Original Article

Time of the First Embryonic Cleavage Indicates Cat Blastocyst Quality

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Abstract

Time of the first cleavage has been used as an indicator to determine developmental rate and quality of embryos in many species. The aim of this study was to examine effect of time of the first cleavage on cat embryo development and blastocyst quality. After *in vitro* fertilization, embryos were observed twice at 27 and 42 hours. Embryos cleaved at 27 hours post-fertilization (pf) were classified as early cleaved embryos and were separated from the remainder which were determined as delayed cleaved embryos (cleaved between 27 and 42 hours). These two groups were separately cultured in the same condition for 7 days to observe embryo developmental and blastocyst formation rates. To evaluate the embryo quality, cells of the blastocyst were classified into inner cell mass (ICM) and trophectoderm cell (TE). The results showed that the percentages of the 8-cell and morula of the early cleaved embryos and the delayed cleaved embryos were not different (100.0 vs. 96.0% and 97.1 vs. 92.0%, respectively; $p>0.05$), whereas the blastocyst rates of the early cleaved embryos were greater than the delayed cleaved embryos (68.4 vs 20.0%; $p<0.05$). The total and ICM cell numbers of the blastocysts derived from the early cleaved embryos were higher than those of the delayed cleaved embryos (85.9 vs 71.6 cells, $p<0.05$). Moreover, the early cleaved embryos had a higher percentage of ICM cells than the delayed cleaved embryos (38.0 vs 31.9%, $p<0.05$). In conclusion, the cat embryos cleaved at early stage likely have the potential to develop to the blastocyst stage with high number of inner cell mass. The time of the first cleavage can be used as an indicator to select good quality of the cat blastocysts.

Keywords: early cleavage, embryo quality, feline, *in vitro* fertilization

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บทคัดย่อ

เวลาการแบ่งเซลล์ระยะแรกของตัวอ่อนบ่งบอกถึงคุณภาพบลาสโตซิสแมว

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ระยะการแบ่งเซลล์ของตัวอ่อนครั้งแรก ถูกนำมาใช้เป็นตัวบ่งชี้เพื่อประเมินการเจริญและคุณภาพตัวอ่อนในหลายสปีชีส์ การศึกษานี้มีวัตถุประสงค์ เพื่อตรวจวัดผลของระยะเวลาในการแบ่งเซลล์ของตัวอ่อนครั้งแรกต่อการเจริญและคุณภาพตัวอ่อนระยะบลาสโตซีสในแมวบ้าน โดยทำการตรวจสอบตัวอ่อน 2 ครั้ง ที่เวลา 27 และ 42 ชั่วโมง (ชม.) หลังจากเริ่มกระบวนการปฏิสนธิภายนอกร่างกาย(ไอวีเอฟ) ตัวอ่อนไอวีเอฟที่เริ่มแบ่งเซลล์ที่ ≤ 27 ชม.ถูกจัดว่าเป็นกลุ่มที่แบ่งตัวก่อนทำการคัดแยกออกจากตัวอ่อนที่แบ่งหลังซึ่งแบ่งตัวที่ > 27-42 ชม. โดยตัวอ่อนไอวีเอฟทั้งสองกลุ่มถูกแยกเลี้ยงในน้ำยาชนิดเดียวกัน และสังเกตการพัฒนาตัวอ่อนเป็นเวลา 7 วัน การประเมินคุณภาพตัวอ่อนเซลล์ของบลาสโตซีสแยกได้เป็นส่วนอินเนอร์เซลล์แมส (ไอซีเอ็ม) และส่วนโทรเฟคโตรเดิร์ม อัตราการเจริญของตัวอ่อนไอวีเอฟไปยังระยะ 8 เซลล์ และระยะมอรูล่าของทั้งสองกลุ่มไม่แตกต่างกัน (100.0 กับ 96.0% และ 97.1 กับ 92.0%, ตามลำดับ, $p>0.05$) ในขณะที่ตัวอ่อนไอวีเอฟแบ่งตัวก่อนมีอัตราการเจริญของระยะบลาสโตซีส (68.4 กับ 20.0%, $p<0.05$) นอกจากนี้ ตัวอ่อนที่แบ่งตัวก่อนยังมีร้อยละของไอซีเอ็มเซลล์มากกว่าตัวอ่อนที่แบ่งตัวหลัง (38.0 vs 31.9%, $p< 0.05$) โดยสรุปตัวอ่อนแมวไอวีเอฟที่แบ่งตัวก่อนมีประสิทธิภาพในการเจริญของบลาสโตซีสที่ให้จำนวนไอซีเอ็มเซลล์สูง และสามารถนำมาใช้เป็นเครื่องบ่งชี้ในการเลือกตัวอ่อนแมวบ้านที่มีคุณภาพได้

