Noninvasive cell lineage tracing in bovine embryos from 2-cell stage up to blastocyst stage

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NONINVASIVE CELL LINEAGE TRACING IN BOVINE EMBRYOS FROM 2-CELL STAGE UP TO BLASTOCYSTS

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INTRODUCTION

The first lineage specification occurs during preimplantation mammalian development. At blastocyst stage two cell lineages can be distinguished: the inner cell mass (ICM) and the trophectoderm (TE). The exact timing when embryo cells are skewed to these lineages is not clearly determined in mammalian species.

In murine embryos, it has been suggested that the first cleavage plane might be related with the embryonic-abembryonic (Em-Ab) axis at blastocyst stage. So the daughter cells of the 2-cell embryo might be already predisposed to a specific cell lineage further on development.

The objective of the present study was to observe, how the first cleavage in bovine embryos may be related to cell lineage allocation at blastocyst stage, using a non-invasive tracing approach.

MATERIALS AND METHODS

Injection of lipophilic tracer DiI on one blastomere of the 2-cell stage bovine *in vitro* fertilized embryos was performed for cell lineage tracing and embryos were left in culture until blastocyst stage and were then classified according to their cell distribution pattern:

- Orthogonal if the borderline between labelled and non-labelled cells was orthogonal (± 30°) to the Em-Ab axis
- Deviant if the borderline was parallel (± 30°)
- Random if labelled and non-labelled cells were intermingled.

Total cell count (TCC) and the ICM/TE ratio was allowed by DNA staining with DAPI and by immunostaining of the ICM with Sox2 antibody. Analysis of variance was performed by one-way ANOVA employing IBM SPSS v21. P values ≤0.05 were considered as significant. All values are reported as mean ± standard error of mean.

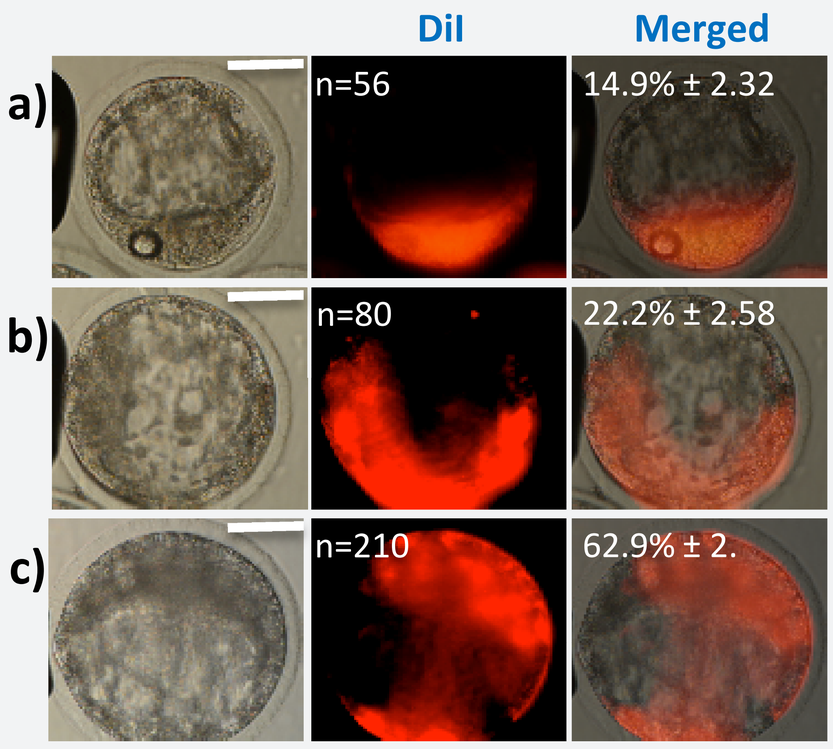

Figure 1. Cell lineage pattern distribution in bovine blastocysts. Where **a)** represents the orthogonal embryos, **b)** deviant and **c)** random. Significant difference was found in the incidence of the random group compared with the orthogonal and deviant. Scale bar 50 µm.

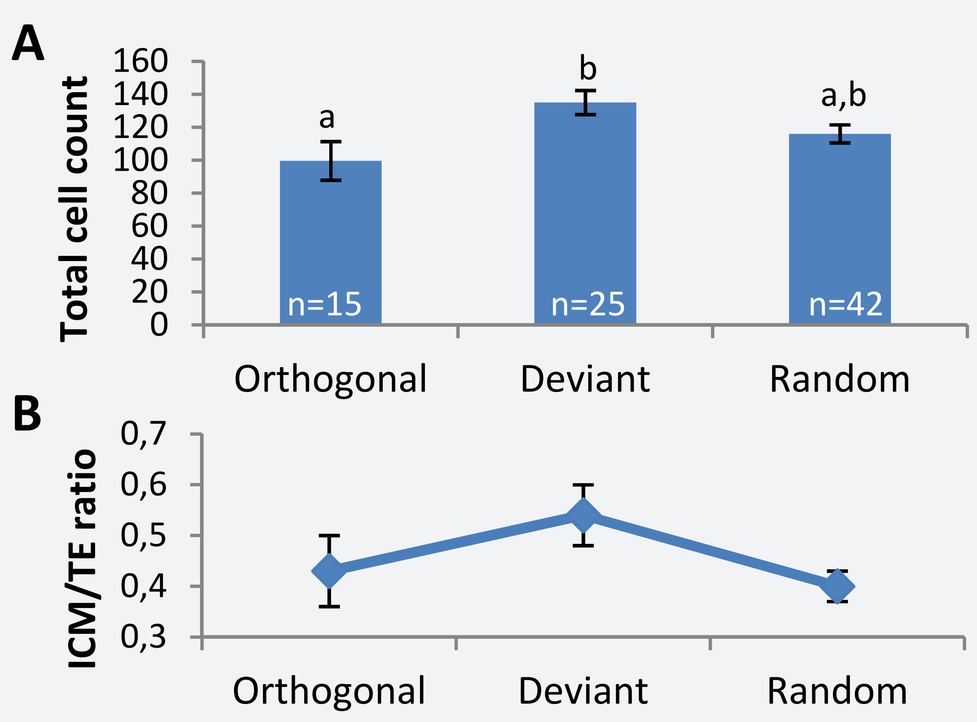

Figure 2. Total cell count and ICM/TE ratio in bovine blastocysts according to cell lineage allocation patterns. A) Total cell count at blastocyst stage. Different letters indicate statistical significant difference. **B)** Inner cell mass/trophectoderm ratio at blastocyst stage.

RESULTS

1. Significant difference was found in the incidence between the random group against the orthogonal and deviant; but not between the last two.
2. Deviant embryos presented a significantly higher number of cells at blastocyst stage compared with the orthogonal group. Nevertheless it was not significantly different than the random group.
3. The ICM/TE ratio was conserved among the groups with no significant differences.

CONCLUSIONS

- Bovine embryos present a marked tendency of a random distribution of the daughter cells derived from the 2-cell blastomeres.
- Around 37% of the bovine blastocysts present a patterned cell division; where the daughter cells remain together through preimplantation development (orthogonal and deviant patterns).
- The impact of cell lineage allocation patterns on implantation and further embryo development needs to be addressed.

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