REVIEW

Morphological criteria of oocyte quality

Kristina Lasienė, Aleksandras Vitkus, Angelija Valančiūtė, Vidmantas Lasys[1]
Department of Histology and Embryology, Kaunas University of Medicine,
[1]Department of Anatomy and Physiology, Lithuanian Veterinary Academy, Lithuania

Key words: *oocytes; quality; morphological criteria.*

Summary. *In vitro fertilization technology consists of the selection and fertilization of oocytes, the production and transplantation of embryos to recipients. The quality of oocytes has a direct impact on the fertilization and developmental competence of oocytes. Criteria that show the quality of oocytes are subdivided into morphological, cellular, and molecular. The aim of this article was to review the morphological criteria that are used for estimation of the quality of oocytes before their fertilization in vitro. These criteria include the evaluation of the structure of oocyte: cumulus complex, oocyte cytoplasm, polar body, perivitelline space, zona pellucida, and meiotic spindle.*

Introduction

The human and animal reproductive technologies (from the maturation and fertilization of oocytes in vitro to the cloning of animals) have been particularly intensively developed in recent decades. The basic object used in the procedures of these technologies is oocytes. The quality of oocytes has the greatest influence on results of the monospermic fertilization, early development, and implantation of embryos. Therefore, the quality of oocytes can be a determining factor in the fertilization of oocytes, culture of high-quality embryos, and treatment of the infertility. The aim of this article was to review the morphological criteria, determining the quality of oocytes.

Oocyte meiotic maturation

Maturation of oocyte includes two interrelated processes: maturation of nucleus and cytoplasm.

Mammalian oocytes are seen within ovarian follicles at the diplotene stage of the first meiotic prophase.

Under stimulation by the excess of pituitary luteinizing hormone (LH) in vivo, the oocyte reinitiates meiosis. The nucleus of oocyte changes its structure. The nuclear membrane of oocyte disappears (1). The microtubules become organized into a bipolar spindle, and all chromosomes align at the cell equator. The first meiotic division continues in oocyte; after this division, the first polar body separates, and it enters the perivitelline space. Then the second meiotic division takes place and stops in the metaphase II. This process is known as the maturation of oocyte nucleus (2).

In order that the oocyte would be successfully fertilized and a new body would develop, the nucleus and cytoplasm of oocyte must be mature at the same time (2, 3). If the cytoplasm of oocytes is immature, the embryo cannot develop normally after fertilization (4). To date, only one method is known to determine the maturity of oocyte cytoplasm. This is the observation of oocyte fertilization and embryo development in vitro (3). Therefore, the influence of various environmental factors and maturation media on the maturity of the oocyte nucleus and cytoplasm in vitro is assessed only by the number of fertilized oocytes and developing embryos.

Influence of oocyte quality on embryo development

Sirard et al. (5) pointed out the influence of the oocytes quality on the resumption of meiosis, cleavage of zygote, embryo development to blastocyst stage, the uterine implantation, and healthy offspring birth. Cytoplasm changes, which accompany the oocyte growth, include mRNA transcription and protein synthesis (6, 7). These processes are necessary for the meiotic maturation of oocyte, activation of the zygotic genome, and blastocyst formation (8, 9). Oocyte is a

Correspondence to K. Lasienė, Department of Histology and Embryology, Kaunas University of Medicine, A. Mickevičiaus, 44307 Kaunas, Lithuania. E-mail: krislasi@itc.kmu.lt

Table. **The criteria showing the quality of oocyte (14)**

Criterion	Parameters	References
Cumulus-oocyte complex	Compactness and thickness of the cumulus investment, brightness of the cytoplasm	Blondin and Sandard 1995; Warriach and Chohan 2004; Nagano et al., 2006
Cytoplasm	Granularity, coloration, regions of organelle clustering	Serhal ir kt., 1997; Balaban et al., 1998; Kahraman et al., 2000
Polar body	Shape (round or ovoid), size (large or small), surface (smooth or rough), cytoplasm (intact or fragmented)	Ebner ir kt., 2000; Ciotti et al., 2004
Zona pellucida	Thickness, structure	Gabrielsen et al., 2001
Perivitelinis tarpas	Size (normal or increased), the presence or absence of grain	De Sutter et al., 1996; Hassan-Ali et al., 1998
Mejozinė verpstė	Location and refraction	Wang et al., 2001; Moon et al., 2003; Rienzi et al., 2003

complex cell with many organelles, each of which must be in the appropriate state for the maturation of cell (10). Any dysfunction or dislocation of oocyte components, such as meiotic spindle, cortical granules, or mitochondria, can decrease the oocyte viability and has a crucial impact on embryo development and quality (11–13).

