

Gene expression analysis in specific cell populations of bovine blastocysts using laser capture microdissection

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Introduction:

The comparison of gene expression profiles between specific cell fractions in early embryos may increase our insight into pathways of initial development. As an example, the separate isolation and analysis of inner cell mass cells (ICM) and trophoblast cells (TB) from single blastocyst will provide more insight into the molecular pathways triggering cellular differentiation and cell fate development.

Here, we describe an optimized protocol for the isolation of ICM and TB cells from bovine blastocysts using Laser capture microdissection (LCM). Histological procedures are hampered by the small size of blastocysts ($\approx 100\mu\text{m}$), and initial results revealed that cryosectioning of blastocysts is not feasible.

Consequently, a procedure was optimized for paraffin embedding of blastocysts prior to microdissection.



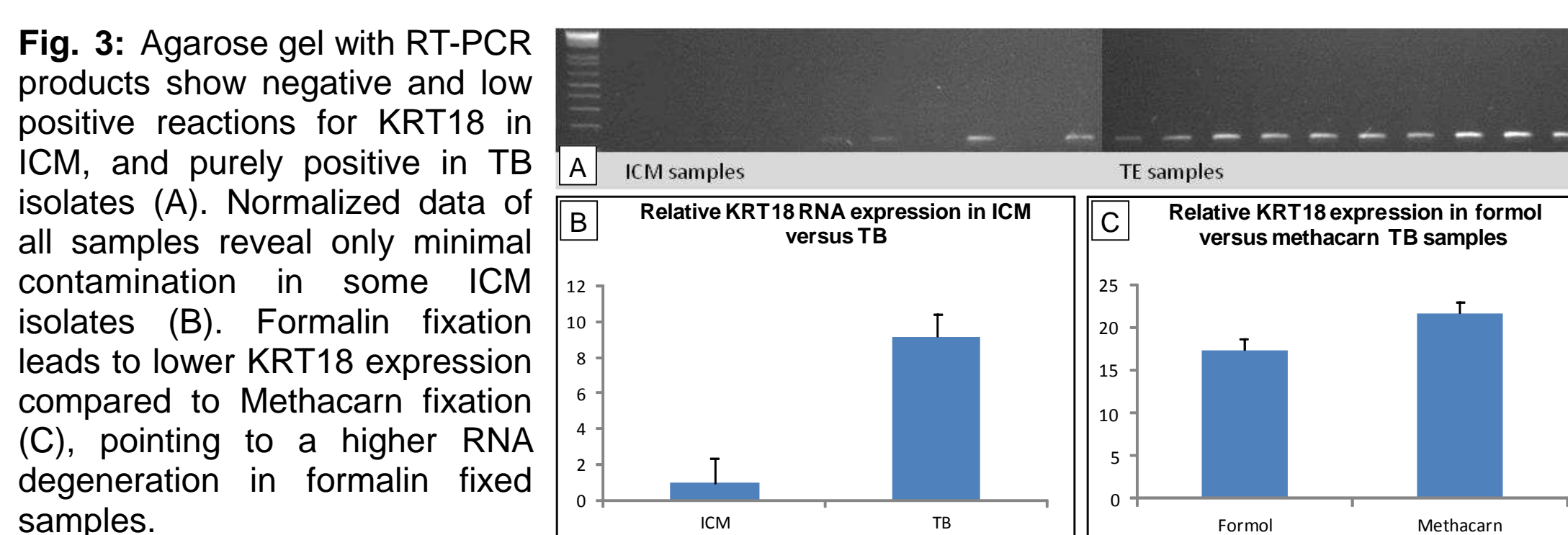
Fig. 1: Micrograph of a section through a blastocyst stained with haematoxylin and eosin after consecutive agarose and paraffin embedding, performed as described below.

Analysis of the quality of the isolates:

To validate this procedure for quantitative analysis of RNA molecules it is important to avoid contamination of TB fractions in ICM isolates and vice versa. In addition, RNA degradation should be kept at a minimum.