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Introduction

In vitro fertilization (IVF) has been a common assisted reproductive technology used to produce kittens after embryo transfer in the cats (Goodrowe et al., 1988). Because most of the 36 felid species are classified as threatened, vulnerable or endangered, the domestic cat is used as a research model for reproductive studies of endangered felid species (Nowell and Jackson, 1996). The IVF technology developed from the domestic cats has been applied successfully to non-domestic felid species such as leopard cats (Goodrowe et al., 1989), tigers (Donoghue et al., 1990) and cheetahs (Donoghue et al., 1992). Pregnancy achievement is predicted from the ability of the embryos to reach blastocyst as well as the blastocyst quality that is not detected until days 6 to 7 of *in vitro* embryo culture in the cats. Recently, many literatures have indicated that timing of the first embryonic cleavage is a simple, practical and non-invasive method to be used as an early indicator for selecting the potentially developed embryos such as in bovine (Dinnyés et al., 1999), human (Lundin et al., 2001) and ovine (Leoni et al., 2006). In human, the 2-cell embryos cleaved at 25-28 hours pf. are usually selected for transfer to improve the chance of achieve pregnancy (Shoukir et al., 1997; Salumets et al., 2003; Windt et al., 2004). Likewise, the time of the first cleavage at 24 hours pf is as reliable as the analyses of metabolic rate of the embryos in the cats (Spindler et al., 2006). In the previous study, the live kittens have been produced from the transfers of early cleaved embryos (24-27 hours pf) (Thongphakdee et al., 2010).

To evaluate the quality of blastocysts, differential cell staining is a common method used in many species such as horses (Bruyas et al., 1993), ferrets (Kidder et al., 1999), pigs (Eckert et al., 1997), cattle (Thouas et al., 2001) and cats (Comizzoli et al., 2004; Tsujioka et al, 2008). The differentiation of blastocyst into inner cell mass (ICM) and trophectoderm (TE) cells is visualized using different fluorescence colors. The high numbers of ICM and TE cells imply good quality of blastocysts. In bovine, the poor morphological embryos had high chance of development to hatched blastocysts with low ICM cell numbers (Van Soom et al., 1997)

The objective of this study was to compare embryo developmental and blastocyst formation rates and percentages of ICM cells of embryos cleaved at ≤27 hours and >27-42 hours pf. The total cell number and percentage of ICM of the blastocysts were examined by differential cell staining.

Materials and Methods

Oocyte recovery: Domestic cat ovaries were obtained after ovariohysterectomy at the Bangkok Metropolis Administration, and from the Small Animal Hospital at Chulalongkorn University. The ovaries were kept

and washed in normal saline containing 100 IU penicillin (Gibco, USA) and 100 µg/ml streptomycin (Gibco, USA), and were subsequently minced by a surgical blade in an oocyte collecting medium; composed of Tissue culture medium 199 (TCM199) (Sigma, St Louis, MO) supplemented with 0.292 g/ml glutamine (Sigma, St Louis, MO), 0.026 g/ml pyruvate (Sigma, St Louis, MO), 0.4% bovine serum albumin (BSA fraction V) (Sigma, St Louis, MO), 100 IU penicillin, 100 µg/ml streptomycin and 10 mM hepes buffer under a stereomicroscope. Oocytes with compact cumulus cells of more than 2 layers and homogenous dark ooplasm were selected.