The scientists considering the importance of the oocyte quality in the development of embryo have started intensively to search for reliable criteria, which were divided into morphological, cellular, and/or molecular. In this article, we will review the morphological features, which are traditionally used to determine the quality of oocytes (Table 1).

Morphological criteria of oocyte quality

Before the fertilization in vitro, the quality of oocytes is usually evaluated according to the structure of cumulus-oocyte complexes. This method is simple and gives information about the quality of oocytes.

The quality of oocyte can be determined more specifically by the evaluation of the characteristics of cumulus-oocyte complex structure, oocyte cytoplasm, polar body, perivitelline space, zona pellucida, and meiotic spindle at the same time (14).

Cumulus-oocyte complex

The cumulus-oocyte complexes collected from ovarian follicles are classified according to the compactness of the cumulus and characteristics of oocyte cytoplasm. For example, the bovine cumulus-oocyte complexes are grouped into three categories of quality:

1. Category A cumulus-oocyte complex. Oocyte is surrounded by a compact cumulus consisting of at least five layers of cells. The oocyte cytoplasm is almost transparent, homogeneous, or a dark ring is seen on the periphery of cytoplasm.
2. Category B cumulus-oocyte complex. Cumulus is less compact. Oocyte has a dark, slightly granular cytoplasm.
3. Category C cumulus-oocyte complex. Cumulus cells are expanded with a dark cytoplasm. Oocyte has a dark, granular cytoplasm (15, 16) (Fig. 1).

Some scientists reported that the oocytes, which are in the cumulus-oocyte complexes of category B, showed the highest developmental competence. After in vitro fertilization, more oocytes developed from the category B cumulus-oocyte complexes than from the category A and C cumulus-oocyte complexes (B>A>C) (17–19), but the reasons for this phenomenon are not clear yet. The number of the cumulus cell layers is a significant factor in determining the quality of oocytes. It is reported that the oocyte quality is better when the oocyte is surrounded by more layers of cells (15, 20–22). We did not find any data in the available literature about the grouping of human cumulus-oocyte complexes according to the quality.

Cytoplasm and polar body

Before the intracytoplasmic sperm injection, after the evaluation of quality of the cumulus-oocyte complex, the

