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Influence of Culture Medium Composition on Relative mRNA Abundances in Domestic Cat Embryos

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Contents

Different culture conditions have been used to produce domestic cat embryos. As part of the *in vitro* procedures, the medium composition significantly affects the quality of the embryo development also. Quality assessments based on cleavage kinetics and blastomere symmetry are useful, but embryos also can differ in their relative gene expression patterns despite similar morphological characteristics. The aim of this study was to compare cat embryos produced with two different *in vitro* culture systems routinely used in two different laboratories [Smithsonian Conservation Biology Institute, Washington D.C., USA (SCBI) and Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany (IZW)]. Specifically, relative mRNA expression patterns of critical genes for pre-implantation embryo development were assessed in both conditions. Embryos were produced in parallel in both culture systems by IVF using frozen-thawed ejaculated semen in the United States and fresh epididymal sperm in Germany. Success of embryo development *in vitro* was recorded as well as relative mRNA abundances [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), beta-actin (ACTB)] in pools of days 4–5 morulae by semi-quantitative RT-PCR assay. Percentages of cleaved embryos were similar ($p > 0.05$) between both culture systems, regardless of the location. OCT4 mRNA abundance was higher ($p < 0.05$) in embryos derived in the SCBI culture system compared with those from the IZW system when epididymal sperm was used for IVF. No clear correlation between the expression pattern and the culture system could be found for all other genes. It is suggested that OCT4 expression might be affected by the media composition in some conditions and can be the indicator of a better embryo quality.

Introduction

The application of assisted reproduction techniques (ART) such as *in vitro* fertilization and embryo transfer are promising tools for the propagation and sustainability of endangered felid populations. An optimal *in vitro* system producing high-quality embryos from *in vitro* oocyte maturation (IVM), fertilization (IVF) and embryo culture (IVC) is an essential prerequisite to the successful application of embryo transfer. However, proper culture systems still remain to be identified through molecular methods previously developed in other species for the evaluation of embryonic quality (Wrenzycki 2007) and also used for the cat in recent publications (Gomez et al. 2008; Imsoonthornruksa et al. 2010). The domestic cat serves not only as a model species for wild felids; furthermore, it is an excellent biomedical model to study the general impact of *in vitro* culture conditions with implications also for human-assisted reproduction (Comizzoli et al. 2010).

Culture system efficiency usually is measured by the developmental competence and morphological criteria of the resulting embryos. Common metrics are the proportions of mature oocytes after IVM, as well as cleaved embryos after IVF, cleavage timing, blastocyst percentages and blastomere numbers. In the domestic cat, it is possible to mature oocytes *in vitro* and obtain 60–85% of oocytes at the metaphase II stage (Spindler and Wildt 1999; Comizzoli et al. 2006; Waurich et al. 2010). Percentage of cleaved embryos after IVM and IVF with fresh epididymal sperm has been reported to be between 41% and 63% (Lengwinat and Blottner 1994; Freistedt et al. 2001; Karja et al. 2002; Ringleb et al. 2004) and between 48% and 52% after IVF with ejaculated sperm (Spindler and Wildt 1999; Comizzoli et al. 2003). Interestingly, despite enormous improvements of *in vitro* embryo production methods, the developmental competence of *in vivo*-derived embryos remains higher than for *in vitro* embryos (Roth et al. 1994).

Although the usefulness of the quality criteria mentioned above is undeniable, more sensitive methods are needed. Expression of critical genes involved in embryo development has been used as a marker of embryo quality in several studies, mainly in cattle (Knijn et al. 2002; Lonergan et al. 2003; Corcoran et al. 2006; Wrenzycki et al. 2006). In cats, the expression pattern of genes involved in epigenetic reprogramming mechanisms were different between IVF-derived and cloned embryos (Imsoonthornruksa et al. 2010). OCT4 (POU5F1) expression was affected in cloned embryos by previous cryopreservation of the donor fibroblast cells (Gomez et al. 2008). Furthermore, OCT4 mRNA levels were found to be significantly increased in *in vivo* blastocysts compared with *in vitro*-derived blastocysts (Filliers et al. 2012). In bovine embryos, gene expression alterations have been found between different media (Wrenzycki et al. 1999; Rizos et al. 2002; Warzych et al. 2007b). Differences in transcript abundances can be detected in embryos even after comparable cleavage proportions and blastocyst yields (Warzych et al. 2007a,b). However, although various culture systems are used to produce cat embryos (Spindler and Wildt 1999; Freistedt et al. 2001; Karja et al. 2002; Gomez et al. 2003; Waurich et al. 2010), the influence of those different compositions on gene expression pattern has not been evaluated yet.

