High Pregnancy and Implantation Rates can be Obtained with Preincubation of Oocytes before Insemination in IVF and ICSI Procedures

Javier I García[1,2]*, Pedro Valdivieso[1], Milton Zambrano[1] and Jorge Carpio[1]

[1]Laboratory of Assisted Reproduction, Alcivar Hospital, Guayaquil, Ecuador, Peru
[2]FERTILAB Laboratory of Assisted Reproduction, Lima, Peru

Abstract

Purpose: Evaluate the effect of preincubation of oocytes prior to IVF or ICSI with embryo transfer at blastocyst stage.

Methods: Retrospective nonrandomized study based on secondary analysis of data.

Setting: Laboratory of Assisted Reproduction of Alcivar Hospital.

Patients: One hundred eighteen cycles of IVF and ICSI were analyzed in the present study. The evaluated groups were formed for those patients whose oocytes, after retrieval, were inseminated at 1-3 h (Group I) or 4-6 h (Group II).

Results: There was no difference in fertilization rate (83.6% and 78.1%), Day 3 cleavage rate (95.1% and 97.1%), and blastocyst formation (31.1% and 39.1%) for groups I and II respectively. Clinical pregnancy rates (PR: 53.0% vs 22.9%) and implantation rates (IR: 38.1% vs 13.0%) were significantly higher in group II versus group I, respectively (P<0.05).

Conclusions: Preincubation of oocytes before insemination is a factor which raises the PR and IR after the blastocyst transfer.

Keywords: Oocytes; Embryo development; Maturation; Blastocyst; ART

Introduction

The science of In Vitro Fertilization (IVF) has improved considerably in the last 25 years since the first "test-tube" baby was born. A greater understanding of the nutritional and environmental requirements of oocytes and embryos led to advances in culture conditions that have improved IVF pregnancy rates.

Oocyte quality impacts early embryonic survival, the establishment and maintenance of pregnancy, fetal development, and even adult disease. The oocytes are considered to be meiotically mature after extrusion of the first polar body, which is characteristic of metaphase II (MII). However, nuclear and cytoplasmic maturation were reached independently during oocyte maturation [1].

Quality, or developmental competence, is acquired during folliculogenesis as the oocyte grows during the period of oocyte maturation. As an oocyte grows and matures, it acquires the abilities to resume and complete meiosis, successfully undergo the fertilization process, and initiate and sustain embryonic development [2]. In the course of acquiring these competences, cytoplasmic changes occur, which may include such cellular processes as mRNA transcription [3-5], protein translation [6], ultrastructural changes [7,8] and post-translational modification of proteins [9], all involved in meiotic progression and cell cycle control [10], and other proteins involved in cellular processes critical for developmental success before and after activation of the zygote genome [11,12].

Trounson et al. [13] postulated that preincubation of Cumulus-Corona-Oocyte Complexes (CCOC) before conventional insemination improves the outcome of IVF. They proposed that a period of culture in vitro is beneficial for the completion of oocyte maturation and to obtain high rates of fertilization and embryo development in vitro.

Several studies evaluating the effect of preincubation period of oocytes in IVF [14] and ICSI procedures [14-16] showed that the fertilization rate significantly increased with a minimal of 3h of preincubation before insemination. Cleavage rates and embryo quality were not affected by the preincubation period. With regard to clinical pregnancy rate, only Falcone et al. [14] observed a significant increase from 2 to 5 h of preincubation with embryo transfer at 48-72 h post insemination [13-16].

Physiologically, the uterus provides a nutritional environment different from than in the fallopian tubes. Therefore, embryo transfer in the cleavage stage would cause homeostatic stress of the embryo and reduction in its implantation potential [17]. Consequently, the transfer in blastocysts stage would allow a better synchronization with rhythm of uterine contractions and the embryo [18,19], and enhance the likelihood of pregnancy [20-22]. Some studies including >100 selected patients have reported high pregnancy rates after single blastocyst transfer on Day 5 [23-25].

We hypothesize that a preincubation period of oocytes after their retrieval from IVF and ICSI procedures will affect embryo quality and clinical outcomes when the embryo transfers are at blastocysts stage. Thus, the objective of this study was to report clinical outcomes

obtained with two preincubation periods of oocytes ($\leq$ 3 h or $\geq$ 4 h) before the insemination procedure with blastocyst stage transfer.

