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1 **Aneuploidy detection and mitochondria quantification in bovine embryos with different**
2 **cleavage onset using Next- generation sequencing-based protocol**

3

4 Miroslav Hornak¹, David Kubicek¹, Petr Broz², Pavlina Hulinska¹, Katerina Hanzalova¹,
5 Darren Griffin³, Marie Machatkova¹, Jiri Rubes¹

6

7 ¹Central European Institute of Technology - Veterinary Research Institute, Brno, Czech
8 Republic

9 ²Institute of Applied Biotechnologies, Prague, Czech Republic

10 ³School of Biosciences, University of Kent, Canterbury, UK

11

12

13 **Correspondence:**

14 Miroslav Hornak, Ph.D.

15 Department of Genetics and Reproduction

16 Veterinary Research Institute

17 Hudcova 70,

18 621 00, Brno,

19 Czech Republic

20 Tel: +420 5 3333 1912

21 Fax: +420 5 4121 1229

22 E-mail: hornak@vri.cz

23

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25

26 **Abstract**

27 Bovine embryos are now routinely used in agricultural systems as a means of disseminating
28 superior genetics worldwide, ultimately with the aim of feeding an ever-growing population.
29 Further investigations common for human IVF embryos are thus a priority to improve cattle
30 IVF and one such area is aneuploidy (abnormal chromosome number). Although the
31 incidence and consequences of aneuploidy are well documented in human preimplantation
32 embryos, they are less well known for the embryos of other animals. To address this, we
33 assessed aneuploidy levels in 30 2-cell bovine embryos derived from early and late cleaving
34 zygotes. Contemporary approaches (whole genome amplification and next generation
35 sequencing) allowed aneuploidy assessment for all chromosomes from oocyte donors aged 4-
36 7 years. We also quantified mitochondrial DNA levels in all blastomeres assessed, thereby
37 testing the hypothesis that they are related to levels of aneuploidy. Overall incidence of
38 aneuploidy in this cohort of bovine embryos was 41.1%, significantly correlated to timing of
39 cleavage (77.8% late cleaved vs. 31.7% early cleaved). Moreover, based on mtDNA sequence
40 read counts, we observed that median mtDNA quantity is significantly lower in late cleaving
41 embryos. The findings further reinforce the use of the bovine system as a model for human
42 IVF studies.

43

44 **Keywords**

45 aneuploidy, cattle, embryo, mitochondria, Next- generation sequencing

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52 **Introduction**

53 Both human and non-human studies indicate that chromosome aneuploidy occurs frequently
54 in early mammalian embryos and leads to embryo developmental arrest, abortions, stillbirths
55 or a birth of affected offspring [Munné et al., 2007; King, 2008]. In pigs, the frequency of
56 aneuploidy in preimplantation embryos varies from 14.3 to 37.3% based on screening of all
57 chromosomes by comparative genomic hybridization (CGH) [Hornak et al., 2009; Hornak et
58 al., 2015]. Studies mainly focus on polyploidy and mixoploidy in cattle, revealing that
59 mixoploidy occurs in 25–90% of bovine embryos [Viuff et al., 1999; Jakobsen et al., 2006;
60 Garcia-Herreros et al., 2010]. It was estimated using FISH that aneuploidy is presented in at
61 least 30% of bovine oocytes [Nicodemo et al., 2010] and 27% of porcine oocytes [Vozdova et
62 al., 2001]. Combined detection of diploidy and aneuploidy by FISH showed that chromosome
63 imbalance might reach 60% in porcine oocytes [Lechniak et al., 2008]. Current evidence
64 suggests that the overall frequency of aneuploidy is higher in in-vitro produced (IVP)
65 embryos compared to embryos generated in-vivo across all farm animal species thus far
66 studied [Viuff et al., 1999; Rambags et al., 2005; Coppola et al., 2007; Lechniak et al., 2007;
67 Ulloa et al., 2008; Hornak et al., 2015]. This, in turn, indicates that sub-optimal oocyte
68 maturation media, maturation and embryo cultivation protocols and/or media supplements
69 may induce aneuploidy at this early stage of development [Demyda-Peyrás et al., 2013].