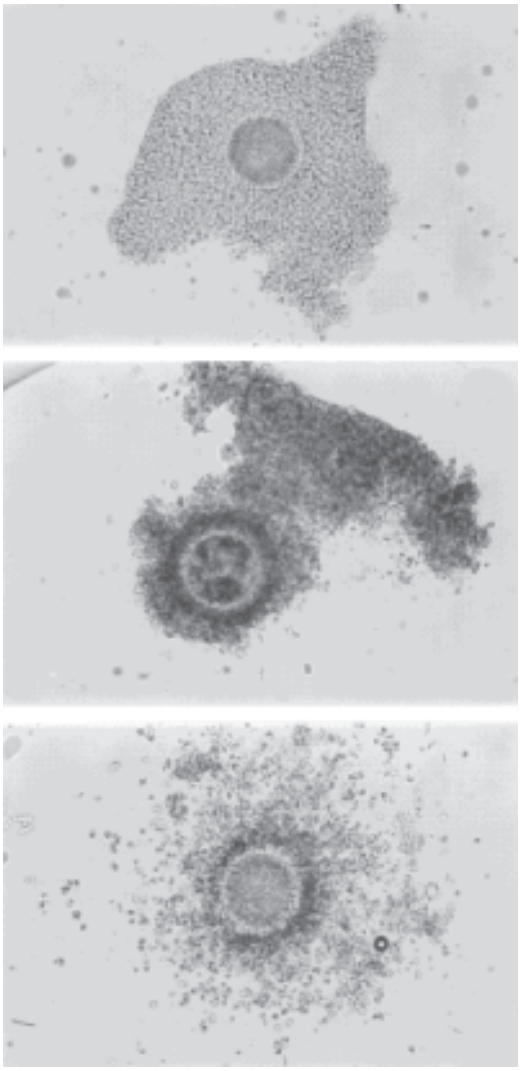


Fig. 1. **Different categories of cumulus-oocyte complexes (17)**

Category A. Oocyte is surrounded by a compact cumulus. The cytoplasm is homogeneous. Category B. Oocyte is surrounded by a less compact and obviously darker cumulus. The cytoplasm is dark and slightly granular. Category C. Oocyte is surrounded by dispersed cumulus cells. Oocyte has a granular cytoplasm.

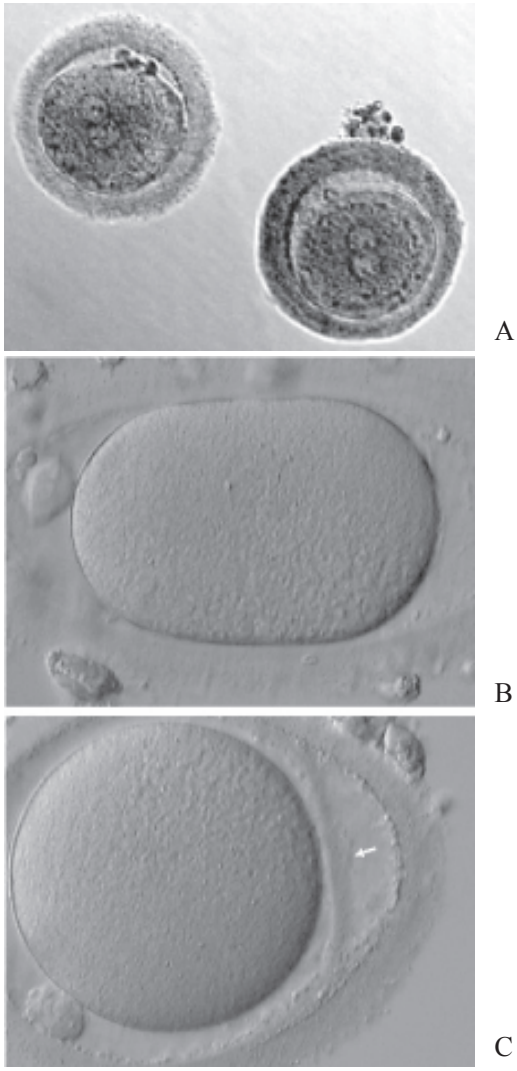


Fig. 2. **Oocytes with a normal structure and oocytes with some structural changes**

The normal fertilized oocyte (A, left). The fertilized oocyte with a dark zona pellucida, large perivitelline space, and a dark granular cytoplasm (A, right) (23). Oocyte with oval zona pellucida and oval cytoplasm (B). Oocyte with a round cytoplasm and oval zona pellucida (C) (26).

cumulus cells are removed from the oocyte. This provides more opportunities for more accurate assessment of oocyte structural features under the light microscope.

The cytoplasm of human oocyte is classified according to coloration, granularity (large or small granules; homogenous or clustering distribution of granules; in center or in the periphery of oocyte), size of perivitelline space, and distribution of organelles (vacuoles, endoplasmic reticulum).

According to these morphological criteria, human oocytes are classified into: 1) normal oocytes, 2) oocytes with extracytoplasmic abnormalities (dark zona pellucida and large perivitelline space), 3) oocytes with intracytoplasmic abnormalities (dark or granular cytoplasm and cytoplasmic fragments), 4) shape abnormalities, and 5) oocytes with multiple abnormalities (23) (Fig. 2).

Some studies have revealed that the embryos developed from the oocytes of normal structure (Group

1) undergo the best uterine implantation. Oocytes with intracytoplasmic abnormalities fertilized worse, and a high frequency of aneuploidy was found in many cells of the developing embryos (aneuploidy is defined as an abnormal number of one or more chromosomes) (23–25, 27).

