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Preimplantation genetic testing for aneuploidy (PGT-A) reveals a high incidence of chromosomal errors in in vivo and in vitro pig embryos

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Chromosome errors in embryos can lead to implantation failure, spontaneous abortions, and birth defects; as such, characterising their incidence is of interest not just in humans but also in domestic animals where embryo production is employed for breeding. Recent studies have found an aneuploidy incidence between 14-24% in cattle embryos; however, information on other domestic species is lacking. Here, we present for the first time a characterisation of chromosome errors in both *in vivo* derived (IVD) and *in vitro* produced (IVP) porcine embryos, using single nucleotide polymorphism based PGT-A. Five sows were inseminated and culled at day 4, 5 or 6 of the oestrous cycle (D0 = onset of oestrous) to collect IVD embryos at different development stages by flushing of the distal portion of the uterine horn. Additionally, IVP blastocysts were produced from 10 sows during three fertilisation rounds. For all embryos, the zona pellucida was removed using 0.5% pronase and the samples were stored at -80 °C. Whole genome amplification was performed by using the REPLI-g Advanced DNA Single Cell Kit (Qiagen, Oslo, Norway), and genotyping was completed on a custom Illumina GeneSeek 25K SNP chip (Lincoln, NE, United States). PGT-A diagnosis were obtained by combining Log R ratio (LRR) and B-allele frequency (BAF) graphs to detect copy number variations, and Karyomapping to trace the parental origin of chromosomal errors (maternal or paternal), and to detect triploidy and uniparental disomy. Proportions between groups were analysed by Fisher's exact test and the threshold for statistical significance was set as $p \leq 0.05$. In IVD embryos, the overall incidence of chromosomal errors was 32% (32/101). Although not significantly different ($p > 0.05$), fewer errors were detected at the blastocyst stage as compared to earlier stages: 40% in 4 cell (10/25), 35% in 6-12 cells and morulae (7/20 and 12/34) and 14% in blastocysts (3/22). Conversely, IVP blastocysts showed an 80% incidence for chromosomal errors (n = 51/64). Even with the low sample size achieved, this provides the indication that IVP blastocysts suffer from a higher incidence of chromosomal errors as compared to IVD blastocysts ($p < 0.001$). Triploidy was the most common chromosomal error in IVD embryos (16%, 16/101), followed by whole chromosome errors (10%, 10/101). Surprisingly, two parthenogenetic embryos and one androgenetic embryo were also identified in the IVD embryos. In IVP blastocysts, parthenogenesis affected one in three embryos (21/64). The parthenogenetic embryos arose from just three sows, across two different IVP rounds, suggesting a possible individual effect. The incidence of triploidy in IVP blastocysts was 25% (16/64), and errors arising from either polyspermy (12/16) or meiotic non-disjunction in the oocyte (4/16) were both detected. Errors with a maternal origin were prevalent in IVP embryos (41/57, $p < 0.01$), whereas IVD embryos presented a similar incidence of errors from either parent (17/36 maternal, $p > 0.05$). In conclusion, PGT-A discovered a high incidence of aneuploidy and triploidy in IVD and IVP embryos, suggesting that the future application of this technology might improve embryo transfer success in the pig.

Keywords: Ploidy, porcine, cytogenetics