70

71 We implemented Next-generation sequencing (NGS) to detect aneuploidy of all chromosomes
72 in farm animals, which is applicable to single cell. The main advantage of this approach is
73 that it might be universally applied to any organism with an available reference genome
74 sequence assembly.

75 Bovine oocytes contain approximately 2.6×10^5 copies of mitochondrial DNA (mtDNA),
76 which is 100 times higher than in somatic cells [Michaels et al., 1982]. This is achieved by
77 approximately 45-fold increase of mtDNA copy number from primordial cell to preovulating
78 oocyte [Smith and Alcivar, 1993]. Mitochondrial maturation, distribution, ATP production
79 and its accumulation are important factors describing oocyte competence, which in turn
80 condition successful embryo development and blastocyst rates [Stojkovic et al., 2001]. It has
81 been established that the quantity of mitochondria correlates with fertilization outcome and
82 serves as an important marker of oocyte quality, explaining fertilization failure in some cases
83 [Reynier et al., 2001; Shourbagy et al., 2006; Santos et al., 2006]. It was shown that in vitro
84 maturation might significantly change distribution pattern of mitochondria in oocytes and was
85 described that higher levels of mtDNA in cumulus cells were observed in good-quality
86 embryos derived from corresponding oocytes [Pawlak et al., 2016; Ogino et al., 2016].
87 Recently, it was also demonstrated that altered levels of mtDNA are associated with female
88 age, aneuploidy, and provide an independent measure of embryonic implantation potential in
89 human IVF programme [Fragouli et al., 2015].

90
91 Current studies in the literature have thus far concentrated mostly on human embryos and, to a
92 lesser extent, porcine ones. Bovine embryos have however been relatively under-studied but
93 they are nonetheless important in that they are used extensively in animal breeding regimes
94 and have been established as an excellent model for human IVF. The purpose of this study
95 was therefore to provide an overview of both aneuploidy levels and mtDNA content in early
96 bovine embryos, establishing the validity of an NGS-based approach to achieve this. We also
97 tested the hypothesis that there are significant differences in aneuploidy and mtDNA content
98 in early vs late cleaving embryos in order to establish whether this early stage of

99 morphokinetics may have a predictive value of the future health of the embryo and
100 subsequent foetus.

101

102

103 **Materials and methods**

104

105 The chemicals used for early embryo production were purchased from Sigma-Aldrich
106 Chemicals Co. (Prague, Czech Republic) unless otherwise stated. The all culture procedures
107 were carried out at 39°C in a humidified atmosphere and 5% CO₂.

108

109 **Oocyte maturation and fertilization**

110 Slaughtered Holstein dairy cows (n = 16), aged 4 to 7 years, in the growth/stagnation or
111 regression phases of folliculogenesis were used as oocyte donors. The oocytes were recovered
112 from 3 to 9 mm sized follicles by ovarian cortex slicing. Only the oocytes with homogenous
113 cytoplasm and compact homogenous cumuli were used for maturation. They were matured in
114 500 µl of TCM-199 medium (Earle's salt) supplemented with 0.20 mM sodium pyruvate,
115 gonadotropins (P.G. 600, 15 IU/ml, Intervet, Boxmeer, Holland), 5% estrus cow serum (ECS,
116 Sevapharma Prague, Czech Republic) and antibiotics (50 IU/ml penicillin, 50 µg/ml
117 streptomycin) in four-well plates (Nunclon Intermed, Roskilde, Denmark) for 24 hours. In our
118 conditions, 92.6% of the oocytes reached MII stage. Only those extruded the first polar body
119 were used for fertilization (n = 139/150).