Nagano with colleagues (28) studied the relation between cytoplasmic features and development of bovine oocytes, derived from small tertiary follicles, after fertilization. They found that a dark cytoplasm indicated an accumulation of lipids and good developmental potential of oocytes after in vitro fertilization. They found that a light-colored cytoplasm indicated a low density of organelles and poor developmental potential. A black cytoplasm indicated aging and low developmental potential.

Morphology of the first polar body indicates the postovulatory age of the human oocyte. The degeneration of the first polar body shows the aged oocyte (29). Some morphological criteria of the first polar body such as the shape (round or ovoid), size (large or small), surface (smooth or rough), and integrity of cytoplasm (intact or fragmented) can be used to predict the oocyte quality (30, 31). Oocytes with a fragmented first polar body developed worse (55.1%) after fertilization than those with a normal polar body (60.3%) (24).

Perivitelline space and zona pellucida

The perivitelline space of human oocytes may vary in size (enlarged or not) and content (presence or absence of the grain) (24, 32). It was estimated that oocytes with a large perivitelline space developed worse after intracytoplasmic sperm injection (37.5%) than those with normal perivitelline space (60.3%) (24). Oocytes, which had the large grains in the perivitelline space, developed worse (59%) after fertilization than those without grains (71.1%) (32).

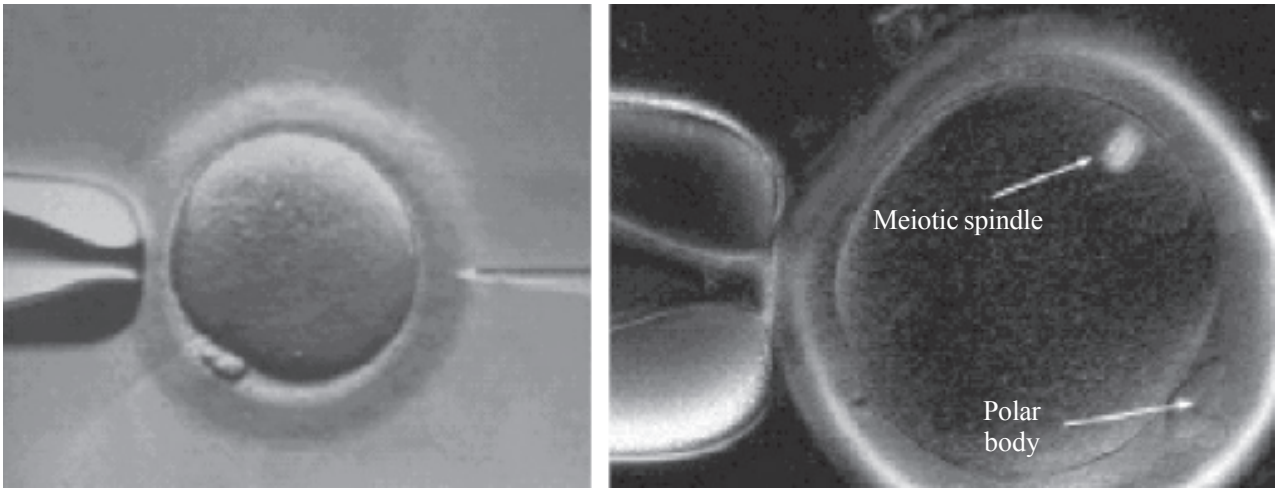
The thickness of the zona pellucida varies from 10 to 31 μm. It is not related to the cytoplasm diameter. The thickness of the zona pellucida influences sperm penetration. The oocytes are fertilized best in vitro when the thickness of the zona pellucida was less than 18.6 μm. The thick zona pellucida (22 μm and thicker) could be an indicator for the use of transplantation of embryos produced by intracytoplasmic sperm injection for infertile patients (33). The thickness of the zona pellucida had no influence on the embryo development after intracytoplasmic sperm injection (34).

Meiotic spindle

Meiotic spindle has a significant influence on the correct alignment of chromosomes in the oocyte and their segregation during meiosis. Parameters of meiotic spindle (location and refraction) are often used to determine the quality of oocytes.

