Detection of cell-free DNA in embryo culture medium: its potential application as a noninvasive method for sex determination in cattle

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Introduction

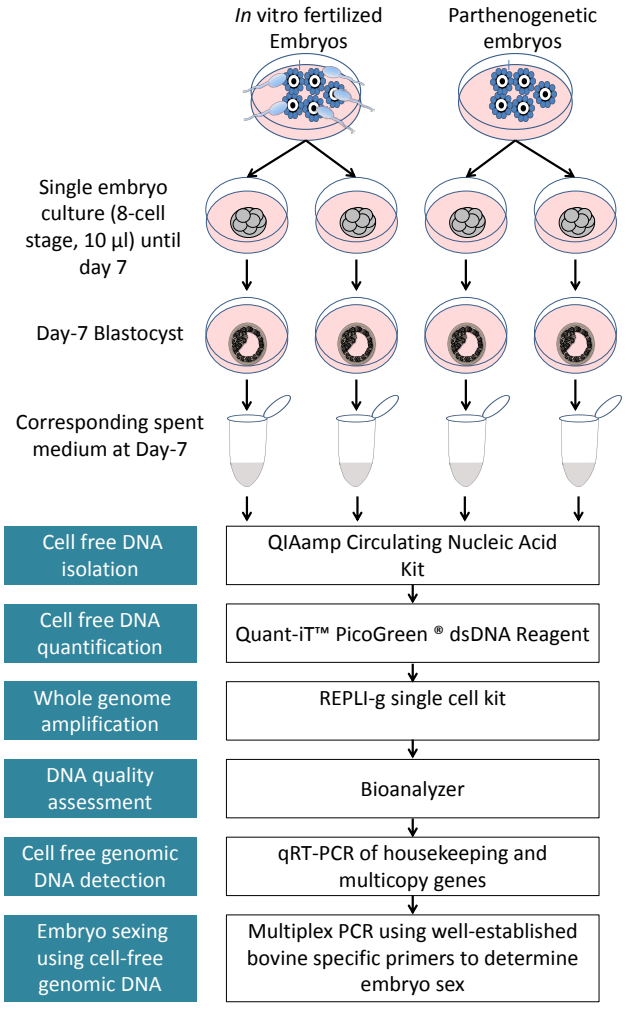
Implementation of embryo sexing allows gender selection according to the owner's desire (females for dairy, males for beef production). The current embryo sexing method relies on an invasive method, which requires embryo biopsy and is reported to affect embryo implantation. Thus, the application of a noninvasive method could be an alternative and would allow the transfer of intact embryos.

Objectives

- To investigate the presence of cell-free DNA molecules released by the embryo to the culture medium
- To develop a noninvasive embryo sexing method using cell-free genomic DNA

Materials and Methods

Superordinated experimental design



Exp 1: Detection of cell-free DNA in spent media

To analyze whether individually cultured bovine embryos release detectable amounts of cell-free DNA to culture, a total of 24 spent media were quantified for the presence of double stranded DNA by Quant-iT™ PicoGreen ® measurement (standard curve method).

Exp 2: Embryo sexing from spent media

To analyze whether the quality of the released cell-free DNA is suitable for embryo sex identification, the corresponding spent media of a total of 15 *in* vitro produced bovine embryos and 6 parthenotes were used for Multiplex PCR after whole genome amplification. In parallel, extraction of DNA from individual blastocyst was performed using blastocyst Lysis buffer

Results

Exp 1: Detection of cell-free DNA in spent media

Table 1: Total cell-free DNAs released by individual embryos to the culture medium

Individual culture medium	Total double stranded DNA (pico gram)
1	146
2	450
3	568
4	634
5	602
6	Not detected
7	224
8	108
9	262
10	732
11	Not detected
12	1086
13	282
14	Not detected
15	684
16	804
17	1432
18	Not detected
19	174
20	1120
21	Not detected
22	648
23	858
24	342

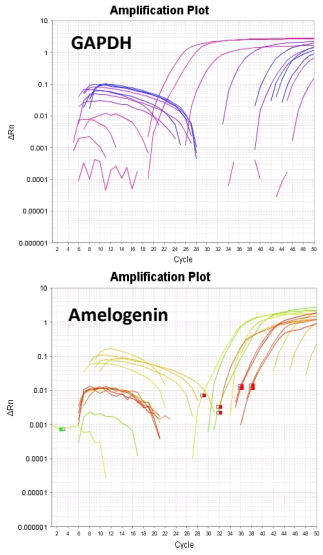


Figure 1: Detection of cell-free genomic DNA using qRT-PCR

80 % of the embryos released cell-free DNA to the spent medium

Housekeeping and multicopy genes were amplified in 52.6 % and 63.2 % of the embryo culture media, respec-tively

Exp 2: Embryo sexing from spent media

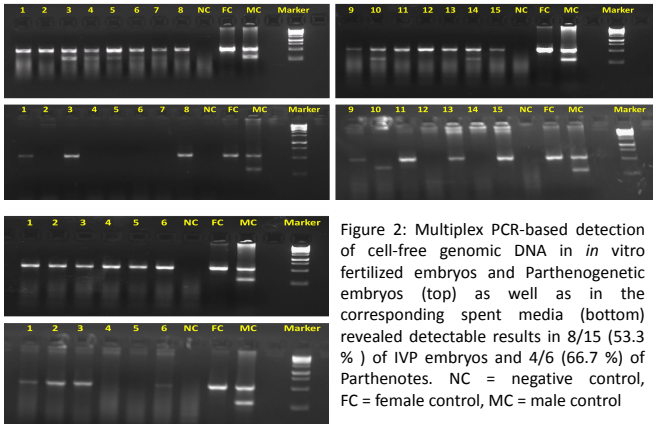


Figure 2: Multiplex PCR-based detection of cell-free genomic DNA in *in* vitro fertilized embryos and Parthenogenetic embryos (top) as well as in the corresponding spent media (bottom) revealed detectable results in 8/15 (53.3 %) of IVP embryos and 4/6 (66.7 %) of Parthenotes. NC = negative control, FC = female control, MC = male control

Accuracy of embryo sexing using spent media analysis

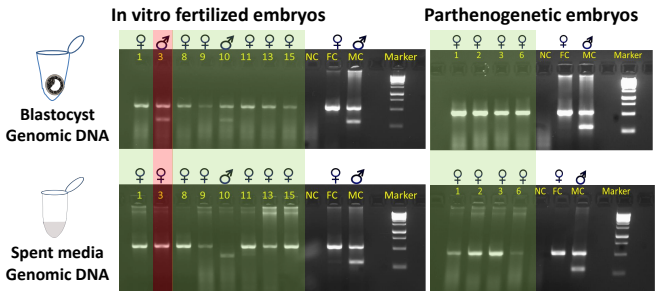


Figure 3: Multiplex PCR-based embryo sexing using blastocyst DNA (top) and the cell-free genomic DNA in the corresponding spent media (bottom) revealed accurate sexing in 7/8 (87.5 %) IVP embryos and 4/4 (100%) Parthenogenetic embryos.

Conclusions

- Most bovine embryos release cell-free DNA to the culture medium, which is enough to be amplified using whole genome amplification
- The cell-free genomic DNA released to the culture medium can potentially be used for embryo sexing prior to embryo transfer
- Further studies should be conducted to improve efficiencies with respect to cell-free DNA isolation and amplification