Materials and Methods

Patients

This is a retrospective nonrandomized study based on secondary analysis of data obtained from 118 cycles of IVF and ICSI at the Laboratory of Assisted Reproduction of Alcivar Hospital (Guayaquil, Ecuador) during the period January 2009 to December 2010. This study was approved by the Institutional Review Board (IRB) and the corresponding Ethics Committee at the Alcivar Hospital (Guayaquil, Ecuador).

The evaluated groups were formed for those patients whose oocytes after retrieval were inseminated at 1-3 h (Group I) or 4-6 h (Group II). The age range of the patients was 26-41 year old in both groups. All embryo transfers were made at blastocyst stage.

Controlled ovarian stimulation and oocyte collection

The menstrual cycles of patients were stimulated using recombinant FSH (rFSH) (Puregon®, Organon Laboratories, Ecuador), HMG (Merional®, IBSA Laboratories, Ecuador) and GnRH antagonist (Orgalutran® Organon Laboratories, Ecuador) according to the stimulation protocols previously established and starting on Day 2 of the menstrual cycle until at the least three follicles reached ~18 mm in diameter. The oocyte retrieval was performed by vaginal ultrasound 36 h after IM application of hCG (Pregnyl® 10,000 UI, Organon Laboratories, Ecuador). For the procedure, the patient was under general anesthesia with 200 mg of Propofol IV (Diprivan® 1% P/V; AstraZeneca laboratories, UK).

During follicular aspiration procedure the oocytes were recovered in Global®-HEPES-buffered medium (IVFonline, Canada) supplemented with 10% vol/vol Serum Substitute Supplement (SSS; Irvine Scientific, USA). After retrieval with the help of two sterile needles, cumulus-oocyte complexes were trimmed of excess cumulus cells to prevent accumulation of debris and red blood cells and the maintained in ~200 µl drops of Global®-Fertilization medium (IVFonline, Canada) plus 10% SSS under oil at 37°C and an atmosphere containing 5.8% CO_2 in air until the recovered oocytes were inseminated or injected. None of the oocytes were damaged by cleaning procedure.

Preparation of spermatozoa

The semen samples were collected by masturbation from the recipients' partners. Motile spermatozoa were separated from the seminal plasma by centrifugation through 1.0 ml 95% and 45% Isolate gradients (Irvine Scientific, USA). For oligospermic samples, the sperm were washed and resuspended in varying amounts of sperm washing medium depending on initial concentration and motility and then placed into 10 µl drops of HEPES-buffered Global medium + 10% SSS for ICSI.

Insemination, fertilization and embryo culture

All oocytes were inseminated or injected, depending about seminal characteristics, with spermatozoa from recipient's husband. After insemination procedure (day 0), all oocytes were cultures at 37°C in an atmosphere of 5.8% CO_2 in air.

In both groups, the fertilization was evaluated 16-18 h after injection by presence of two pronuclei (day 1). The zygotes were individually cultured under mineral oil, in 10 µl droplets of Global® medium (IVFonline, Canada) supplemented with 10% vol/vol SSS from day 1 to day 3. On day 3, the embryos were moved to fresh 10 µl droplets of Global® medium +10% SSS.

Embryo quality

On day 3 the embryos were evaluated for cell number, fragmentation, and multinucleation. Good quality day 3 embryos were defined as those with 6-8 cell and $\leq$ 10% of fragmentation. Good quality blastocysts were defined as having an Inner Cell Mass (ICM) and trophoectoderm type A or B [26]. The ICM score was evaluated as follow: type A=compact area, many cells present; type B=cells are loosely grouped. The trophoectoderm was scored as follows: type A=many cells forming a tight epithelial network of cells; type B=few cells forming a loose network of cells.

Blastocyst transfer

Blastocysts were transferred on day 5 in all recipients. There were sixty nine and one hundred seventy one blastocyst transfers in groups I and II respectively. In group I, the rate of transfer was 1.97 ± 0.29 blastocyst/patient (Mean ± SD) and in group II, 2.06 ± 0.29 blastocyst/patient (Mean ± SD). The blastocysts that were not transferred were cryopreserved or discarded according to their morphology. The blastocyst transfer was performed with a Labotech Embryo Transfer Catheter Set® (Labotect, Germany) that had been previously washed with culture medium. The catheter was completely filled with culture medium and the blastocysts filled in the last 10 µl of the catheter. All transfers were performed according to the methods previously described by Mansour [27].