The aim of this study was to compare the mRNA expression in embryos produced in two different culture

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systems routinely used for a long time at the Smithsonian Conservation Biology Institute (SCBI), Washington, DC, USA (Spindler and Wildt 1999; Comizzoli et al. 2003) or at Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany (Freistedt et al. 2001; Ringleb et al. 2004, 2011; Waurich et al. 2010). As previously published, both culture systems were suitable for cat embryo development because the proportion of blastocysts relative to the total number of cleaved embryos was up to 43% in United States (Comizzoli et al. 2003, 2006) and approximately 25% in Germany (Waurich et al. 2010). Expression pattern of critical genes involved in several essential processes of the early embryo [DNA methylation: DNA methyltransferases (DNMT1 and DNMT3A); compaction and cell-to-cell communication: gap junction protein alpha 1 (GJA1); transcription regulation and maintenance of pluripotency: octamer-binding transcription factor 4 (OCT4); metabolism in terms of growth factor reception: insulin-like growth factor receptors (IGF1R and IGF2R); cell structure formation: beta-actin (ACTB)] were examined in embryos produced in parallel in both culture systems.

Table 1. Differences between culture media formulated at the Smithsonian Conservation Biology Institute, Washington D.C., USA (SCBI) and Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany (IZW)

	SCBI culture system	IZW culture system
IVM	MEM Eagle based	M199 based
	–	5.7 mM HEPES
	0.4% BSA (w/v)	0.3% BSA (w/v)
	1 mM pyruvate	2.2 mM pyruvate
	0.02 mg/ml L-cysteine	0.1 mg/ml L-cysteine
	0.1 mg/ml streptomycin	0.055 mg/ml gentamicin
	100 IU/ml penicillin	–
	1.64 IU/ml ovine FSH	0.02 IU/ml porcine FSH
	1.06 IU/ml ovine LH	0.05 IU/ml human LH
	(both NHPP, Rockville, MD)	(both Sigma-Aldrich)
	1 µg/ml 17ß-estradiol	–
IVF	Ham's F10 based	Tyrode based
	–	5 mM HEPES
	5% FBS (v/v)	0.6% BSA (w/v)
	–	10 µg/ml heparin
	1 mM L-glutamine	–
	0.1 mg/ml streptomycin	–
	100 IU/ml penicillin	–
IVC	Ham's F10 based	M16 based
	5% FBS (v/v)	10% FBS (v/v)
	1 mM L-glutamine	–
	–	1x MEM non-essential amino acids
	0.1 mg/ml streptomycin	0.03 mg/ml gentamicin
	100 IU/ml penicillin	–

Materials and Methods

In vitro production of cat embryos

All experiments involved in this study were performed according to the ethical guidelines of both institutes (Smithsonian Directive 607 related to Responsible Conduct of Research; ethics committee for animal welfare, approval number: 2008-11-01).

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Sigma-Aldrich (Taufkirchen, Germany) unless stated otherwise and were of the highest purity available.

Domestic cat embryos were produced in two different laboratories (United States and Germany) and in two different media systems designated as SCBI culture system (previously described by Spindler and Wildt 1999) and IZW culture system (previously reported by Freistedt et al. 2001, with modifications) and described in the following. Table 1 summarizes the differences between the two *in vitro* systems regarding their *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and embryo culture media.