Meiotic spindle is studied by a confocal microscope, after the fixation and staining of oocyte with some fluorescent dyes, and this causes the death of oocyte. Therefore, in the previous experiments of intracytoplasmic sperm injection, the angle of the needle was adjusted in relation to the position of the first polar body to prevent the injury of meiotic spindle and chromosomes. However, the location of the first polar body is not a specific indicator of position of the metaphase spindle in oocytes (35–37).

Nowadays, the meiotic spindle can be examined and its location can be determined by means of polarizing microscopy avoiding the damage to oocyte.



A B

Fig. 3. **Meiotic spindle and polar body in human oocytes imaged with the light microscope (A) and the Polscope (B) (39)**

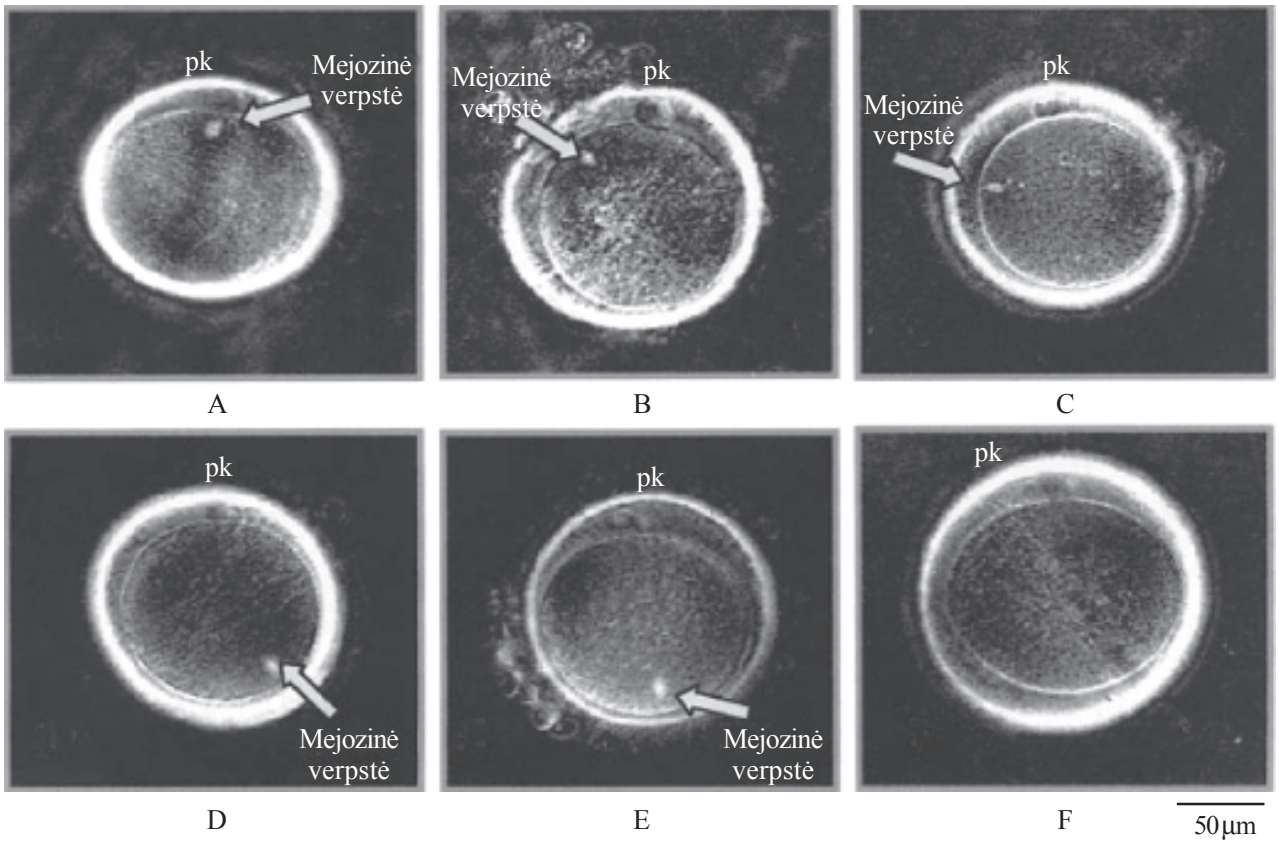


Fig. 4. **Different location of meiotic spindle in oocytes (40)**

A – oocyte has the spindle located under the first polar body; B – oocyte has the spindle located between 0° and 60° relative to the polar body; C – oocyte has the spindle located between 60° and 120° relative to the polar body; D – oocyte has the spindle located between 120° and 180° relative to the polar body; E – oocytes had the spindle located exactly at 180° relative to the polar body; F – oocyte has no visible spindle. PB – polar body. Arrow indicates the spindle. Bar, 50 µm.