Pregnancy assessment

The biochemical pregnancy was assessed 12 days after the blastocyst transfer by measuring the Human Chorionic Gonadotropin beta subunit (hCG-b) in blood. The clinical pregnancy was determined by transvaginal ultrasonography to detect gestational sacs and fetal heartbeats at approximately 21 and 28 days after transfer respectively.

Statistical analysis

Data were statistically analyzed using the $\chi 2$ test and Student's t-test as appropriate and differences were considered to be significant at $P<0.05$. All statistical analysis was carried out using the statistic package Stata 10 (StataCorp, College Station, TX).

The normal fertilization was calculated from the number of zygotes with two pronuclei divided by the number of mature oocytes inseminated times 100. Clinical pregnancy rate per transfer was calculated from the number of patients with blastocyst transfer with at least one gestational sac divided by the total blastocyst transfers times 100. Implantation rate was calculated by dividing the number of gestational sacs (observed by ultrasound at the 21st day post transfer) by the total number of blastocysts transferred times 100. Miscarriages were defined as the number of pregnancies with total loss of the gestational sacs before the 20 weeks of gestation over the number of pregnancies times 100.

Results

The ages of patients were similar in both evaluated groups (34.31 ± 3.59 vs. 33.39 ± 3.78 years). Laboratory results obtained from group I (1-3 h) and control group (4-6 h) are shown in Table 1. A total of 314 and 950 oocytes were collected from patients of group I and group II respectively. Two hundred seventy nine oocytes from group I and

six hundred ninety six oocytes from group II were inseminated. There was no difference in the number of inseminated (IVF) or injected (ICSI) oocytes in both group evaluated (data no shown). The normal fertilization (2PN) was similar in both evaluated groups (Group I: 83.6% vs. Group II: 78.1%; P:NS). In group I, the cleavage rate by embryo at Day 3 was 95.1%, whereas in group II it was 97.1% (P:NS). Embryos from group I had mean cell numbers at Day 3 significantly higher compared to those embryos from group II (P<0.05). Embryo quality derived from oocytes that were inseminated 1-3 h was similar to those that were inseminated at 4-6 h (76.0% vs. 80.1%). Blastocyst development rates were similar in both study groups (31.1% vs. 39.1%). In addition, embryos reaching the blastocyst stage were morphologically similar in both groups.

Clinical outcomes are shown in Table 2. The mean value for blastocysts was significantly lower for the patients of group I than those for the patients of group II (2.41 ± 1.78 vs. 3.37 ± 2.03; P<0.05). In group I, a total of sixty nine blastocysts were transferred to 35 patients with a mean value of 1.97 blastocysts. In group II, a total of one hundred seventy one blastocysts were transferred to 35 patients with a mean value of 2.06 blastocysts. The mean quality values of blastocysts transferred was similar in both analyzed groups (P:NS).

The patients of group I compared with group II had significantly higher clinical PR (22.9% vs. 53.0%; P<0.05) and IR (13% vs. 38.1%; P<0.05) respectively. Miscarriage and biochemical pregnancy rates were similar in both groups (P:NS). For group I, one and two gestational sacs were observed in seven (87.5%) and one (12.5%) patients respectively. For group II, one and two gestational sacs were observed in twenty eight (63.6%) and sixteen (36.4%) patients respectively. These percentages were similar in both evaluated groups (P:NS).

Discussion

Complete nuclear and cytoplasmic maturation of oocytes is essential for the activation of oocytes at fertilization and the development of embryos [28]. An oocyte is considered to reach nuclear maturity when its meiosis is arrested again at MII with the presence of an extruded first polar body. However, nuclear and cytoplasmic maturation are acquired independently during oocyte maturation [1,29].

