



Relative abundance of mRNA transcripts in *in vitro* produced early embryos of the domestic cat (*Felis catus*) – first results

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BACKGROUND



Since all wild cat species are endangered, assisted reproduction methods (ART) are required to encourage their survival. Production of *in vitro* embryos with a high developmental potency is essential for successful transfer, implantation, pregnancy and birth. The basis for a healthy early development is the accurate temporal and quantitative embryonic gene activation (EGA). To get a first impression of the temporal pattern of EGA in the cat embryo, relative mRNA expression of genes with developmental importance (DNA methyltransferases 1 and 3a, gap junction protein alpha 1, octamer binding transcription factor 4, insulin-like growth factor 1 and 2 receptors) as well as one housekeeping gene (β -Actin) was examined by RT-PCR techniques in different cleavage stages of preimplantative *in vitro* embryos. The domestic cat serves as a model organism for wild felids.

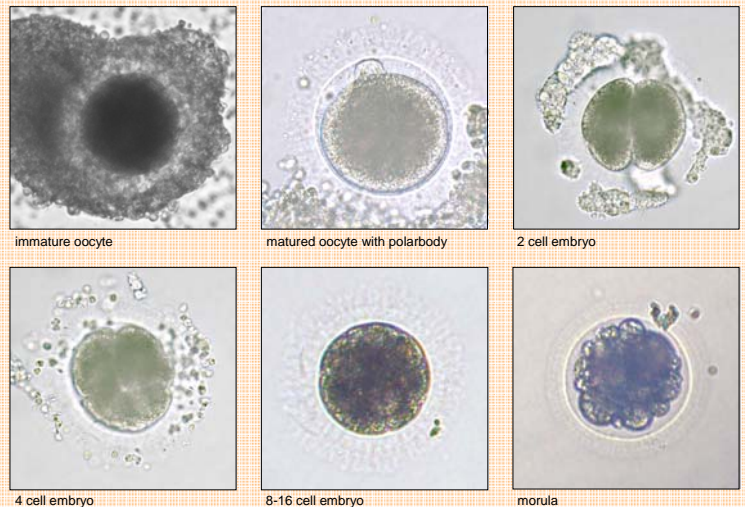
MATERIALS AND METHODS

1. Embryo production

Ovaries and testes from free-ranging as well as house-kept cats were obtained from local veterinary clinics. Oocytes were isolated and matured *in vitro*. Spermatozoa were recovered from epididymis and used for *in vitro* fertilization.

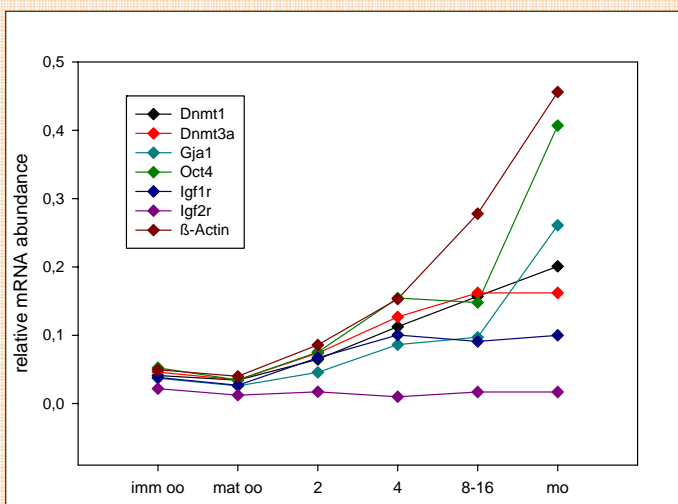
2. Relative mRNA abundance

RNA was isolated from pools of oocytes or embryos* and transformed by reverse transcription into cDNA. Target-specific PCR amplification with primers designed based on alignments of several known non-felid sequences (online databank) followed. Primers were tested in feline ovarian cDNA, products were sequenced. Relative abundance of specific fragments was measured by gel electrophoresis and gel band analysis by means of an internal standard.



* RNA isolation from oocytes and embryos occurred after total removal of surrounding cumulus cells

RESULTS



We examined specific relative mRNA abundances in immature oocytes (imm oo), matured oocytes (mat oo), 2-cell (2), 4-cell (4), 8-16-cell embryos (8-16) as well as morulae (mo).

While relative mRNA amounts decreased during oocyte maturation, we observed an increase during the first cleavage, except for **Igf2r**, what implies that the cat embryo is able to transcribe already in this early stage of development. While relative mRNA abundance increased further until the 4 cell embryo, the pattern of EGA varied from that stage on between the examined genes. Whereas relative mRNA amounts remained constant for **Gja1**, **Oct4** and **Igf1r** until 8-16 cell embryo, it increased steadily for **Dnmt1** and **Dnmt3a** as well as **β -Actin**. On further progress to morula stage, we found a further constant increase of relative mRNA abundance for **Dnmt1**, **Dnmt3a** and **β -Actin** as well as a heavy increase for **Gja1** and **Oct4**, whereas relative **Dnmt3a** and **Igf1r** mRNA abundances stagnated. Relative mRNA amount of **Igf2r** remained unchanged during the analysed period.

Expression patterns differ remarkably between the examined genes even in these early embryonic stages, possibly reflecting the varying needs for specific transcripts.

PERSPECTIVES

The presented results give an impression of temporal relative mRNA expression patterns in preimplantative domestic cat embryos. In future experiments mRNA amounts will be compared in embryos derived by different fertilization methods (IVF vs. ICSI) and sperm sources (fresh vs. frozen-thawed) to evaluate the influence of these artificial reproduction techniques. Quantification is to be made by real time PCR techniques. The examination of *in vitro* embryos is strongly required to determine the target state and to complete the picture of reprogramming in the domestic cat and make it a marker for its developmental potency.