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Evaluation of two IVP bovine embryo sexing techniques according to their ability to preserve embryo viability after vitrification/warming

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Trophectoderm (TE) biopsies are frequently used for embryo genotyping, although they are invasive and harmful to further embryonic development. Cell-free DNA (cfDNA) found in blastocoele fluid (BF) can be considered as a non-invasive sexing method. The purpose of this study was to compare the accuracy of a non-invasive method using cfDNA present in BF, to the biopsy procedure in terms of determining the embryo's sex and its effect on embryo survival following vitrification/warming. Expanded Day 7 IVP embryos were randomly assigned to two groups: VIT-Collapsed (n=37), blastocysts artificially collapsed by aspiration of BF with an ICSI pipette; VIT-Biopsied (n=56): blastocysts biopsied by cutting off a small portion of the TE using a microblade. After sample collection, all embryos were vitrified/warmed by the Cryotop method and individually cultured *in vitro*. Intact embryos individually cultured (VIT-Single) (n=58) or cultured in group (VIT-Control) (n=56) after vitrification/warming were used as vitrification controls, whereas intact non-vitrified embryos were used as fresh controls (n=45). The survival of vitrified blastocysts was assessed as re-expansion and hatching rates at 24 h post-warming. Sex identification was performed in BF or biopsies as well as in the corresponding surviving embryos of VIT-Collapsed and VIT-Biopsied groups. BF samples underwent a whole genome amplification using REPLI-g single cell kit (Qiagen, Germantown, MD, USA), whereas biopsies and blastocysts were lysed by incubation with 100 µg/mL proteinase K at 55°C for 2h. Embryo sex was analyzed by PCR using two sets of primers: Y-chromosome specific primer (*BRY4a*) and bovine specific satellite sequence primer (*SAT1*). Products were visualized on a SafeView stained 2% agarose gel. Samples with *BRY4a/SAT* bands were considered male, while samples with only the *SAT1* band were assigned as female. Data were analyzed with a one-way ANOVA (P≤0.05). VIT-Collapsed blastocysts showed similar post-warming survival rates (87.55±16.1%) to those of fresh non-vitrified blastocysts (100%) and significantly higher than blastocysts from the VIT-Single and VIT-Control groups (79.0±9.1% and 72.0±14.3%, respectively). Blastocysts vitrified after biopsy showed the lowest (P≤0.05) survival rate (53.5±12.6%). No differences (P>0.05) between the two sources of DNA were observed either in their amplification efficiency (72.0% (18/25) in BF samples; 79.3% (23/29) biopsies) or in their accuracy in sex diagnosis (83.3% (15/18) in BF samples; 82.6% (19/23) in biopsies). In conclusion, the results of this study indicate that cell-free DNA analysis is an efficient and minimally invasive approach to sex IVP cattle embryos. Moreover, artificial collapse of blastocoel had a positive effect on embryo viability after vitrification/warming. Further studies to improve the efficiency of cell-free DNA collection and amplification are guaranteed.

Keywords: Cell-free DNA, Blastocoele collapse, trophectoderm biopsy

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