	Group I(1-3 h)	Group II(4-6 h)	P
Patients	35	83	-
No. total oocytes (Mean ± SD)	314 (8.97 ± 4.03)	950 (11.45 ± 4.98)	-
No. total inseminated/injected oocytes (Mean ± SD)	269 (7.69 ± 3.75)	696 (10.14 ± 4.43)	-
No. total fertilized oocytes (2PN) (%)	225 (83.6)	657 (78.1)	0.51
Cleavage rate by embryo at Day 3 (%)	95.1	97.1	0.85
No. cell/embryo at Day 3 (Mean ± SD)	6.68 ± 0.87	7.09 ± 0.86	0.02
Good-quality embryos at Day 3 (%)	76.0	80.1	0.66
No. total blastocyst (%)	70 (31.1)	257 (39.1)	0.14
Good-quality blastocysts (%)	72.7	74.3	0.90
Early blastocyst (%)	25.7	23.7	0.79
Full blastocyst (%)	40.0	45.1	0.63
Expanded blastocyst (%)	32.6	24.1	0.27
Hatching blastocyst (%)	0	5.1	0.06

Table 1: Comparison of laboratory results between both evaluated groups

	Group I(1-3 h)	Group II(4-6 h)	P
Patients	35	83	
No. of inseminated oocytes/patient (Mean ± SD)	7.69 ± 3.75	10.14 ± 4.43	0.01
No. of fertilized oocytes/patient (Mean ± SD)	6.43 ± 3.12	7.92 ± 3.79	0.02
No. of blastocyst/patient (Mean ± SD)	2.41 ± 1.78	3.37± 2.03	0.01
No. total blastocyst transferred (Mean ± SD)	69(1.97 ± 0.29)	171(2.06 ± 0.29)	0.06
Pregnancy rate (%)	22.9	53.0	0.04
Implantation rate (%)	13.0	38.1	0.01
Single pregnancy (%)	87.5	63.6	0.58
Twin pregnancy (%)	12.5	36.4	0.31
Miscarriages (%)	0	6.8	0.46
Biochemical pregnancy rate (%)	12.5	2.3	0.19

Table 2: Clinical outcomes in those patients whose oocytes were inseminated at ≤3 h or ≥4 h after oocyte retrieval.

Cytoplasmic maturation encompasses a wide array of metabolic and structural modifications, including events that ensure the occurrence of normal fertilization, meiotic to mitotic cell cycle progression, and activation of pathways required for genetic and epigenetic programmes of preimplantation embryonic development [1,30-33]. On the other hand, deficient cytoplasmic maturity may be reflected by certain cytoplasmic abnormalities such as cytoplasmic inclusions, vacuoles, smooth endoplasmic reticulum (sER) clustering visible at light microscope level [34]. In previous studies, the incidence of vacuoles in MII oocytes varied between 5.7% [35] and 12.4% [36]. De Sutter et al. [35] showed a severely reduced fertilization rate in vacuolized oocytes (40%) compared with gametes without vacuolization (69.9%). Several studies evaluating the presence of SER in human oocytes have shown no differences on fertilization and cell division rates [37]; however, negative effects on the blastocyst formation rate, clinical pregnancy rate [37-39] and implantation rate were evident [40].

Our data suggests that overall fertilization rate is not influenced by preincubation time of oocytes before insemination. However, we observed a significantly greater cell number in embryos at Day 3 achieved from group II oocytes, which had a longer culture time (4-6 h) compared to group I (1-3 h), indicating a better preimplantation embryonic development under the same culture conditions, and similar results were obtained for Falcone et al. [14]. The present results confirm that the quality of embryo cleavage depends on the period of oocyte preincubation before insemination. Additionally, cleavage rate, cleavage stage embryo morphology, cytoplasmic fragmentation and multinucleation have been shown to be important markers of embryo quality and viability that may be observed over time during in vitro culture. After the advent of extended embryo culturing, higher IRs have been reported because of better embryo selection compared with earlier developmental stages and because of better synchronization between the embryo developmental stage and uterine environment.

Previous studies have shown that during the growth phase, arrested oocytes accumulate a large store of messenger RNA (mRNA) and proteins that function after fertilization to support and regulate preimplantation embryonic development [41,42], and whose oocytes with deficient mRNA or protein accumulation cannot complete the cytoplasmic maturation process [43-45]. On the other hand, preimplantation mammalian embryos display an impressive capacity to adapt to the pressures that suboptimal culture environments place

upon them. The embryo can compensate, at least partially, for missing components or offset the presence of deleterious components by adjusting its developmental program [46-48].