120

121 The motile spermatozoa were separated from frozen-thawed semen of the IVF tested bull by
122 the swim-up method using modified Tyrode's medium (SP-TALP). The oocytes were
123 inseminated with 1×10^6 spermatozoa/ml using modified Tyrode's medium (IVF-TALP) with
124 10 µg/ml heparin. The adequate number of inseminated oocytes was checked at 24 hours for
125 fertilization efficiency. They were fixed with 2.5% glutaraldehyde solution (v/v) at 4°C
126 overnight, stained with 33258 Hoechst in citrate buffer for 10 minutes and examined by
127 epifluorescence at a magnification of 400×.

128

129 Embryo cultivation and cleavage assessment

130 The preliminary zygotes were denuded from cumulus cells by vortex, rinsed in Dulbecco-PBS
131 and cultured in 500 μ l of MEM-medium supplemented with 5% ECS. The two-cell embryos
132 were collected either at 26–31 h (early onset cleavage) or 32–36 h (late onset cleavage) after
133 insemination. Only equally cleaved embryos were used for aneuploidy and mtDNA content
134 examination. They were incubated in PBS with 0.1% pronase and 0.1% polyvinylalcohol to
135 dissolve the zona pellucida and to separate mechanically individual blastomeres. The
136 blastomeres were washed in several droplets of PBS to prevent DNA contamination. The each
137 blastomere was individually transferred into PCR tube in 1 μ l of 1 \times PBS without Ca & Mg²⁺
138 and stored at -80°C until examination.

139

140 NGS validation

141 In order to validate a new NGS based protocol we reanalysed the WGA product of the
142 aneuploid porcine embryos previously examined by CGH in the recent study [Hornak et al.,
143 2015]. In total, 22 chromosome abnormalities observed in 7 aneuploid embryos by CGH were
144 reanalysed by NGS protocol and we observed full concordance for chromosome losses and
145 gains between both methods (Supplementary Fig. S1).

146

147 Genome Amplification and next-generation sequencing (NGS) to detect aneuploidy

148 One of two individually tubed blastomeres underwent Whole Genome Amplification (WGA)
149 using the Single cell WGA kit (New England Biolabs) according to the manufacturer's
150 protocol. Amplified samples were checked on 1.5% agarose / 0.5 \times TBE buffer for the
151 presence of a 200-2000bp DNA smear, which indicated successful amplification. Stringent
152 precautions against DNA contamination during the whole WGA process were taken; WGA

153 was performed in a UV-illuminated PCR box with a dedicated set of sterile PCR tubes,
154 pipettes and filter tips. Amplified DNA was quantified using Qubit dsDNA HS (High
155 Sensitivity) Assay Kit (Life Technologies). DNA library containing sequencing adaptors and
156 unique indices (barcodes) was prepared for each sample using Nextera XT DNA Sample Prep
157 Kit and Nextera® XT Index Kit (Illumina) according to manufacturer's manual without any
158 modification. Up to 48 individual samples were barcoded and pooled. The final pooled library
159 was cleaned up using Agencourt AMPure XP magnetic beads (Beckman-Coulter) and
160 requantified with Qubit dsDNA HS Assay Kit (Life Technologies). The library was diluted to
161 12.5 pM using HT1 hybridization buffer (Illumina) and sequenced on MiSeq sequencing
162 system (Illumina, Inc.). A sequencing run with optimal cluster density (1.200–1.400k/mm²)
163 produced typically 25–30 millions of 36 bp-long reads with Q30 base quality $\geq 95\%$.

