



Transcriptional onset and influences of ICSI and sperm cryopreservation on mRNA expression in early domestic cat embryos

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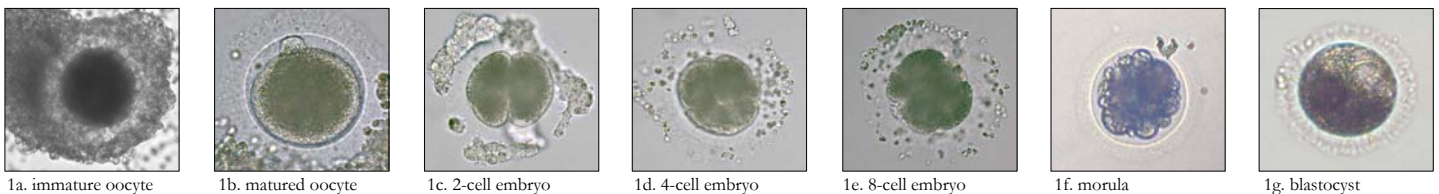
Introduction

Sperm cryopreservation and intracytoplasmic sperm cell injection (ICSI) are widely used techniques in assisted reproduction (ART) for various species. They are also crucial for endangered felid species because they provide tools for long-term storage of sperm for use at an appropriate time as well as assisted fertilization despite low sperm concentration or quality. For embryo transfer as future purpose, production of high quality in vitro embryos is essential. Next to morphology and cleavage timing, mRNA expression patterns are used as markers for embryo quality since early embryonic development is characterized by a well-orchestrated gene expression onset and regulation.

This study aimed to investigate the temporal pattern of embryonic gene activation (EGA) in terms of relative mRNA abundance in early in vitro derived domestic cat embryos. Furthermore, the influence of the fertilization method (in vitro fertilization vs. ICSI) and sperm source (fresh vs. cryopreserved) on relative mRNA expression of developmental important genes was examined.

Materials and Methods

Ovaries and testes were obtained from local veterinary clinics. After recovery, oocytes were matured and fertilized by i.) IVF with fresh sperm, ii.) IVF with cryopreserved sperm, iii.) ICSI with fresh sperm or iv.) ICSI with cryopreserved sperm. Oocytes and embryos were stored in PBS at -80°C.



Total RNA was isolated from pools of immature oocytes (imm oo), matured oocytes (mat oo), and different stage embryos (Fig. 1a-g). Reverse transcription and sequence-specific amplification was performed for DNA methyltransferases 1 and 3a (Dnmt1, Dnmt3a), Gap junction protein alpha 1 (Gja1), Octamer binding transcription factor 4 (Oct4), Insulin-like growth factors 1 and 2 receptors (Igf1r, Igf2r) and β -Actin. Relative mRNA abundance was determined by gel band intensities normalized by an internal standard.

Results

Temporal pattern of embryonic gene activation in vitro

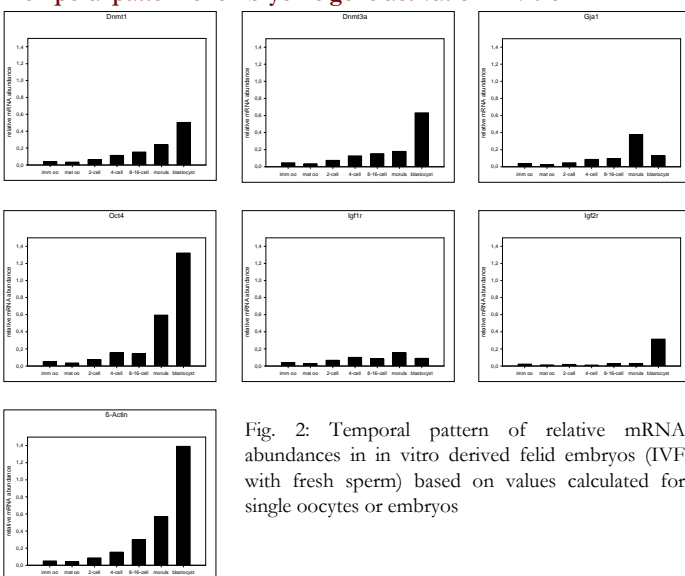


Fig. 2: Temporal pattern of relative mRNA abundances in in vitro derived felid embryos (IVF with fresh sperm) based on values calculated for single oocytes or embryos

Comparison of fertilization methods and sperm sources

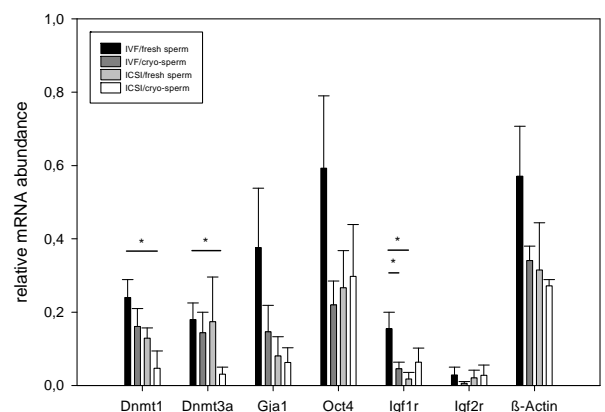


Fig. 3: Relative mRNA abundances in morulae derived by IVF with fresh sperm (IVF_fr, n=6), IVF with cryo-sperm (IVF_cr, n=6), ICSI with fresh sperm (ICSI_fr, n=4) and ICSI with cryo-sperm (ICSI_cr, n=3), values are shown as mean \pm SEM, significant differences (p < 0.05, Mann-Whitney U-test) are indicated by asterisks

Conclusion and Perspectives

In the 2-cell embryo we found elevated levels of relative mRNA abundance compared to matured oocyte, indicating that cat embryos are able to transcribe shortly after fertilization. The varying patterns of gene activation after 4 cell stage may due to the embryo's distinct needs for specific transcripts during different developmental stages. The effects of ICSI and sperm cryopreservation on the relative mRNA abundances observed in our study imply a sperm impact on early embryo gene activation. However, elevation of sample sizes in the ICSI-groups is required.