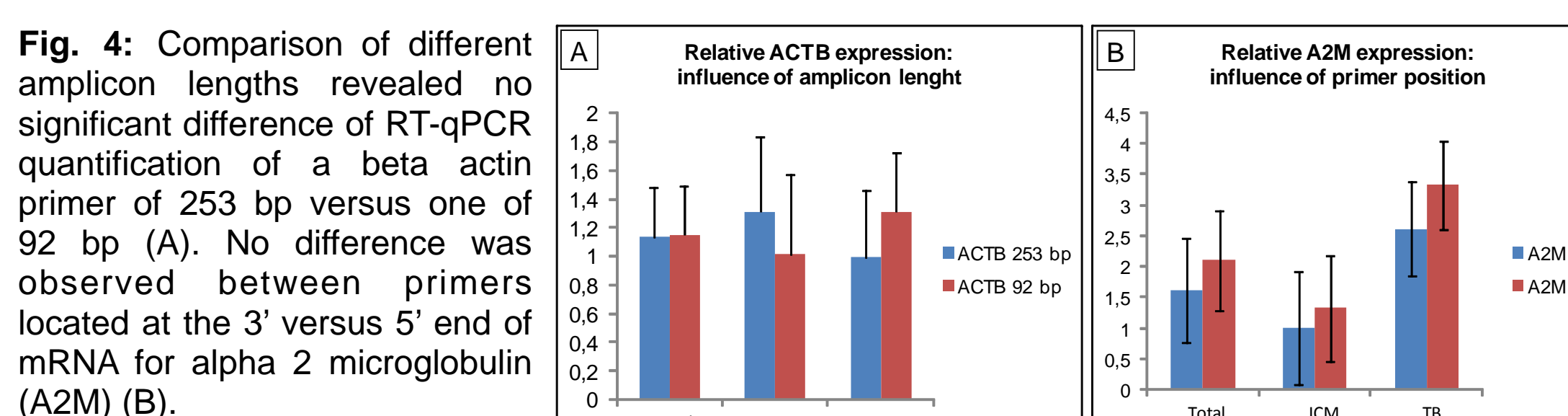
Purity of the isolates

Keratin 18 (KRT18) is specifically expressed in TB cells and was used as a marker for contamination of ICM isolates. Minimal to no expression of KRT18 was detected in ICM isolates, indicating neglectable contamination of ICM isolates (Fig. 3)



RNA quality

Due to the low amount of isolated RNA, the RNA quality could not be assessed with automated electrophoresis. Consequently, two PCR based assays to investigate RNA quality were used (Fig. 4). These revealed minimal influence of RNA quality on RT-qPCR.



Tissue processing and microdissection:

Blastocysts were produced by coincubating bovine cumulus-oocyte-complexes with bovine sperm, followed by in vitro embryo culture. On day 8, blastocysts with an intact zona pelucida were washed in phosphate buffered saline and fixed in modified methacarn (8 parts methanol and 1 part acetic acid).

After 6 hours fixation, the blastocysts were collected and suspended in a 2% agarose solution at $>60^\circ\text{C}$. This step embeds the blastocysts in a mold that allows paraffin embedding and facilitates manual handling.

Serial section of $10\mu\text{m}$ were cut, and stained with 0.1% cresyl violet solution in 85% ethanol, to ease visual recognition of the blastocysts while minimizing RNA decay.

Finally, the ICM and TB fractions of the blastocysts were isolated using laser capture microdissection