As human embryo gene expression is switched on at around the 8-cell stage before compaction [49], only embryos that undergo the transition from maternal to embryonic genome might reach the blastocyst stage. Furthermore, there are both cytoplasmic and nuclear reasons why a large proportion of embryos stop developing before implantation. For example, blastocyst formation in the mouse is dependent on the relocation of cytoplasmic organelles, alterations to membrane transport system and transcripts in the oocyte generated before fertilization during cytoplasmic maturation process [32,45,50]. Gross abnormalities in nuclear and cytoplasmic maturation process generally interrupt the meiotic cycle or block fertilization, but more subtle imperfections during oocyte cytoplasmic maturation may be manifested during the late cleavage or blastocyst stage.

Many reports have underlined the difficulties of correctly selecting the best embryo on Day 2 [51] or on Day 3 [52,53]. The aim of extending embryo culture to blastocyst stage is to select an embryo with increased probability of implantation rather than to improve embryo quality. In our study all transfers were made at blastocyst stage and similar blastocyst formation rate in both groups was evident, but patients whose oocytes were preincubated 4-6 h prior insemination procedure had higher implantation and pregnancy rates than those whose oocytes were incubated 1-3 h ($P<0.05$), suggesting that the oocyte maturation is an important process required to achieve optimal oocyte quality and a subsequent genetically normal embryo. The aneuploidy rate has been reported to be lower for blastocysts compared with top quality Day 3 embryos, even when genetic abnormalities have not prevented development to the blastocyst stage [54-56].

To conclude, our data indicates that a period of preincubation $\geq 4h$ before insemination improves oocyte quality, thus allowing adequate nuclear and cytoplasmic maturations to take place, which will be evident during embryo development and will be reflected in implantation and pregnancy rates. Additionally, we suggest more studies randomized controlled on the effect of preincubation time before the process of oocyte insemination with blastocyst transfer in cycles of IVF and ICSI.

References

1. Eppig JJ, Schultz RM, O'Brien M, Chesnel F (1994) Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. Dev Biol 164: 1-9.

2. First NL, Leibfried-Rutledge ML, Sirard MA (1988) Cytoplasmic control of oocyte maturation and species differences in the development of maturational competence. Prog Clin Biol Res 267: 1-46.

3. Hunter AG, Moor RM (1987) Stage-dependent effects of inhibiting ribonucleic acids and protein synthesis on meiotic maturation of bovine oocytes in vitro. J Dairy Sci 70: 1646-1651.

4. Kastrop PM, Bevers MM, Destree OHJ, Kruip THAM (1991) Protein synthesis and phosphorylation patterns of bovine oocytes matured in vivo. Mol Reprod Dev 29: 271-275.

5. Farin CE, Yang L (1992) Inhibition of germinal vesicle breakdown by 5,6-dichlorobenzimidazole riboside in bovine oocytes matured in vitro. Theriogenology 37: 208.

6. Sirard MA, Florman HM, Leibfried-Rutledge ML, Barnes FL, Sims ML, et al. (1989) Timing of nuclear progression and protein synthesis necessary for meiotic maturation of bovine oocytes. Biol Reprod 40: 1257-1263.

7. Kruip TAM, Cran DG, van Beneden TH, Dieleman SJ (1983) Structural changes in bovine oocytes during final maturation in vivo. Gamete Res 8: 29-47.

8. Hyttel P, Xu KP, Smith S, Greve T (1986) Ultrastructure of in-vitro oocyte maturation in cattle. J Reprod Fertil 78: 615-625.

9. Levesque JT, Sirard MA (1995) Effects of different kinases and phosphatases on nuclear and cytoplasmic maturation of bovine oocytes. Mol Reprod Dev 42: 114-121.

10. Wickramasinghe D, Albertini DF (1993) Cell cycle control during mammalian oogenesis. Curr Top Dev Biol 28: 125-153.

11. Barron DJ, Valdimarsson G, Paul DL, Kidder GM (1989) Connexin 32, a gap junction protein, is a persistent oogenetic product through preimplantation development of the mouse. Dev Genet 10: 318-323.