In vitro oocyte maturation: The selected oocytes were cultured in an oocyte culture medium (30-50 COCs/800 µl) which was composed of TCM199 supplemented with 0.292 g/ml glutamine, 0.026 g/ml pyruvate, 0.4% BSA, 100 IU penicillin, 100 µg/ml streptomycin, 1 µg/ml porcine lutienizing hormone, 10 µg/ml porcine follicle stimulating hormone (FSH; Folltropin-V, Belleville, ON, Canada), 1 µg/ml estradiol (Sigma, St Louis, MO) and 25 ng/ml epidermal growth factor (EGF; Sigma, St Louis, MO), at 38.5°C, under 5% CO_2 in air, for 24-27 hours.

Sperm recovery and cryopreservation: Cat semen was collected from an adult tom cat by an artificial vagina and cryopreserved using the protocol described by Chatdarong et al. (2007). Briefly, when the tom cat is allowed to mount an estrous female, the artificial vagina which made from a rubber bulb and a small test tube was slipped over the glans penis while he thrusted and searched for the vulva. The fresh semen was evaluated and centrifuged at 600 ×g for 6 min. Thereafter, the supernatant was removed and the sperm pellet was resuspended with an extender A containing 2.4% (w/v) Tris (Fluka, Buenos Airez, Argentina), 1.4% (w/v) citric acid (Fluka, Buenos Airez, Argentina), 0.8% (w/v) glucose (BDH, VWR Internatonal, Poole, England), 3% (v/v) glycerol (Fluka, Buenos Airez, Argentina), 20% (v/v) egg yolk, 0.06% (w/v) Na-benzylpenicillin (M&H Manufacturing, Samudprakan, Thailand), and 0.1% (w/v) streptomycin sulphate (M&H Manufacturing, Samudprakan, Thailand) in distilled water (pH 6.5, 842 mOsm). The extended sample was placed in a bench cooler and cooled to 4^0C within 45 min. After cooling for 1 hour, the extender B which had the same composition as that of extender A except that it contained 7% glycerol (v/v) and 1% (v/v) Equex STM paste (Nova Chemical Sales, Scituate, Inc., MA, USA) (pH 6.5, 1,352 mOsm was added 1:1 into the cooled sample. The extended semen was then half filled in a 0.25 ml-straw. The straws were put into goblets and cranes. The goblet was placed into a canister which was subsequently lowered into an Apollo SX-18 liquid nitrogen tank filled with a level of 16-18 cm of liquid nitrogen (MVE Cryogenetics, New Prague, MN, USA) in three steps, with the top of the goblet held 7, 13, and 20 cm below the opening of the tank, for 2, 2, and 1 min, respectively.

In vitro fertilization and embryo culture: Frozen semen was thawed in a water bath at 70^0C for 6 sec and immediately diluted 1:1 in a Tris buffered solution. Then, the semen was evaluated and washed by centrifugation at 600xg, for 10 min, in an IVF medium comprising synthetic oviductal fluid plus 1% essential and 1% non-essential amino acids (SOFaa) (Freistedt et al., 2001) supplemented with 0.6% bovine serum albumin, penicillin and streptomycin (Freistedt et al., 2001). The supernatant was discarded and the spermatozoa were allowed to swim up in the IVF medium overlaid on the sperm pellet, at 38.5^0C for 2-3 hours. The supernatant was collected and subsequently adjusted to a final concentration of $5x10^5$ spermatozoa/ml.

The 24-27 hours-IVM oocytes were washed in the IVF medium for two times and were subsequently co-incubated with the sperm for 18 hours, at 38.5^0C, under 5% CO_2 and humidified atmosphere. Subsequently, the cumulus cells surrounding the oocytes (Day-1 embryos) were removed and washed 3 times before cultured in SOFaa supplemented with 0.4% BSA, penicillin and streptomycin, at 38.5^0C, under 5% CO2 under humidified atmosphere. The cleavage rate was evaluated twice; at 27 hours pf and 42 hours pf. At 27 hours pf, the cleaved embryos were cultured separately from uncleaved oocytes. At 42 hours pf, the uncleaved oocytes were investigated for their development and cleaved embryos were collected and cultured.