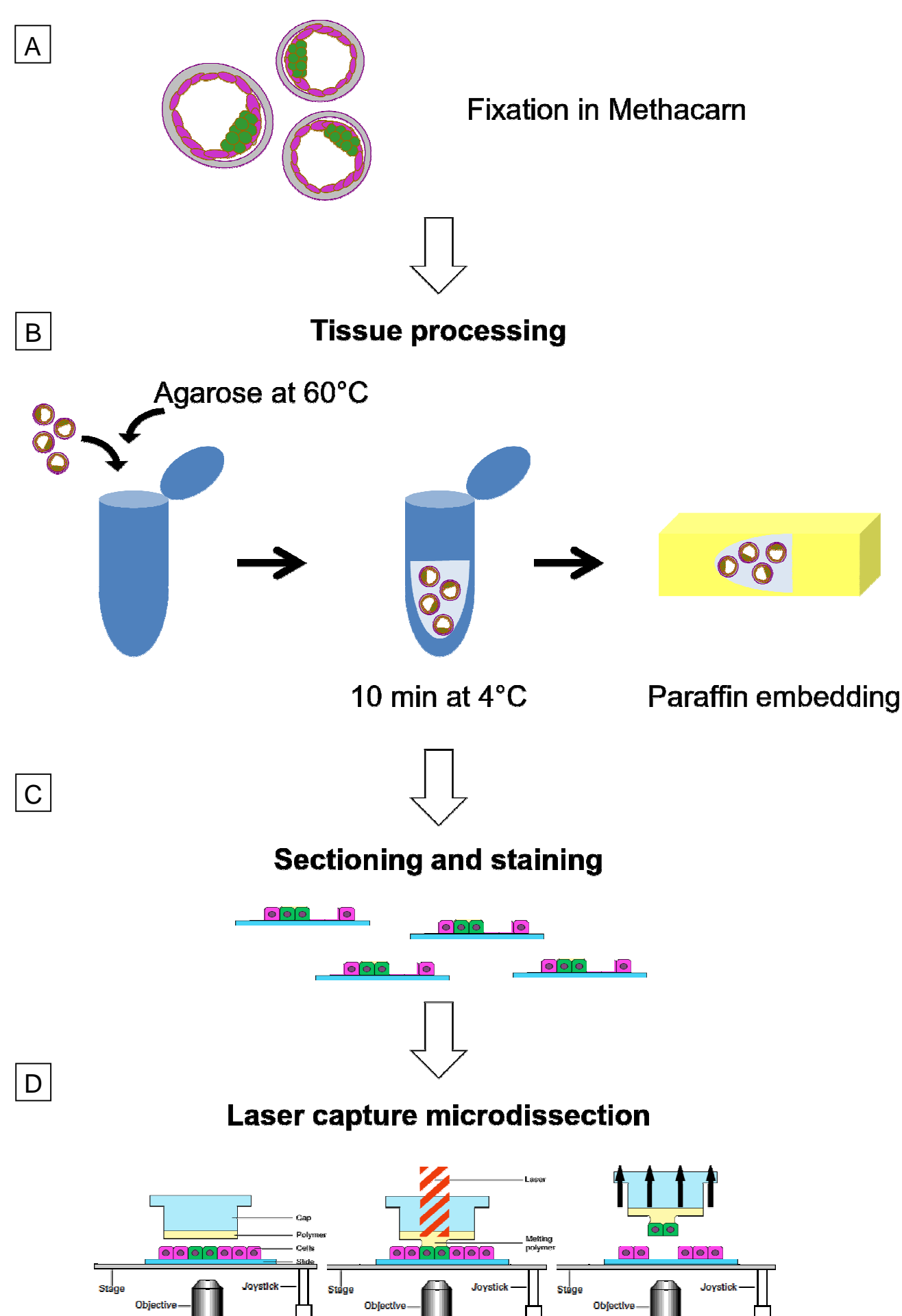


Fig. 2: A-D, Illustration of the fixation (A), processing (B), sectioning (C), and microdissection (D) of blastocysts. E-F, Micrographs of a blastocyst before LCM showing the ICM (white arrow) and the TB cells (black arrows; E), after LCM the ICM is removed (F), and the captured ICM is found on the cap (G).

Applications:

The presented method has been successfully applied with quantitative PCR for the quantification of mRNA and micro RNA (miRNA) (Fig 5). Current work is ongoing to optimize a procedure for deep sequencing to allow a comparison of the whole transcriptome between ICM and TB fractions.

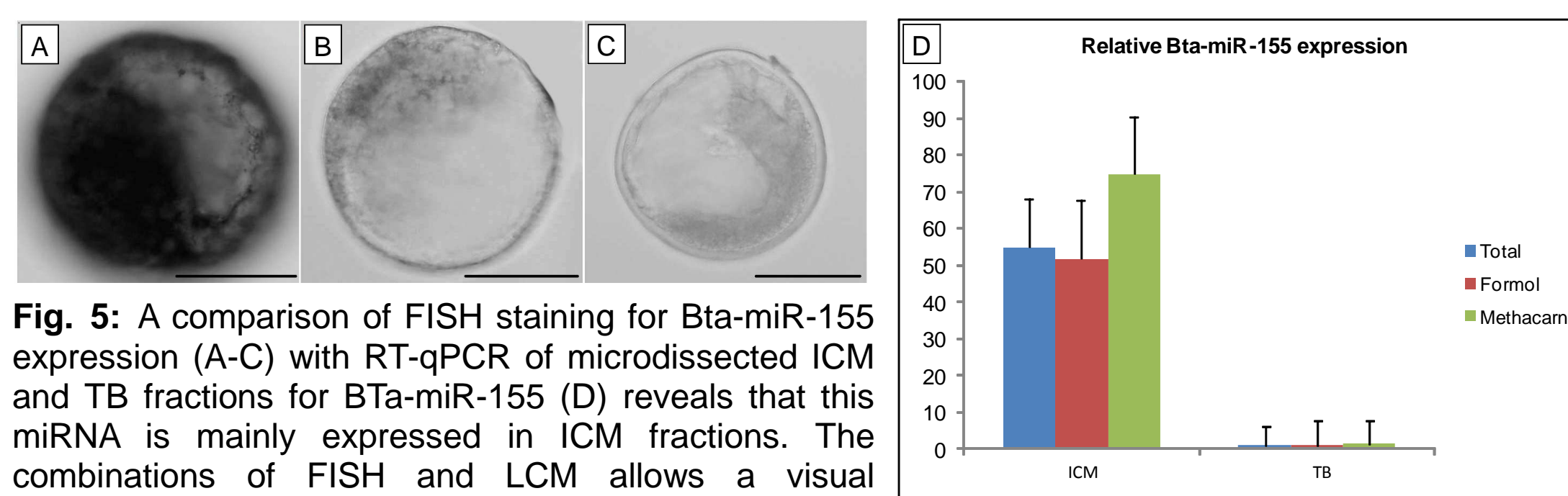


Fig. 5: A comparison of FISH staining for Bta-miR-155 expression (A-C) with RT-qPCR of microdissected ICM and TB fractions for Bta-miR-155 (D) reveals that this miRNA is mainly expressed in ICM fractions. The combinations of FISH and LCM allows a visual evaluation with a quantitative analysis respectively.

Conclusion:

The present method to isolate specific cellular populations from small embryonic tissues provides the possibility to quantitatively assess differential gene expression between the different cell populations.

This tool will help future research to explore the molecular pathways behind pluripotency and stem cell differentiation.

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References:

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