12. Watson AJ, Westhusin ME, De Sousa PA, Betts DH, Barcroft LC (1999) Gene expression regulating blastocyst formation. Theriogenology 51: 117-133.

13. Trounson AO, Mohr LR, Wood C, Leeton JF (1982) Effect of delayed insemination on in-vitro fertilization, culture and transfer of human embryos. J Reprod Fertil 64: 285-294.

14. Falcone P, Gambera L, Pisoni M, Lofiego V, De Leo V, et al. (2008) Correlation between oocyte preincubation time and pregnancy rate after intracytoplasmic sperm injection. Gynecol Endocrinol 24: 295-299.

15. Rienzi L, Ubaldi F, Anniballo R, Cerulo G, Greco E (1998) Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod 13: 1014-1019.

16. Ho JY, Chen MJ, Yi YC, Guu HF, Ho ES (2003) The effect of preincubation period of oocytes on nuclear maturity, fertilization rate, embryo quality, and pregnancy outcome in IVF and ICSI. J Assist Reprod Genet 20: 358-364.

17. Gardner DK, Lane M, Calderon I, Leeton J (1996) Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil Steril 65: 349-353.

18. Fanchin R, Ayoubi JM, Righini C, Olivennes F, Schonauer LM, et al. (2001) Uterine contractility decreases at the time of blastocyst transfers. Hum Reprod 16: 1115-1119.

19. Quea G, Romero K, García-Velasco JA (2007) Extended embryo culture to increase implantation rate. Reprod Biomed Online 14: 375-383.

20. Marek D, Langley M, Gardner DK, Doody KM, Confer N, et al. (1999) Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. Fertil Steril 72: 1035-1040.

21. Gardner DK, Lane M, Stevens J, Schlenker T (2000) Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 73: 1155-1158.

22. Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, et al. (2004) Single blastocyst transfer: a prospective randomized study. Fertil Steril 81: 551-555.

23. Henman M, Catt JW, Wood T, Bowman MC, De Boer KA, et al. (2005) Elective transfer of single fresh blastocysts and later transfer of cryostored blastocysts reduces the twin pregnancy rate and can improve the in vitro fertilization live birth rate in younger women. Fertil Steril 84: 1620-1627.

24. Nilsson S, Waldenström U, Engström AB, Hellberg D (2005) Promising results with 306 single blastocyst transfers. Fertil Steril 83: 1849-1841.

25. Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, et al. (2006) In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. N Engl J Med 354: 1139-1146.

26. Gardner DK, Schoolcraft WB (1999) In vitro culture of human blastocysts Towards Reproductive Certainty: Infertility and Genetics Beyond 1999: the Plenary Proceedings of the 11th World Congress on In Vitro Fertilization and Human Reproductive Genetics. Parthenon Press 378-388.

27. Mansour R (2005) Minimizing embryo expulsion after embryo transfer: A randomized controlled study. Hum Reprod 20: 170-174.

28. Zenzes MT, Belkien L, Bordt J, Kan I, Schneider HP, et al. (1985) Cytological investigation of human in vitro fertilization failures. Fertil Steril 43: 883-891.

29. Kubiak JZ (1989) Mouse oocytes gradually develop the capacity for activation during the metaphase II arrest. Dev Biol 136: 537-545.

30. Eppig JJ, O'Brien MJ (1996) Development in vitro of mouse oocytes from primordial follicles. Biol Reprod 54: 197-207.

31. Heikinheimo O, Gibbons WE (1998) The molecular mechanisms of oocyte

maturation and early embryonic development are unveiling new insights into reproductive medicine. Mol Hum Reprod 4: 745-756.

32. Moor RM, Dai Y, Lee C, Fulka J Jr (1998) Oocyte maturation and embryonic failure. Hum Reprod Update 4: 223-236.

33. Trounson A, Anderiesz C, Jones G (2001) Maturation of human oocytes in vitro and their developmental competence. Reproduction 121: 51-75.

34. Ebner T, Moser M, Sommergruber M, Puchner M, Wiesinger R, et al. (2003) Developmental competence of oocytes showing increased cytoplasmic viscosity. Hum Reprod 18: 1294-1298.