164

165 **Bioinformatic pipeline for aneuploidy detection**

166 A raw bcl files were demultiplexed by their barcodes and FASTQ files were created for each
167 sample. Each FASTQ file was trimmed by Phred Quality Score which falls under Q=30
168 threshold. FASTQ files were aligned to reference genome of *Bos taurus* and *Sus scrofa*
169 (*SusScr3* and *BosTau8* UCSC build; downloaded from Illumina's iGenomes). Alignment was
170 performed using Burrows-Wheeler Aligner algorithm [Li and Durbin, 2010], allowing a
171 maximum of gaps opens [parameter = 1], Mismatch penalty [parameter = 3]. Output BAM
172 files were filtered strictly by mapping quality [parameter = 15]. Unmapped reads and multi-
173 mapped reads were filtered out. In final filtration step PCR and Optical duplicates were
174 removed. The programming language AWK [Aho et al., 1987] was used for read counting and
175 GC content calculation after each filtration were use. Then we split chromosomes into 10 Mb
176 bins and calculate GC content and number of reads in each bin using bedtools and
177 programming language AWK. Bin read counts were normalized to the total read count for

178 each sample and were compared to the bin read counts in an amplified male euploid reference
179 samples. Sample:reference bin read counts ratio >1.3 and <0.7 was indicative of chromosomal
180 region gain and loss, respectively. All the NGS results were plotted in statistical computing
181 and graphics program R (freeware, available from <https://www.r-project.org/>). Standard
182 library packages and functions were used for plotting in R.

183

184 **Mitochondrial DNA quantification in early embryos**

185 A quantification of mtDNA in one blastomere using NGS involved determination of the
186 number of DNA sequence reads corresponding to the mitochondrial genome. Then the
187 quantity of mtDNA was calculated as a fraction of mtDNA sequence reads related to the total
188 reads obtained from the sample.

189

190 **Statistical analysis**

191 The data were analyzed with the chi-square test, Student's t-test using ANOVA and the
192 Fischer's exact test, SPSS Version 11.5 for Windows (SPSS, Inc. Chicago, IL, USA).

193

194

195 **Results**

196 **Early embryo development**

197 In order to validate efficiency of fertilization and cleavage in our conditions, these parameters
198 were at first assessed using oocyte control group. The mean proportions of fertilized and
199 cleaved oocytes from those inseminated in control group are shown in Table 1. Start of
200 embryonal development was different in terms of the first cleavage onset of embryos. The
201 significantly higher (chi-square test; $P < 0.01$) proportion of embryos was cleaved from 26 to

202 31 h (early cleavage) than from 32 to 36 h (late cleavage). On the basis of first cleavage time,
203 the 2-cell embryos were divided into two categories: “early cleaved” and “late cleaved.”

204

205 **Aneuploidy in 2-cell bovine embryos**

206 After successful validation of the NGS protocol we analysed copy numbers of each one of
207 chromosomes in 31 single blastomeres from both the early and late cleaved 2-cell embryos
208 (Table 2). The overall incidence of aneuploidy when merging both embryo categories was
209 41.1%. The significantly lower proportion of embryos with aneuploidy was found in the early
210 cleaved embryos compared with the late cleaved embryos (Fischer’s exact; $P < 0.05$). The
211 aneuploidies in both embryo categories were described. No significant difference in ratio of
212 male and female sex was found between the early and late cleaved embryos (Table 2).

213 Examples of aneuploidies detected in embryos by NGS are shown in Fig. 1.

214

215 **Mitochondrial DNA (mtDNA) quantification in early embryos**

216 Based on mtDNA sequence read counts in all 31 blastomeres examined for aneuploidy we
217 also quantified mtDNA. When mtDNA was calculated for early and late cleaved embryo
218 group, we have observed that median mtDNA quantity was significantly decreased (Student’s
219 t-test; $P < 0.01$) in the late cleaved embryos compared with the early cleaved embryos (Fig. 2).