Differential cell staining: Blastocysts at Day 7 pf. were stained to determine total cell number and ICM and TE cell ratio using protocol described by Tsujioka et al. (2008). In short, to stain the TE cells the blastocysts were incubated for 30 sec. at room temperature in phosphate buffered saline solution containing 1% Triton-X (Sigma) and 100 µg/ml propidium iodide (Sigma). To stain the ICM cells the blastocysts were further incubated for 3-5 hours at 4^0C in ethanol with 25 µg/ml bisbenzimide (Hoechst 33342). Then, the blastocysts were mounted on a slide glass with glycerol (Sigma, St Louis, MO) under a cover glass. The cells were counted under a fluorescence microscope at 320-350 nm and the percentage of ICM cells was calculated based on the total number of blastocyst cells. The ICM cells were stained blue while the TE cells were stained pink.

Experimental design: The effect of time at the first embryonic cleavage between ≤27 and >27-42 hours pf on embryo development and blastocyst formation rate were evaluated. The matured oocytes were co-cultured with frozen-thawed semen (IVF). The cleaved embryos at 27 hours pf. were separated from the uncleaved. The embryos cleaved between 27 to 42 hours pf. were collected. The two groups were cultured in the same condition for 7 days to observe embryo development and blastocyst formation. Total cell number and percentage of ICM cell indicating quality of blastocysts derived from both of the two groups was assessed by differential cell staining.

Table 1. Developmental rates of cat embryos first cleaved at ≤ 27 and >27 to 42 hours after *in vitro* fertilization (mean ± SD, %).

Time of first cleaved embryos (hours)	n	8-cell	morula	blastocyst
≤ 27	136	136 (100)[a]	132 (97.06)[a]	93 (68.38)[a]
> 27-42	25	24 (96)[a]	23 (92)[a]	5 (20)[b]

[a, b] Values with different superscripts within columns are significantly different (p<0.05)

Table 2. Numbers of total cells and inner cell mass (ICM) of cat blastocysts cleaved at ≤27 and >27 to 42 hours after *in vitro* fertilization (mean ± SD)

Time of first cleaved embryos (hours)	n	Total cell number	Number of ICM	Percentage of ICM
≤27	10	85.9±22.7[a]	32±8.2[a]	38±8.4[a]
> 27-42	5	71.6±29.3[b]	22.4±9.3[b]	30.9±2.9[b]

[a, b] Values with different superscripts within column are significantly different (p<0.05)

Statistical analysis: The developmental competence and blastocyst rate of embryos cleaved at ≤27 hours and >27-42 hours pf. was analysed by chi-square. Mean total number of blastocyst cells and percentage of ICM cells between ≤27 hours and >27-42 hours pf cleaved embryos were compared by one-way ANOVA. A p-value of <0.05 was considered significant.

Results

A total of 161 oocytes were collected. The developmental rates of the cat embryos first cleave at <27 and >27 to 42 hours pf illustrated in Table 1. The embryo cleavage rate at 27 hours pf was 43.6% while the corresponding rate at 42 hours pf. was 51.6% (Fig 1). The percentages of the 8-cell and morula of ≤27 hours pf. cleaved embryos (n=136) and >27 to 42 hours pf. (n=25) were not different (100.0 vs. 96.0% and 97.1 vs. 92.0%, respectively; p>0.05). However, the blastocyst rates of the ≤27 hours pf. cleaved embryos were greater than those of >27 to 42 hours pf. cleaved embryos (68.4 vs 20.0%; p<0.05) (Table 2). The overall 8-cell, morula and blastocyst development of cleaved embryos (≤42 hours pf.) was 99.4, 96.3 and 60.9%, respectively (Fig 2).

As shown in Table 2, the mean numbers of total cells and ICM cells of the blastocysts derived from ≤27 hours pf. cleaved embryos were higher than those of the embryos cleaved at >27 to 42 hours pf. (85.9 vs 71.6 cells; 32 vs 22.4, p<0.05). Moreover, the percentages of ICM of blastocysts of ≤27 hours pf cleaved embryos were higher than that of >27 to 42 hours pf cleaved embryos (38 vs 31.93%, p<0.05).