The birefringence of meiotic spindle can be studied by a Polscope microscope (Polscope, Cambridge, MA, USA) (Fig. 3). The scientists estimated that the oocytes with birefringent spindle had higher developmental potential after fertilization in vitro or intracytoplasmic sperm injection than oocytes without birefringent spindle (37, 39–42).

Moon et al. (2003) estimated by Polscope that the location of meiotic spindle could vary in oocyte (Fig. 4). Therefore, it can be damaged when the "blind" intracytoplasmic sperm injection is made into human oocytes. However, no relationship was found between the deviation of the meiotic spindle from the polar body within oocytes and oocyte developmental competence (37, 40, 44).

Battaglia et al. (1996) reported that the number of oocytes with spindle abnormalities (abnormal placement of tubulin) increased with increasing women's age (40 year olds and older). This abnormality determines displacement of one or more chromosomes from the metaphase plate during the second meiotic division. This process is a contributing factor to aneuploidy in the cells of embryos (43). It was proposed that the meiotic spindle disrupts in the "old" oocytes (37).

Therefore, the precise analysis of oocyte morphology, in combination with spindle visualization using the Polscope, could be an informative, noninvasive, and reliable factor in the evaluation of oocyte quality and embryonic developmental competence.

Conclusion

Before any manipulation procedures and fertilization of oocytes, the quality of oocytes must be estimated exactly, because this has a high influence on embryo development. The suitability of oocytes for fertilization in vitro must be estimated most precisely using a complex evaluation of the characteristics of cumulus-oocyte complex structure, oocyte cytoplasm, polar body, perivitelline space, zona pellucida, and meiotic spindle at the same time.

Oocitų kokybės morfologiniai požymiai

Kristina Lasienė, Aleksandras Vitkus, Angelija Valančiūtė, Vidmantas Lasys[1]
Kauno medicinos universiteto Histologijos ir embriologijos katedra,
[1]Lietuvos veterinarijos akademijos Anatomijos ir fiziologijos katedra

Raktažodžiai: oocitai, kokybė, morfologiniai požymiai.

Santrauka. Pagalbinio apvaisinimo technologija apima oocitų atrinkimą, jų apvaisinimą in vitro, embrionų auginimą bei jų transplantaciją į gimdą. Nuo oocitų kokybės tiesiogiai priklauso jų apvaisinimo ir tolesnio embrionų auginimo in vitro sėkmė. Apie oocito tinkamumą tolesnėms procedūroms sprendžiama pagal jo požymius, kurie skirstomi į morfologinius, ląstelinius bei molekulinius. Šiame straipsnyje siekiame apžvelgti morfologinius kriterijus, kurie galėtų būti taikomi oocitų kokybei įvertinti prieš atliekant jų apvaisinimą in vitro – tai oocito – spindulinio vainiko komplekso struktūros, oocito citoplazmos, polocito, perivitelinio tarpo, skaidriosios zonos ir mejozinės verpstės įvertinimas.

Adresas susirašinėti: K. Lasienė, KMU Histologijos ir embriologijos katedra, A. Mickevičiaus 9, 44307 Kaunas
El. paštas: krislasi@itc.kmu.lt