Gonads were obtained from adult cats ovariectomized or castrated by local veterinary clinics (United States) or at animal shelter (Germany) and shipped at 4°C to the laboratory. Ovaries were collected from healthy adult females that were not pregnant. Ovaries were either in inactive, intermediate or follicular stage, but ovaries with large CL were discarded. Ovaries were kept in PBS (United States) or modified MEM HEPES modification (Germany) as described previously (Spindler and Wildt 1999; Waurich et al. 2010). In both culture systems, mature oocytes were fertilized with frozen-thawed electro-ejaculated sperm of normospermic tomcats in the United States and with epididymal fresh sperm in Germany. Straws with ejaculated semen (electro-ejaculated as described in Howard et al. (1990)) were thawed for 10 s at room temperature followed by 30 s at 38°C. Epididymal sperm cells were isolated by mincing the spermatic duct and the cauda epididymis. The final sperm concentration in the fertilization drops was 2×10^5 motile sperm ml and 1×10^6 motile spermatozoa ml for fresh epididymal spermatozoa and frozen-thawed ejaculated spermatozoa, respectively. Co-incubation of oocytes and spermatozoa occurred for approximately 18 h in both culture systems. The progress of embryonic development was monitored daily, and normal speed embryos at morula stage were collected for RNA isolation at days 4 and 5. Before that, all residual cumulus cells were removed by aspirating each embryo into a narrow pipette. All embryos were washed at least three times in droplets of PBS and then frozen in 10–20 µl of RNA later (Applied Biosystems/Ambion, Austin, TX, USA; Qiagen, Hilden, Germany, respectively) at −80°C.

SCBI culture system

After slicing the ovaries, grade I cumulus oocyte complexes (COCs) were washed in modified H-MEM (MEM Eagle containing Hank's salts, supplemented with 0.4% BSA (w/v), 0.02 mg/ml L-cysteine, 25 mM HEPES, 1 mM sodium pryruvate, 1 mM L-glutamine, 0.1 mg/ml streptomycin and 100 IU/ml penicillin). For IVM, oocytes were placed into 400 µl of supplemented MEM Eagle's (MEM Eagle containing Earle's salts, supplemented with 0.4 % BSA, 0.02 mg/ml L-cysteine, 1 mM sodium pryruvate, 1 mM L-glutamine, 0.1 mg/ml streptomycin and 100 IU/ml penicillin, 1.64 IU/ml FSH, 1.06 IU/ml LH and 1 µg/ml 17ß-estradiol). Maturation proceeded at 38.5°C and 5% CO_2 in a humidified air atmosphere. For IVF, motile sperm were

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selected by swim-up processing for 30 min in complete HEPES-Ham's (Modified Ham's F10 Basal Medium-HEPES supplemented with 5% fetal bovine serum (FBS, v/v) (Irvine Scientific, Santa Ana, CA, USA), 1 mM sodium pyruvate, 1 mM L-glutamine, 0.1 mg/ml streptomycin and 100 IU/ml penicillin). IVF was performed after 24–26 h of IVM in 50 μl drops of complete Ham's without HEPES (Modified Ham'S F10 Basal Medium supplemented with 5% FBS (v/v), 1 mM sodium pryruvate, 1 mM L-glutamine, 0.1 mg/ml streptomycin and 100 IU/ml penicillin). Embryo culture was performed at 38.5°C in air with 5% CO_2 in complete Ham's without HEPES.

IZW culture system

Washing of obtained COCs occurred in TCM199 (Medium 199 containing Earle's salts, supplemented with 0.3% BSA (w/v), 0.01% L-cysteine (w/v), 5.7 mM HEPES, 2.2 mM sodium pyruvate, 1 mM L-glutamine and 0.055 mg/ml gentamicin). For IVM, oocytes were placed into TCM199 supplemented with 0.02 IU/ml FSH and 0.05 IU/ml LH under 400 μl mineral oil. Maturation proceeded at 38.5°C and 5% CO_2 in a humidified air atmosphere.

Semen preparation and swim up for 30 min for following IVF occurred in supplemented Tyrode's salts (with 0.6% BSA (w/v), 5 mM HEPES, 1 mM L-glutamine and 0.1 mM sodium pyruvate). IVF proceeded in supplemented Tyrode's salts with 10 μg/ml heparin at 38.5°C and 5% CO_2 in an air atmosphere. After 18 h of sperm-oocyte co-incubation, presumptive embryos were cultured in supplemented M16 (with 0.03 mg/ml gentamycin and 0.1 mM MEM non-essential amino acids), to which 10% FBS (v/v) was added 48 h after IVF.