220

221 **Discussion**

222 **Next-generation sequencing protocol**

223 Cattle (*Bos taurus*; $2n = 60$) with 29 acrocentric autosomes and one sex chromosome pair
224 represent significant challenge for standard karyotyping. Several studies have focused on
225 aneuploidy detection in bovine embryos however most only yield information about
226 polyploidy or mixoploidy [King et al., 1987; Kawarsky et al., 1996; Viuff et al., 1999]. Other
227 studies used FISH in order to detect aneuploidy of individual bovine chromosomes, however,
228 in those studies only a few chromosomes were analysed [Nicodemo et al., 2010]. Using NGS-
229 based protocol however we were able to detect copy number of all bovine chromosomes from
230 single blastomere. Every chromosome was sub-divided into approximately 10Mb intervals
231 (bins), which were plotted individually at NGS charts. This allowed us to increase resolution
232 of the platform and detect segmental chromosomal abnormalities. By direct comparison with
233 arrayCGH technology we demonstrated that NGS offers increased sensitivity when
234 embryonic biopsies were analysed during preimplantation genetic diagnosis [Fiorentino et al.,
235 2014; Yang et al., 2015]. We subsequently performed a validation study of our NGS-based
236 protocol by a comparison of metaphase-CGH and NGS results from the same WGA product
237 of the previously analysed porcine blastomeres using metaphase-CGH [Hornak et al., 2015].
238 The metaphase-CGH and NGS charts are shown in Supplementary material (S1). From the
239 validation study we conclude that, for whole chromosome aneuploidy, the sensitivity of both
240 platforms is comparable, however for segmental chromosome abnormalities the NGS analysis
241 was more accurate and offered better resolution. NGS technology is perceived as expensive
242 one, however this is a relative statement. For example, NGS might be coupled with barcoding
243 technology, where different samples might be multiplexed with a use of specific indices
244 before sequencing run. In our study up to 48 samples were barcoded and sequenced in a
245 massively parallel fashion. The final cost per sample was comparable with the metaphase-

246 CGH technology used in our previous study [Hornak et al., 2015]. The disadvantage of NGS
247 technology is its inability to detect polyploidy.

248

249 **Aneuploidy frequency in bovine embryos**

250 As well as in humans, in cattle and pigs, timing of the first cleavage is also a valuable
251 indicator of intrinsic quality of early embryos and their developmental competence [Loneragan
252 et al., 1999; Loneragan et al., 2000; Lundin et al., 2001; Brevini et al., 2002]. Early cleaved
253 porcine embryos are more likely to develop to blastocysts than their late cleaved counterparts
254 [Isom et al., 2012]. The timing of first zygotic cleavage is associated with many factors
255 including transcript level of developmentally important genes and chromosomal aberrations
256 [Lechniak et al., 2008]. In the present study we established that bovine early cleaved embryos
257 show lower levels of aneuploidy than late cleaved ones (31.8% vs 77.8%). Our findings thus
258 provide strong evidence that embryo selection based on the time of zygotic cleavage may
259 increase proportion of euploid embryos. Our data are supported by time lapse studies of
260 human IVF embryos showing that any delay in cleavage times, incorrect divisions or lower
261 morphology grade are linked with higher aneuploidy levels [Chawla et al., 2014; Coates et al.,
262 2015].

263

264 Aneuploidy frequency varies, between individuals of the same species and is probably
265 affected by the different age of animals used, the protocols employed and/or whether in-vitro
266 in-vivo production is used. Here we report that the frequency of aneuploidy in 2-cell stage
267 IVP bovine embryos obtained from 4–7 years old oocyte donors employing whole
268 chromosome screening by NGS-based protocol is 41%. This frequency is comparable to
269 similar study focusing on porcine 2-cell stage IVP embryos where we observed 37% of the
270 embryos to be aneuploid [Hornak et al., 2015]. Nicodemo et al., [2010] reported that

271 approximately 30% of IVM bovine oocytes are aneuploid, by applying FISH for the X
272 chromosome and chromosome 5, and extrapolating data to the rest of the karyotype. The
273 incidence of aneuploidy in porcine and bovine cleavage embryos seems to be lower compared
274 to human embryos evaluated by preimplantation genetic screening, where aneuploidy
275 typically affects more than 50% of IVF produced embryos [Hellani et al., 2008; Mantzouratou
276 and Delhanty, 2011; Chavez et al., 2012]. However, it is important to emphasize that the
277 majority of human IVF embryos derives from infertile couples and such a cohort of embryos
278 might, in general, display elevated frequency of chromosomal abnormalities.