35. De Sutter P, Dozortsev D, Qian C, Dhont M (1996) Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. Hum Reprod 11: 595-597.

36. Alikani M, Palermo G, Adler A, Bertoli M, Blake M, et al. (1995) Intracytoplasmic sperm injection in dysmorphic human oocytes. Zygote 3: 283-288.

37. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H (2004) The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod 19: 1591-1597.

38. Serhal PF, Ranieri DM, Kinis A, Marchant S, Davies M, et al. (1997) Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod 12: 1267-1270.

39. Loutradis D, Drakakis P, Kallianidis K, Milingos S, Dendrinos S, et al. (1999) Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. Fertil Steril 72: 240-244.

40. Meriano JS, Alexis J, Visram-Zaver S, Cruz M, Casper RF (2001) Tracking of oocyte dysmorphisms for ICSI patients may prove relevant to the outcome in subsequent patient cycles. Hum Reprod 16: 2118-2123.

41. Krisher RL (2004) The effect of oocyte quality on development. J Anim Sci 82: E14-E23.

42. Sirard MA, Desrosier S, Assidi M (2007) In vivo and in vitro effects of FSH on oocyte maturation and developmental competence. Theriogenology 68: S71-S76.

43. Pavlok A, Kopecny V, Lucas-Hahn A, Niemann H (1993) Transcriptional activity and nuclear Ultrastructure of 8-cell bovine embryos developed by in vitro maturation and fertilization of oocytes from different growth categories of antral follicles. Mol Reprod Dev 35: 233-243.

44. Lonergan P, Monaghan P, Rizos D, Boland MP, Gordon I (1994) Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and culture in vitro. Mol Reprod Dev 37: 48-53.

45. De Sousa PA, Caveney A, Westhusin ME, Watson AJ (1998) Temporal patterns of embryonic gene expression and their dependence on oogenic factors. Theriogenology 49: 115-128.

46. Fair T, Murphy M, Rizos D, Moss C, Martin F, et al. (2004) Analysis of differential maternal mRNA expression in developmentally competent and incompetent bovine two-cell embryos. Mol Reprod Dev 67: 136-144.

47. Pennetier S, Uzbekova S, Perreau C, Papillier P, Mermillod P, et al. (2004) Spatio-temporal expression of the germ cell marker genes MATER, ZAR1, GFD9, BMP15, and VASA in adult bovine tissues, oocytes, and preimplantation embryos. Biol Reprod 71: 1359-1366.

48. Alizadeh Z, Kageyama S, Aoki F (2005) Degradation of maternal mRNA in mouse embryos: Selective degradation of specific mRNAs after fertilization. Mol Reprod Dev 72: 281-290.

49. Braude P, Bolton V, Moore S (1988) Human gene expression first occurs between the four and eight-cell stages of preimplantation development. Nature 332: 459-461.

50. Renard JP, Baldacci P, Richoux-Duranthon V, Pournin S, Babinet C (1994) A maternal factor affecting mouse blastocyst formation. Development 120: 797-802.

51. Guerif F, Le Gouge A, Giraudeau B, Poindron J, Bidault R, et al. (2007) Limited value of morphological assessment at days 1 and 2 to predict blastocyst development potential: a prospective study based on 4042 embryos. Hum Reprod 22: 1973-1981.

52. Rijnders PM, Jansen CA (1998) The predictive value of day 3 embryo morphology regarding blastocyst formation, pregnancy and implantation rate after day 5 transfer following in-vitro fertilization or intracytoplasmic sperm injection. Hum Reprod 13: 2869-2873.

53. Milki AA, Hinckley MD, Gebhardt J, Dasig D, Westphal LM, et al. (2002) Accuracy of day 3 criteria for selecting the best embryo. Fertil Steril 77: 1191-1195.

54. Magli MC, Jones GM, Gras L, Gianaroli L, Korman I, et al. (2000) Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts in vitro. Hum Reprod 15: 1781-1786.

55. Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, et al. (2004) Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod 19: 2849-2858.

56. García JI, Noriega-Portella L, Noriega-Hoces L (2011) Effect of vitrification procedure on chromosomal status of embryos achieved from vitrified and fresh oocytes. Health 3: 467-476.