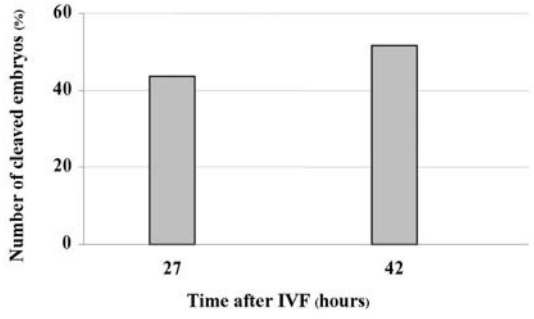

Figure 1 Percentages of cleaved embryos at 27 and 42 hours after *in vitro* fertilization (n=161 oocytes).

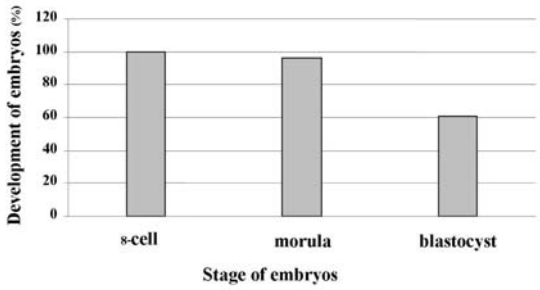

Figure 2 Development of fertilized embryos to 8-cell, morula and blastocyst observed at Day 3, 5 and 7, respectively (n=161 oocytes from 3 replicates).

Discussion

In the present study, the time of the first embryonic cleavage was related with the blastocyst rate and embryo quality measured by the total cell number and percentage of ICM cells. The early cleaved embryos (≤27 hours pf) had a higher blastocyst rate and quality than the delayed cleaved embryos (>27 hours pf). These results were in accordance with the previous literatures in several species such as bovine (Majerus et al., 2000), human (Fenwick et al., 2002) and ovine (Leoni et al., 2006). There are various techniques that have been used to predict the embryo viability and pregnancy rate in human such as the assessment of embryo features at the pronuclear stage (Arroyo et al., 2007), blastocyst development (Gardner et al., 2000) and cleavage stage of embryos at 25-29 hours pf (Fu et al., 2009). In the previous study, the cat embryos selected by the time of the first cleavage at 24 hours pf had similar potential of development to those selected by the metabolic rate analyses (Spindler et al., 2006). The pig embryos cleaved earlier at a specific time performed better developmental rate and quality than those cleaved faster due to the insufficient reprogramming of the embryonic or somatic cell nuclei, and slower due to the delayed repair of damaged DNA (Kawakami et al., 2008). Thus, the reprogramming and DNA repairing of the cat oocytes seemed to be completed within 27 hours pf.

The embryos cleaved ≤ 27 hours were proven of better quality than those cleaved >27 to 42 hours by the higher total cell number and percentage

of ICM in the present study. Perhaps, there were particular intrinsic factors in the oocytes that modulated the embryo cleavage. Maternal proteins such as nuclear lamins that synthesized during oogenesis and involved in fertilization (Zhang et al., 2009) might play a role in this case. The lamins were stored in an inactive form in the oocytes until they become activated in a stage-specific manner during oocyte maturation (Ralle et al., 1999). The good quality of blastocysts implied the potential of achieving pregnancy after embryo transfer (Van Soom et al., 2001). Moreover, the time of blastocyst formation was correlated with freezing resistant (Dinnyes et al., 1999; Tsujioka et al., 2008). Thus, selection of early cleaved embryos to be cultured is suggested for embryo production technology in the cats.

In this study, the oocytes were retrieved from the cats of unknown reproductive history. The delayed cleaved embryos might be derived from ovaries of cats of old age. Liu and Keefe (2002) reported that the ageing-associated oocyte in infertile humans resulted from the spindle disruption and/or the chromosome misalignments at both MI and MII stages. However, the oocyte quality of some species is not affected by the age of donors. In goat, there are no differences in the rates of cleavage and blastocyst development between oocytes obtained from pre-pubertal and adult goat (Mogas et al., 1997; Izquierdo et al., 2002).

In conclusion, the time of the first cleavage can be used to select good quality of embryos and may be used as early and non-invasive indicator for cat embryo development *in vitro* as well as embryo viability *in vivo* after transfer. Moreover, the differential staining is a potential technique for assessment of blastocyst quality.

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References

Arroyo, G., Veiga, A., Santaló, J. and Barri, P.N. 2007. Developmental prognosis for zygotes based on pronuclear pattern: usefulness of pronuclear scoring. J Assist Reprod Genet. 24(5): 173-181.