With small modifications, the same chemicals were used for media preparations in both laboratories. Exceptions were penicillin (USA: penicillin G potassium salt, P4687; Germany: penicillin G potassium salt, P7794), HEPES (USA: HEPES buffer solution, Gibco 15630; Germany: HEPES solution, H0887), L-cysteine (USA: L-cysteine hydrochloride, C1276; Germany: L-cysteine, C7352), serum (USA: FBS, 3000A, Irvine Scientific, Santa Ana, CA, USA; Germany: FBS, F7524) and 17ß-estradiol (USA: 17ß-estradiol, E4389; Germany: 17ß-estradiol, E2257). Furthermore, in Germany, a concentration of 0.05 mg/ml streptomycin was used compared with 0.1 mg/ml in the United States for the preparation of the SCBI culture medium.

Determination of relative mRNA abundance by RT-PCR

RNA isolation, cDNA preparation and gene expression analysis occurred in the IZW laboratory in Germany. For that, embryos produced in the United States were shipped to Germany (stored in RNA later). Relative mRNA abundances of the genes of interest were determined as published previously (Waurich et al. 2010). Total RNA was isolated from each pool (2 morulae/pool, at least nine replicates per group) of embryos with RNeasy Micro Kit (Qiagen) after the addition of 1 pg rabbit globin RNA (non-felid standard RNA) to each sample. Reverse transcription into cDNA was carried out with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas GmbH, St. Leon-Rot, Germany) and Oligo(dT) primers in a total volume of 40 μl.

Amplification occurred in a G-Storm GS1 Thermocycler (Gene Technologies Ltd., Essex, England) in a total volume of 25 μl using the Fast Start Taq Polymerase dNTP Pack (Roche Diagnostics GmbH, Mannheim, Germany). The reactions occurred in 1 × PCR buffer, 200 μM dNTPs, 0.8 μM forward and reverse primer each and 1 U Taq polymerase. The PCR programme consisted of an initial denaturation step of 6 min at 95°C, 40 cycles of 30 s denaturation at 95°C, 30 s annealing at different temperatures and 30 s elongation at 72°C. After a terminal elongation step of 7 min at 72°C, PCR products were stored at −20°C. The sequences of specific primers as well as annealing temperatures and fragment lengths were described previously (Waurich et al. 2010) and are shown in Table 2.

Amplified specific PCR products were analysed genewise on a 1.2% agarose gel (Peqlab Biotechnology GmbH, Erlangen, Germany) in 1 × TBE (90 mM Tris base, 90 mM boric acid, 2 mM EDTA) containing 0.25 × Gelred Nucleic Acid Stain (Biotrend Chemikalien GmbH, Köln, Germany). The relative mRNA amounts were calculated by dividing the specific fragment gel band intensity of each template by the intensity of the corresponding globin band. For signal intensity

Table 2. Primer sequences, annealing temperatures and length of each amplified fragment (Waurich et al. 2010)

	Primer sequence	Annealing temperature	Fragment length
DNMT1	Fw: GCG GCT CAA AGA TTT GGA AAG Rev: GGA TTT GAC TTT AGC CAG GTA	54.6°C	175 bp
DNMT3a	Fw: GAT CAT GTA CGT CGG GGA C Rev: GCT GGT CTT TGC CCT GCT T	56.2°C	502 bp
GJA1	Fw: CCA TCT TCA TCA TCT TCA TGC Rev: CTT GTA CCC AGG AGG AGA CA	56.2°C	242 bp
OCT4	Fw: GAA CAT GTG TAA GCT GCG GC Rev: CTT GAT CGT TTG CCC TTC TG	56.2°C	282 bp
IGF1r	Fw: CAG AGT GGA TAA CAA GGA GAG Rev: CAT TAG AAT CAG TCC ATT GGG	56.2°C	255 bp
IGF2r	Fw: GTC GTG GAG GAA ACT GGT AC Rev: CAC CAT GGG ACC CGT CTT TG	56.2°C	212 bp
ACTB	Fw: CTG GTA TTG TCA TGG ACT CTG Rev: CTC CAG GGA GGA CGA GGA C	59.8°C	266 bp
Globin	Fw: GCA GCC ACG GTG GCG AGT AT Rev: GTG GGA CAG GAG CTT GAA AT	59.8°C	257 bp

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measurement, ImageJ (National Institutes of Health, http://rsbweb.nih.gov/ij/) was used.

