (EVE treatment: 95 and 98%, respectively, for open and closed vitrification protocols) when compared with the high oxygen environment (Thermo treatment: 86 and 87%, respectively, for open and closed systems); $P < 0.05$. *In vitro* culture in a reduced-oxygen environment improves blastocysts competence after vitrification.

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133 NONINVASIVE ANALYSIS OF EMBRYO METABOLITES USING NUCLEAR MAGNETIC RESONANCE

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Wide-spread use of IVF has significantly increased the number of multiple births (Janvier *et al.* 2011 J. Pediatr. **159**, 409–413). A potential solution to this problem is to develop improved methods for embryo selection to permit single-embryo transfer. Identification of a noninvasive technique to assess embryo implantation potential in assisted reproduction would greatly increase success rates and lead to more efficient single-embryo transfer. The aim of this study was to assess whether there are metabolic differences among embryos produced by IVF and embryos obtained by