Bruyas, J.F., Bézard, J., Lagneaux, D. and Palmer, E. 1993. Quantitative analysis of morphological modifications of day 6.5 horse embryos after cryopreservation: differential effects on inner cell mass and trophoblast cells. J Reprod Fertil. 99(1): 15-23.

Chatdarong, K., Axnér, E., Manee-In, S., Thuwanut, P. and Linde-Forsberg, C. 2007. Pregnancy in the domestic cat after vaginal or transcervical insemination with fresh and frozen semen. Theriogenology. 68(9): 1326-1333.

Comizzoli, P., Wildt, D.E. and Pukazhenthi, B.S. 2004. Effect of 1,2-propanediol versus 1,2-ethanediol on subsequent oocyte maturation, spindle integrity, fertilization, and embryo development in vitro in the domestic cat. Biol Reprod. 71(2): 598-604.

Dinnyés, A., Lonergan, P., Fair, T., Boland, M.P. and Yang, X. 1999. Timing of the first cleavage posr-fertilization affects cryosurvival of *in vitro*-produced bovine blastocysts. Mol Reprod Dev. 53(3): 318-324.

Donoghue, A.M., Johnston, L.A., Seal, U.S., Armstrong, D.L., Tilson, R.L., Wolf, P., Petrini, K., Simmons, L.G., Gross, T. and Wildt, D.E. 1990. *In vitro* fertilization and embryo development *in vitro* and *in vivo* in the tiger (*Panthera tigris*). Biol Reprod. 43(5): 733-744.

Donoghue, A.M., Howard, J.G., Byers, A.P., Goodrowe, K.L., Bush, M., Blumer, E., Lukas, J., Stover, J., Snodgrass, K. and Wildt, D.E. 1992. Correlation of sperm viability with gamete interaction and fertilization *in vitro* in the cheetah (*Acinonyx jubatus*). Biol Reprod. 46(6): 1047-1056.

Eckert, J., Tao, T. and Niemann, H. 1997. Ratio of inner cell mass and trophoblastic cells in blastocysts derived from porcine 4- and 8-cell embryos and isolated blastomeres cultured in vitro in the presence or absence of protein and human leukemia inhibitory factor. Biol Reprod. 57(3): 552-560.

Fenwick, J., Platteau, P., Murdoch, A.P. and Herbert, M. 2002. Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos *in vitro*. Hum Reprod. 17(2): 407-412.

Freistedt, P., Stojkovic, M. and Wolf, E. 2001. Efficient in vitro production of cat embryos in modified synthetic oviduct fluid medium: Effects of season and ovarian status. Biol Reprod. 65(1): 9-13.

Fu, J., Wang, X.J., Wang, Y.W., Sun, J., Gemzell-Danielsson, K. and Sun, X.X. 2009. The influence of early cleavage on embryo developmental potential and IVF/ICSI outcome. J Assist Reprod Genet. 26(8): 437-441.

Gardner, R.L. 1989. Cell allocation and lineage in the early mouse embryo. Ciba Found Symp. 144: 172-181

Goodrowe, K.L., Wall, R.J., O'Brien, S.J., Schmidt, P.M. and Wildt, D.E. 1988. Developmental competence of domestic cat follicular oocytes after fertilization *in vitro*. Biol Reprod. 39(2): 355-372.

Goodrowe, K.L., Miller, A.M. and Wildt, D.E. 1989. *In vitro* fertilization of gonadotropin-stimulated leopard cat (*Felis bengalensis*) follicular oocytes. J Exp Zool. 252(1): 89-95.

Izquierdo, D., Villamediana, P., López-Bejar, M., Paramio, M.T. 2002. Effect of in vitro and in vivo culture on embryo development from prepubertal goat IVM-IVF oocytes. Theriogenology. 57(5): 143-141.

Kawakami, M., Kato, Y. and Tsunoda, Y. 2008. The

effects of time of first cleavage, developmental stage, and delipidation of nuclear-transferred porcine blastocysts on survival following vitrification. Anim Reprod Sci. 106(3-4): 402-411.