Experimental design

The aim of this study was to examine the influence of the culture system on relative mRNA abundances in early cat embryos. For that, embryos were produced in the two IVF systems in two different parallel approaches:

1 Morulae were generated by IVF with frozen-thawed ejaculated sperm in the United States (4 IVF treatments, SCBI system: n = 26 morulae, IZW system: n = 18).
2 Morulae were generated by IVF with fresh epididymal sperm in Germany (6 IVF treatments, SCBI system: n = 18 morulae, IZW system: n = 18).

Cleavage success and proportions of obtained morulae were recorded, and RNA was extracted from pools of two morulae each.

Statistical analysis

Cleavage success and morula outcome in the two different culture systems (SCBI and IZW system) were compared by Mann–Whitney test. Independent samples *t*-test was used to evaluate the influence of the culture system on relative mRNA expression. Both tests were performed by PSAW Statistics 18.0 (SPSS, Inc., IBM Corporation, Armonk, NY, USA).

Results

Influence of the culture system on the proportion of cleaved embryos

On average, 3.2 ± 0.3 (2.0–5.6) and 3.7 ± 0.3 (2.0–6.0) immature oocytes per ovary were recovered in the United States and in Germany, respectively. Percentages of cleavage after IVF did not differ ($p > 0.05$) between the two culture systems regardless of the location (Table 3). IVF with frozen-thawed ejaculated sperm in both culture systems in the United States, resulted in 41.8% ± 7.8% (30–64%) in the SCBI system and 47.8% ± 8.6% (36–73%) cleaved oocytes in the IZW system. 58.9% ± 14.0% and 52.9% ± 13.9% of the cleaved oocytes developed into morulae in the SCBI and IZW system, respectively. In Germany, where both systems were compared using fresh epididymal sperm for IVF, 58.5% ± 4.6% (43.5–70%) and 53.9% ± 6.3% (31.3–77.8%) of the oocytes cleaved in the SCBI culture system and in the IZW culture system, respectively. Proportions of obtained morulae were 36.6% ± 16.4% for the SCBI system and 48.1% ± 12.9% for the IZW system.

Table 3. Proportions of cleaved oocytes and morulae in the two culture systems depending on embryo production method, sperm source and location; values as mean ± SEM

Location	Sperm source	Culture system	Total number of oocytes	Cleaved oocytes/total oocytes	Morulae/cleaved oocytes
USA	Ejaculated,	SCBI	130	41.8 ± 7.8	58.9 ± 14.0
	frozen-thawed	IZW	178	47.8 ± 8.6	52.9 ± 13.9
Germany	Epididymal,	SCBI	82	58.5 ± 4.6	36.6 ± 16.4
	fresh	IZW	78	53.9 ± 6.3	48.1 ± 12.9

Influence of the media on the relative mRNA abundances

Regarding embryos produced by IVF with frozen-thawed ejaculated sperm in the United States, there were no significant differences ($p > 0.05$) between the SCBI and the IZW system. Relative abundances of DNMT1-, GJA1- and ACTB-mRNA were similar in both groups. By trend, higher OCT4- as well as lower DNMT3A-, IGF1R- and IGF2R-mRNA abundances were detected in embryos derived from the SCBI system compared with the IZW system, but variation within groups was too high for any significant effect (Fig. 1).

In embryos produced by IVF with fresh epididymal sperm in Germany, a significant difference regarding the OCT4 expression level between the two culture systems could be verified (Fig. 2). Embryos produced in the SCBI culture system had significant higher OCT4 mRNA abundances than morulae from the IZW system (independent samples *t*-test: $t = 2.249$, $df = 16$, $p = 0.039$). DNMT1- and GJA1-levels were similar in both systems. For all other genes (DNMT3A, IGF1R, IGF2R, ACTB) higher relative mRNA abundances were found in morulae from the SCBI system compared with those from the IZW system (IGF1R: $p = 0.066$, ACTB: $p = 0.079$), but because of high variation within the groups, these differences were not significant.