279
280 Chromosomal differences in length, centromere position, pericentromeric and other repetitive
281 sequences, recombination patterns and chromatin characteristics might all be related to a
282 differential susceptibility to aneuploidy [Warburton and Kinney, 1996]. In human oocytes and
283 embryos, there is shown tendency that chromosomes equal to or smaller than chromosome 13
284 are involved most frequently in aneuploidy [Fragouli et al., 2010; Gabriel et al., 2011;
285 Alfarawati et al., 2011]. It is important to stress that within the above-mentioned
286 chromosomes frequently found in aneuploidy are involved all human acrocentric
287 chromosomes. Similarly, in porcine embryos, the acrocentric chromosomes were relatively
288 frequently involved in aneuploidy [Hornak et al., 2012]. In the present study of bovine
289 embryos, no striking difference between the size of chromosome was noticed (Table 1),
290 moreover all bovine autosomes are acrocentric. Thus, the observation on the type of
291 chromosomes involved in aneuploidy might indicate that not the smallest, but rather
292 acrocentric chromosomes are more prone to error during meiotic/mitotic divisions. However,
293 from human and animal studies it is well documented that aneuploidy can affect any
294 chromosome [Hassold et al., 2007; Hornak et al., 2012].

295

296 **Mitochondrial content in early bovine embryos**

297 In the present study, single blastomeres derived from 2-cell stage bovine embryos were
298 analysed, thus the ratio between the read count mapped to mtDNA and the total read count
299 reflects relative difference in number of mitochondria in analysed embryos. The majority of
300 DNA sequence reads were derived from the nuclear genome and the mtDNA represented
301 approximately 1.5–2% of all DNA sequence reads. We observed that in a group of late
302 cleaved embryos there was significantly lower numbers of mitochondria compared to the
303 early cleaved group. This might be explained by decreased ATP reserves in embryos coming
304 from oocytes with lower mitochondria counts. Indeed, in a cohort of human oocytes there was
305 observed that oocytes with higher ATP content displayed increased potential for continued
306 embryogenesis and implantation [Blerkom et al., 1995]. However, in our study late embryos
307 displayed significantly higher level of aneuploidies compared to early ones. This observation
308 indicates that the aneuploid 2-cell stage embryos contain less mtDNA compared to euploid
309 ones. As a possible explanation it was shown that ATP levels influence spindle formation and
310 chromosome alignment during meiosis [Zhang et al., 2006; Johnson et al., 2007]. In contrast,
311 comparable study using NGS technology showed that, on average, biopsy specimens derived
312 from aneuploid blastocysts contained significantly greater amounts of mtDNA than samples
313 from embryos that were euploid [Fragouli et al., 2015]. These contradictory findings between
314 present study and the study of Fragouli et al., [2015] very probably relates to different stages
315 of embryos analysed. In the present study the mtDNA reflects copy number of mitochondria
316 coming from oocytes, but in the latter study the trophectoderm biopsies of human blastocysts
317 were analysed. It is known that in the trophectoderm of blastocyst stage embryos the
318 replication of mitochondria is initialized in order to fulfil ATP demands of preimplantation
319 embryos [John et al., 2010]. So the increased mtDNA in trophectoderm of aneuploid embryos
320 might reflect enhanced energy requirements of chromosomally abnormal embryos, which

321 might be caused, for example, by overexpression of DNA repair signalling pathway genes in
322 such embryos [Bazrgar et al., 2014]. However, whether and what relationship between
323 aneuploidy and mtDNA exist cannot be clearly answered at the moment and there are more
324 studies needed to clarify the issue.