Kidder, J.D., Giles, J.R., Foote, R.H., Richmond, M.E. and Salerno, M. 1999. Allocation of inner cell mass and trophectoderm cells to the preimplantation blastocyst of the domestic ferret, *Mustela putorius furo*. J Exp Zool. 283(2): 202-209.

Leoni, G.G., Succu, S., Berlinguer, F., Rosati, I., Bebbere, D., Bogliolo, L., Ledda, S. and Naitana, S. 2006. Delay on the in vitro kinetic development of prepubertal ovine embryos. Anim Reprod Sci. 92(3-4): 373-383.

Liu, L. and Keefe, D.L. 2002. Aging-associated aberration in meiosis of oocytes from senescence-accelerated mice. Hum Reprod. 17(10): 2678-2685.

Lundin, K., Bergh, C. and Hardarson, T. 2001. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod. 16(12): 2652-2657.

Majerus, V., Lequarré, A.S., Ferguson, E.M., Kaidi, S., Massip, A., Dessy, F. and Donnay, I. 2000. Characterization of embryos derived from calf oocytes: kinetics of cleavage, cell allocation to inner cell mass, and trophectoderm and lipid metabolism. Mol Reprod Dev. 57(4): 346-352.

Mogas, T., Palomo, M.J., Izquierdo, M.D. and Paramio, M.T. 1997. Morphological events during *in vitro* fertilization of prepubertal goat oocytes matured *in vitro*. Theriogenology. 48(5): 815-829.

Nowell, P. and Jackson, P. 1996. Status survey and conservation action plan: wild cats. Gland, Switzerland: IUNC: 149-179.

Ralle, T., Gremmels, D. and Stick, R. 1999. Translational control of nuclear lamin B1 mRNA during oogenesis and early development of Xenopus. Mech Dev. 84(1-2): 89-101.

Salumets, A., Hydén-Granskog, C., Mäkinen, S., Suikkari, A.M., Tiitinen, A. and Tuuri, T. 2003. Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures. Hum Reprod. 18(4): 821-825.

Shoukir, Y., Campana, A., Farley, T. and Sakkas, D. 1997. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. Hum Reprod. 12(7): 1531-1536.

Spindler, R.E., Crichton, E.G., Agca, Y., Loskutoff, N., Critser, J., Gardner, D.K. and Wildt, D.E. 2006. Improved felid embryo development by group culture is maintained with heterospecific companions. Theriogenology. 66(1): 82-92.

Thongphakdee, A., Siriaroonrat, B., Manee-in, S., Klincumhom, N., Kamolnorranath, S., Chatdarong, K. and Techakumphu, M. 2010. Intergeneric somatic cell nucleus transfer in marbled cat and flat-headed cat. Theriogenology. 73(1): 120-128.

Thouas, G.A., Korfiatis, N.A., French, A.J., Jones, G.M. and Trounson, A.O. 2001. Simplified technique for differential staining of inner cell mass and trophectoderm cells of mouse and bovine blastocysts. Reprod Biomed Online. 3(1): 25-29.

Tsujioka, T., Otzdorff, C., Braun, J. and Hochi, S. 2008. Effect of post-IVF developmental kinetics on *in vitro* survival of vitrified-warmed domestic cat blastocysts. Reprod Domest Anim. 43(3): 323-327.

Van Soom, A., Ysebaert, M.T. and de Kruif, A. 1997. Relationship between timing of development, morula morphology, and cell allocation to inner cell mass and trophectoderm in *in vitro*-produced bovine embryos. Mol Reprod Dev. 47(1): 47-56.

Van Soom, A., Vanroose, G. and de Kruif, A. 2001. Blastocyst evaluation by means of differential staining: a practical approach. Reprod Domest Anim. 36(1): 29-35.

Windt, M.L., Kruger, T.F., Coetzee, K. and Lombard, C.J. 2004. Comparative analysis of pregnancy rates after the transfer of early dividing embryos versus slower dividing embryos. Hum Reprod. 19(5): 1155-1162.

Zhang, P., Ni, X., Guo, Y., Guo, X., Wang, Y., Zhou, Z., Huo, R. and Sha, J. 2009. Proteomic-based identification of maternal proteins in mature mouse oocytes. BMC Genomics. 10: 348.