Discussion

This study is the first in which relative mRNA abundances of potential marker genes, in addition to common criteria, were used to compare two different culture systems [Smithsonian Conservation Biology Institute (SCBI), Washington DC, USA, and Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany] routinely used for a long time and proven to

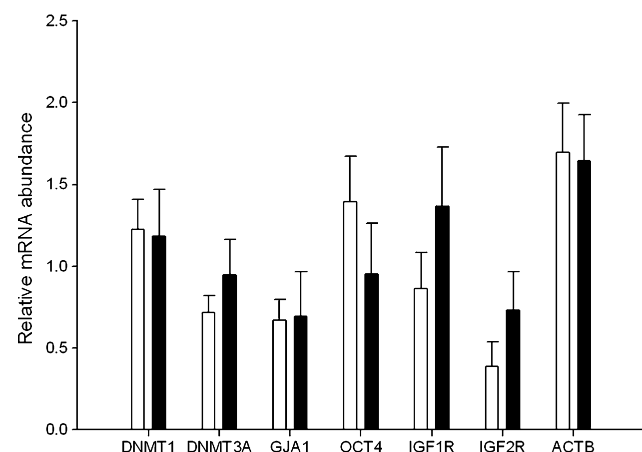

Fig. 1. Relative mRNA abundances in days 4–5 morulae derived by IVF with frozen-thawed ejaculated sperm in the United States in the two different culture systems, white bars: SCBI culture system, black bars: IZW culture system; values are shown as mean ± SEM

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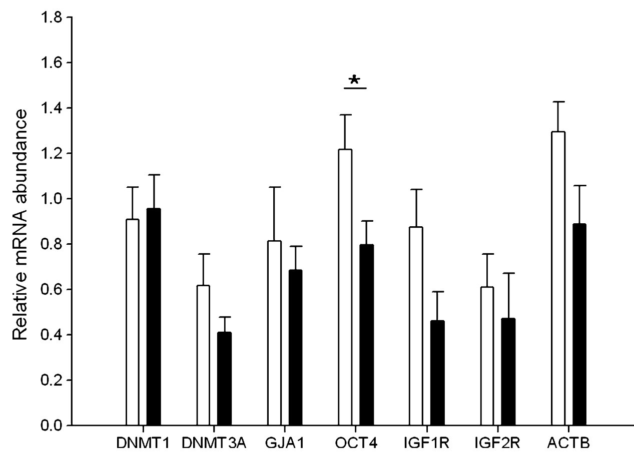

Fig. 2. Relative mRNA abundances in days 4–5 morulae derived by IVF with fresh epididymal sperm in Germany in the two different culture systems, white bars: SCBI culture system, black bars: IZW system; values are shown as mean ± SEM, and asterisks indicate significant differences with $p < 0.05$ (independent samples *t*-test)

be able to produce high-quality cat blastocysts. The comparison occurred in two different experiments: by comparing relative mRNA abundances in morulae derived by 1. IVF with frozen-thawed ejaculated sperm in the United States and 2. IVF with fresh epididymal sperm in Germany.

As discussed previously (Waurich et al. 2010), the morula stage plays a key role in cats. Under suboptimal culture conditions, cat embryos arrest in the morula stage and fail to develop further into blastocysts (Swanson et al. 1996). The examination of the temporal pattern of embryonic gene expression (Waurich et al. 2010) also demonstrated that in the morula stage either the expression is intensified (for GJA1, OCT4) in comparison with the previous stages or expression habits tend to change in the morula or shortly after on the way to blastocyst stage (GJA1, DNMT3A, IGF2R). Furthermore, the expression of most investigated genes related to pluripotency and differentiation, among others OCT4, increased markedly at the morula stage (Filliers et al. 2012), demonstrating the important role of the morula stage for embryonic genome activation in the domestic cat. It seems that during the morula stage the decision about the fate of a cat embryo is made. For those reasons, the morula stage was chosen as the most informative stage for the examination of potential influencing factors.