325 The role of mitochondria seems to be important also for fertilization outcomes. In porcine
326 oocytes, data suggest that a correlation exists between increased cytoplasmic volume and
327 increased mtDNA content, and that appears to be factors positively influencing fertilization
328 outcomes [Shourbagy et al., 2006; Santos et al., 2006]. However, to confirm this observation
329 was beyond the scope of the research reported here.

330

331 **Declaration of interest**

332 The authors declare that there is no conflict of interest and have nothing to disclose.

333

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338 Agency of the Ministry of Agriculture of the Czech Republic.

339

340 **Figure legends**

341 **Figure 1. Detection of chromosome aneuploidies in single blastomeres derived from**
342 **2-cell stage bovine IVF embryos by Next-generation sequencing-based protocol (NGS)**
343 NGS charts showing copy number of each bovine chromosome. (A) a male euploid bovine
344 embryo blastomere with 2 copies of all autosomes and 1 copy of chromosome X and Y; (B)
345 afemale blastomere with monosomy of chromosome 6; (C) a male blastomere with

346 monosomy of chromosome 4 and partial gain of chromosome 13 (15–84Mb); (D) a female
347 blastomere with monosomy of chromosome 19.

348 **Figure 2. Comparison of mtDNA quantity between single bovine blastomeres derived**
349 **from early and late cleaved embryos by Next-generation sequencing-based protocol**
350 **(NGS)**

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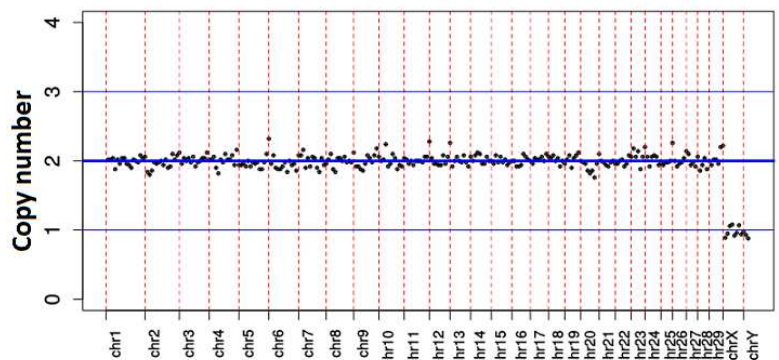
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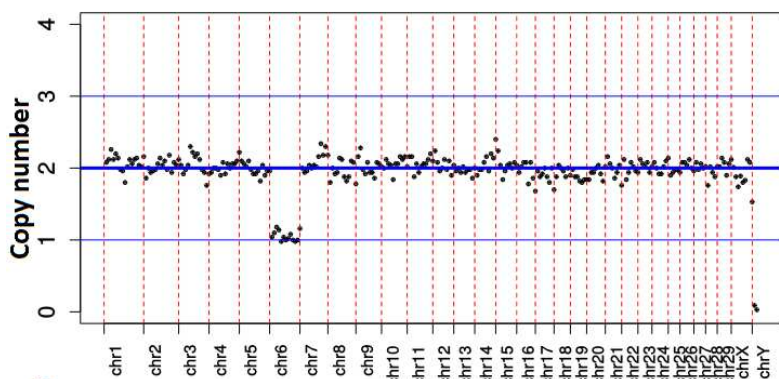
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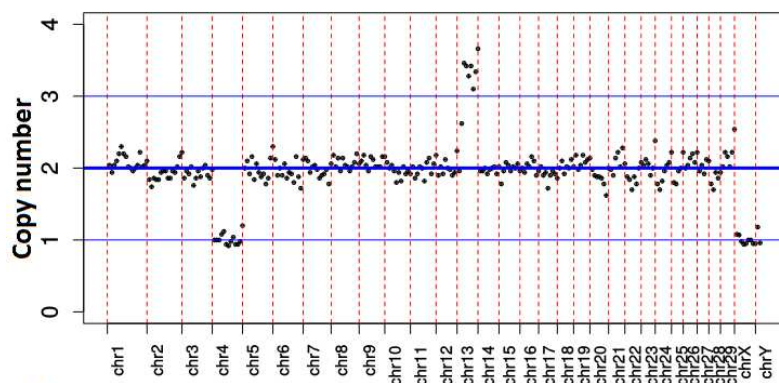
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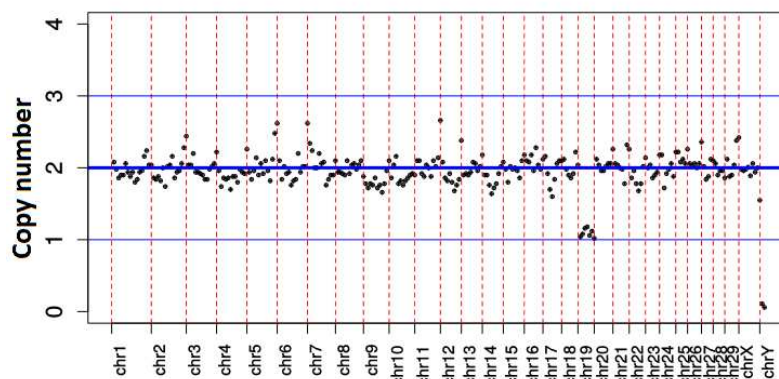
A Chromosomal position