References

1. Fan HY, Sun QY. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. Biol Reprod 2004;70:535-47.
2. Sun QY, Nagai T. Molecular mechanisms underlying pig oocyte maturation and fertilization. J Reprod Dev 2003;49: 347-59.
3. Krisher RL. The effect of oocyte quality on development. J Anim Sci 2004;82:E14-23.
4. Vassena R, Mapletoft RJ, Allodi S, Singh J, Adams GP. Morphology and developmental competence of bovine oocytes relative to follicular status. Theriogenology 2003;60:923-32.
5. Sirard MA, Richard F, Blondin P, Robert C. Contribution of the oocyte to embryo quality. Theriogenology 2006;65:126-36.
6. Sirard MA, Florman HM, Leibfried-Rutledge ML, Barnes FL, Sims ML, First NL. Timing of nuclear progression and protein synthesis necessary for meiotic maturation of bovine oocytes. Biol Reprod 1989;40:1257-63.
7. Kastrop PM, Bevers MM, Destree OH, Kruip TA. Protein synthesis and phosphorylation patterns of bovine oocytes maturing in vivo. Mol Reprod Dev 1991;29:271-5.
8. Barnes FL, First NL. Embryonic transcription in in vitro cultured bovine embryos. Mol Reprod Dev 1991;29:117-23.
9. De Sousa PA, Caveney A, Westhusin ME, Watson AJ. Temporal patterns of embryonic gene expression and their dependence on oogenetic factors. Theriogenology 1998;49: 115-28.
10. Trimarchi JR, Keefe DL. Assessing the quality of oocytes derived from in vitro maturation: are we looking under the lamppost? Fertil Steril 2006;85:839-40.
11. Sun QY, Wu GM, Lai L, Park KW, Cabot R, Cheong HT, et al. Translocation of active mitochondria during pig oocyte maturation, fertilization and early embryo development in vitro. Reproduction 2001;122:155-63.
12. Coticchio G, Sereni E, Serrao L, Mazzone S, Iadarola I, Borini A. What criteria for the definition of oocyte quality? Ann NY Acad Sci 2004;1034:132-44.
13. Combelles CM, Racowsky C. Assessment and optimization of oocyte quality during assisted reproductive technology treatment. Semin Reprod Med 2005;23:277-84.
14. Wang Q, Sun QY. Evaluation of oocyte quality: morphological, cellular and molecular predictors. Reprod Fertil Dev 2007; 19:1-12.
15. Blondin P, Sirard MA. Oocyte and follicular morphology as determining characteristics for developmental competence in bovine oocytes. Mol Reprod Dev 1995;41:54-62.
16. Mayes MA, Sirard MA. The influence of cumulus-oocyte complex morphology and meiotic inhibitors on the kinetics of nuclear maturation in cattle. Theriogenology 2001;55:911-22.
17. De Wit AA, Wurth YA, Kruip TA. Effect of ovarian phase and follicle quality on morphology and developmental capacity of the bovine cumulus-oocyte complex. J Anim Sci 2000;78: 1277-83.
18. De Wit AA, Kruip TA. Bovine cumulus-oocyte-complex-quality is reflected in sensitivity for alpha-amanitin, oocyte-diameter and developmental capacity. Anim Reprod Sci 2001;65:51-65.
19. Boni R, Cuomo A, Tosti E. Developmental potential in bovine oocytes is related to cumulus-oocyte complex grade, calcium current activity, and calcium stores. Biol Reprod 2002;66:836-42.
20. Zeuner A, Muller K, Reguszynski K, Jewgenow K. Apoptosis within bovine follicular cells and its effect on oocyte development during in vitro maturation. Theriogenology 2003;59: 1421-33.
21. Warriach HM, Chohan KR. Thickness of cumulus cell layer is a significant factor in meiotic competence of buffalo oocytes. J Vet Sci 2004;5:247-51.
22. Yuan YQ, Van Soom A, Leroy JL, Dewulf J, Van Zeveren A, de Kruif A, et al. Apoptosis in cumulus cells, but not in oocytes, may influence bovine embryonic developmental competence. Theriogenology 2005;63:2147-63.
23. Balaban B, Urman B, Sertac A, Alatas C, Aksoy S, Mercan R. Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. Hum Reprod 1998;13(12):3431-3.
24. Xia P. Intracytoplasmic sperm injection: correlation of oo-

cyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. Hum Reprod 1997;12:1750-5.
25. Kahraman S, Yakin K, Donmez E, Samli H, Bahce M, Cengiz G, et al. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Hum Reprod 2000;15:2390-3.
26. Ebner T, Shebl O, Moser M, Sommergruber M, Tews G. Developmental fate of ovoid oocytes. Hum Reprod 2007;22(12): 3077-82.
27. Serhal PF, Ranieri DM, Kinis A, Marchant S, Davies M, Khadum IM. Oocyte morphology predicts outcome of intracytoplasmic sperm injection. Hum Reprod 1997;12:1267-70.
28. Nagano M, Katagiri S, Takahashi Y. Relationship between bovine oocyte morphology and in vitro developmental potential. Zygote 2006;14:53-61.
29. Eichenlaub-Ritter U, Schmiady H, Kentenich H, Soewarto D. Recurrent failure in polar body formation and premature chromosome condensation in oocytes from a human patient: indicators of asynchrony in nuclear and cytoplasmic maturation. Hum Reprod 1995;10:2343-9.
30. Ebner T, Yaman C, Moser M, Sommergruber M, Feichtinger O, Tews G. Prognostic value of first polar body morphology on fertilization rate and embryo quality in intracytoplasmic sperm injection. Hum Reprod 2000;15:427-30.
31. Ebner T, Moser M, Sommergruber C, Yaman C, Pfleger U, Tews G. First polar body morphology and blastocyst formation rate in ICSI patients. Hum Reprod 2002;17:2415–8.
32. Hassan-Ali H, Hisham-Saleh A, El-Gezeiry D, Baghdady I, Ismaeil I, Mandelbaum J. Perivitelline space granularity: a sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. Hum Reprod 1998;13:3425-30.
33. Bertrand E, Van den Bergh M, Englert Y. Does zona pellucida thickness influence the fertilization rate? Hum Reprod 1995; 10(5):1189-93.
34. Gabrielsen A, Lindenberg S, Petersen, K. The impact of the zona pellucida thickness variation of human embryos on pregnancy outcome in relation to suboptimal embryo development. A prospective randomized controlled study. Hum Reprod 2001;16:2166-70.
35. Cooke S, Tyler JP, Driscoll GL. Meiotic spindle location and identification and its effect on embryonic cleavage plane and early development. Hum Reprod 2003;18:2397-405.
36. Silva CP, Kommineni K, Oldenbourg R, Keefe DL. The first polar body does not predict accurately the location of the metaphase 2 meiotic spindle in mammalian oocytes. Fertil Steril 1999;71:719-21.
37. Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. The spindle observation and its relationship with fertilization after intracytoplasmic sperm injection in living human oocytes. Fertil Steril 2001;75:348-53.
38. Fletcher-Holmes DW, Boutin C, Hoyt C. Assessing oocyte quality using birefringence imaging. Oosight™ imaging system. 2002. Available from: URL: http://www.cri-inc.com/files/Oosight%20WP%20Assessing%20Oocyte%20Qual%20w%20Biref%20Img%2002.pdf
39. Wang WH, Meng L, Hackett RJ, Keefe DL. Developmental ability of human oocytes with or without birefringent spindles imaged by Polscope before insemination. Hum Reprod 2001; 16:1464-8.
40. Moon JH, Hyun CS, Lee SW, Son WY, Yoon SH, Lim JH. Visualization of the metaphase II meiotic spindle in living human oocytes using the Polscope enables the prediction of embryonic developmental competence after ICSI. Hum Reprod 2003;18:817-20.
41. Shen Y, Stalf T, Mehnert C, De Santis L, Cino I, Tinneberg HR, et al. Light retardance by human oocyte spindle is positively related to pronuclear score after ICSI. Reprod Biomed Online 2006;12(6):737-51.
42. Fang C, Tang M, Li T, Peng WL, Zhou CQ, Zhuang GL, et al. Visualization of meiotic spindle and subsequent embryonic development in in vitro and in vivo matured human oocytes. J Assist Reprod Genet 2007;24(11):495-9.
43. Battaglia DE, Goodwin P, Klein NA, Soules MR. Influence of maternal age on meiotic spindle in oocytes from naturally cycling women. Hum Reprod 1996;11:2217–22.
44. Rienzi L, Ubaldi F, Martinez F, Iacobelli M, Minasi MG, Ferrero S, et al. Relationship between meiotic spindle location with regard to the polar body position and oocyte developmental potential after ICSI. Hum Reprod 2003;18:1289-93.

Received 23 November 2007, accepted 3 July 2009