We expected that if the two *in vitro* systems would differ regarding their potential to produce high-quality embryos, this difference would be characterized by diverging cleavage and developmental success. Furthermore, because media composition and even slight alterations in media supplementation have an impact on gene expression in bovine embryos (Ho et al. 1995; Wrenzycki et al. 1999; Rizos et al. 2003; Warzych et al. 2007b), different quality of culture systems should be also reflected in different mRNA abundances in the cat.

Results revealed no significant differences in proportions of cleaved oocytes as well as morula outcome between the two examined culture systems, neither in the experiment in the United States nor in Germany. This shows that despite the tremendous differences in media composition both systems are comparable effective in cat embryo production.

One gene, OCT4, was found to be significantly different expressed between the SCBI and the IZW culture system in embryos produced by fertilization with fresh epididymal semen in Germany. Also, OCT4 mRNA abundances tended to be higher in embryos produced in the SCBI culture system compared with those from the IZW system after IVF with ejaculated sperm. However, those differences were not significant. Among others, aberrant expression of OCT4 is supposed to be responsible for the suppressed developmental potency of cloned embryos (Boiani et al. 2002; Bortvin et al. 2003). Both increased and repressed expression cause differentiation in embryonic stem cells, and thus, an accurate expression level is required to sustain pluripotency (Niwa et al. 2000). OCT4 plays a role in transcription regulation of genes that are important in early embryonic development (Ovitt and Scholer 1998). In the previous studies, OCT4 expression was different between IVF and cloned cat embryos (Imsoonthornruksa et al. 2010) and also was found to be affected by the cryopreservation of the fibroblast donor cells (Gomez et al. 2008). In case of the study by Imsoonthornruksa et al. (2010), the most profound differences between cloned and IVF domestic cat embryos were found during the morula stage, the same developmental stage that was the object of this study. Furthermore, OCT4 expression levels were significantly higher in *in vivo*-derived domestic cat blastocysts compared with *in vitro*-produced embryos (Filliers et al. 2012). According to those results, the difference between the two examined culture systems in OCT4 expression might indicate the differences in embryo quality. Higher OCT4 expression in embryos from the SCBI culture system could represent more *in vivo*-like conditions compared with the lower expression in IZW embryos.

Using ejaculated sperm, there was only the tendency of higher OCT4 mRNA abundances in the SCBI culture system in comparison with the IZW culture system. As suggested in a previous study (Waurich et al. 2010), an influence of the sperm source could be a possible explanation. In contrast to ejaculated sperm, epididymal sperm does not complete the epididymal transit and are less mature. This is demonstrated by distal cytoplasmic drops in epididymal sperm that gets lost during ejaculation (Axner 2006). Furthermore, in contrast to fresh epididymal semen, only cryopreserved ejaculated semen was utilized for fertilization. These differences in sperm treatments could be another reason for the differing results obtained in gene expression pattern in different culture media.

Expression of all other examined genes was not found to be dependent on the used culture system. Here, variation was too high to estimate any significant difference. These results are consistent with those of Filliers et al. (2012) who also observed OCT4 as the only influenced gene in their investigation.

In conclusion, this study revealed important insights into gene expression pattern in early cat embryos. It clearly demonstrated that different culture media

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systems are able to produce embryos of similar - developmental potential and molecular features. Furthermore, OCT4 was identified as a potential molecular marker for embryo quality. But also, regarding the significant differences in OCT4 expression accompanied by similar cleavage and developmental success rates, the exclusive use of conventional criteria for the evaluation of embryo quality should be reconsidered and possibly supplemented by the more sensitive molecular features. On the basis of the comparison of *in vitro*- and *in vivo*-produced embryos by others (Filliers et al. 2012), it can be suggested that the higher level found in SCBI embryos reflects *in vivo* conditions better. Finally, embryo-transfer experiments could assess which culture system is the best for the production of high-quality cat embryos that result in healthy offspring.

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Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contributions

All four authors contributed to the study design. Romy Hribal produced the embryos examined in this study, with support of Pierre Comizzoli. Also, she analyzed the data with support in molecular methods by Beate C. Braun. Romy Hribal, Katarina Jewgenow and Pierre Comizzoli drafted the paper, with contributions of Beate C. Braun.

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