B Chromosomal position



C Chromosomal position



D Chromosomal position

Figure 1



| Group | N | Min | Q1 | Median | Q3 | Max |
|---------------|----|------|-------|--------|------|------|
| Early cleaved | 22 | 1.26 | 1.94 | 2.19 | 2.46 | 2.86 |
| Late cleaved | 9 | 0.83 | 1.285 | 1.54 | 1.83 | 2.11 |

Figure 2

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496 **Table 1. Early embryos developed from bovine oocytes fertilized in vitro (control group)**

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| Oocytes examined n | fertilized/inseminated n (%) | cleaved/inseminated n (%) | Cleaved embryos | |
|--------------------------|---------------------------------|------------------------------|---------------------------|---------------------------|
| | | | early/total n (%) | late/total n (%) |
| 139 | 27/31 (87.1) | 92/108 (85.2) | 75/92 (81.5) ^a | 17/92 (18.5) ^b |

498 Values with different superscripts are statistically different (a-b; P<0.01).

499 Early cleaved embryos: cleavage onset at 26–31 h post insemination.

500 Late cleaved embryos: cleavage onset at 32–36 h post insemination.

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Table 2. Chromosome abnormalities in early bovine embryos developed in vitro

| Embryo cleavage category | Embryos examined n | Aneuploidy n (%) | No. of embryo | Description of aneuploidy | Sex of embryo | |
|--------------------------|-----------------------|------------------------|---------------|-----------------------------------|------------------------|-----------------------|
| | | | | | XY n (%) | XX n (%) |
| early | 22 | 7 (31.8%) ^a | 1. | -20 | 13 (59.1) ^a | 9 (40.9) ^a |
| | | | 2. | -6 | | |
| | | | 3. | +21 | | |
| | | | 4. | -13,+17 | | |
| | | | 5. | +8,-10,-19,+25,+26,-27 | | |
| | | | 6. | -6 | | |
| | | | 7. | -4,+13part.(15-84Mb) | | |
| late | 9 | 7 (77.8%) ^b | 1. | +2part.(65-141Mb) | 6 (66.7) ^a | 3 (33.3) ^a |
| | | | 2. | +23,-24 | | |
| | | | 3. | -6,+11 | | |
| | | | 4. | -14part.(32-81Mb) | | |
| | | | 5. | +20 | | |
| | | | 6. | -5part.(0-22Mb),+5part.(22-125Mb) | | |
| | | | 7. | -19 | | |

Values with different superscripts are statistically different (a-b; $P < 0.05$).

+/- stands for trisomy or monosomy of particular chromosome

part. = gain or loss of chromosome part (length in megabases)

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