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The predictive value of pronuclear morphology of zygotes in the assessment of human embryo quality

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BACKGROUND: Recent studies have shown that zygote morphology could be used for the assessment of human embryo quality. Pronuclear (PN) morphology is based on certain distinct features seen in zygotes 16–18 h after fertilization. In the present study PN stage morphology was assessed and combined with a single embryo transfer in order to investigate whether currently used zygote classifications are able to predict embryo quality and implantation rates. METHODS AND RESULTS: Zygotes were analysed according to two different classification systems. In the first, a total of 764 zygotes was analysed according to the degree of polarization of nucleolar precursor bodies (NPB). Zygotes with unpolarized PN (i.e. scattered localization of NPB) showed significantly slower ($P < 0.005$) cleavage rates (38.9%) than zygotes having at least one pronucleus polarized (57.3% and 54%). However, there was no difference in the pregnancy rate in 105 single embryo transfers between the groups. The appearance of a cytoplasmic halo was related to embryo morphology. Embryos derived from halo-positive zygotes had significantly better ($P < 0.05$) morphology (60.9%) compared to halo-negative derived embryos (52.2%), but in terms of pregnancy rates no difference was found. A total of 1520 zygotes was analysed according to a second classification system, which was based on the number and distribution of NPB. In the comparative analysis, none of the six different classes produced superior quality embryos or higher pregnancy rates in 144 single embryo transfers. CONCLUSIONS: Our results indicate that there are no significant differences in embryo quality or implantation/pregnancy rates between proposed zygote classes.

Key words: embryo quality/human/IVF/zygote

Introduction

Various embryo scoring systems have been described to assess the developmental potential of human day 2 or day 3 preimplantation embryos. In the most commonly used systems the blastomere cleavage rate (i.e. number of blastomeres), the shape and size of the blastomeres and the amount of anucleated fragments are estimated. Several studies have shown that regardless of minor fragmentation, the optimal cleavage rate would be the most important criteria when selecting embryos for transfer (Ziebe *et al*., 1997; Sakkas *et al*., 1998). Variation in zona thickness, embryo symmetry and the presence of multinucleated blastomeres have also been shown to affect implantation rates (Cohen *et al*., 1989; Ziebe *et al*., 1997; Pelinck *et al*., 1998).

Recently, some investigators have sought reliable non-invasive scoring systems for zygotes (Scott and Smith, 1998; Tesarik and Greco, 1999; Scott *et al*., 2000). The scoring system would be valuable in countries where legislation prohibits the culture of more than three embryos at a time and the actual selection of the transferable embryos is done at pronuclear (PN) stage (Ludwig *et al*., 2000). The zygote scoring systems are based on morphological aspects and localization of the PN as well as the cytoplasmic appearance of the zygote. The main characteristics analysed are the position and the number of nucleolar precursor bodies (NPB) and the existence of a cytoplasmic halo (Scott and Smith, 1998; Tesarik and Greco, 1999).

In 1998, Scott and Smith reported a zygote scoring system based predominantly on the appearance of cytoplasmic haloes and the polarization stage of NPB (Scott and Smith, 1998). Their results indicated that the implantation rate of halo-positive zygotes with polarized distribution of NPB and progression to 2-cell stage 24–26 h after fertilization was higher than the implantation rate of embryos with scattered distribution of NPB and homogenous cytoplasm. Similar results were later obtained by a German group (Ludwig *et al*., 2000).

Another classification system proposed by Tesarik and Greco (1999) included six different zygote categories according to the number and distribution of NPB. Using this classification, zygotes were divided into one normal (pattern 0) and five potentially abnormal (patterns 1–5) groups. Zygotes in the normal group had both PN at the same developmental stage, i.e. all NPB are either polarized or unpolarized at the time of evaluation. They reported a clinical pregnancy rate of 50% when at least one normal embryo was transferred and 9% when only abnormal embryos were transferred (Tesarik and Greco, 1999). Subsequent studies by them and others have supported this hypothesis (Tesarik *et al.*, 2000; Wittemer *et al.*, 2000).

The main problem associated with all afore-mentioned studies is that two or more embryos have been transferred at a time. Therefore, the correct estimation of the implantation ability of different embryos is not possible. We have recently published good results in elective single embryo transfers (Vilska *et al.*, 1999). In patients who had at least two embryos available and only one embryo was selected for transfer, the pregnancy rate was 29.7% which was comparable with the pregnancy rate in two embryo transfers (29.4%) (Vilska *et al.*, 1999). A high proportion of elective single embryo transfers (at present up to 50%) allows us to evaluate correctly different NPB scoring systems. The aim of this study was to find out whether the development of good quality embryos could be predicted by a single microscopic observation at the PN stage.

Materials and methods

A total of 1520 zygotes from 245 IVF and intracytoplasmic sperm injection (ICSI) cycles was investigated in the Infertility Clinic of the Family Federation of Finland (Clinic A) and Helsinki University Central Hospital (Clinic B) between September 1999 and August 2000. In 191 cases (78%) only one embryo was selected for transfer. The selection of couples for single embryo transfer was based on their infertility history, previous treatments, age and quality of embryos achieved. For calculation of pregnancy rates only single embryo transfers were used.

Ovarian stimulation regimen, oocyte collection, IVF and ICSI procedure and embryo transfer were similar in both clinics and have been reported elsewhere (Vilska *et al.*, 1999). Culture media for zygotes and embryos were IVF medium (MediCult, Denmark) and Sydney IVF Fertilization and Cleavage Medium (Cook IVF, Australia). The oocytes were checked for the presence of PN and polar bodies 16–18 h after the microinjection or insemination. The PN morphology was analysed using a ×20 objective on Nikon Diaphot® TMD inverted microscope with Hoffman modulation contrast optics. For accurate estimation of zygote morphology, each fertilized oocyte was rotated using a needle connected to the Narishige micromanipulator MO 188® until both PN were clearly visible. Images of zygotes were recorded and later evaluated independently by three embryologists. Only oocytes having two equally sized PN were included in this study. For each fertilized oocyte, the localization and the number of NPB and the existence of clear cortical cytoplasm (cytoplasmic halo) were recorded. The fertilized oocytes and embryos were maintained in separate 20 µl drops of culture medium.

Two different classification systems were used to describe the polarization and number of NPB. The first scoring system has been described by Scott and Smith and includes only the polarization of NPB and the appearance of a cytoplasmic halo (Scott and Smith, 1998). In class I all NPB in both PN are polarized. In class II, NPB in one pronucleus are polarized, whereas NPB in the other pronucleus are scattered and in class III the NPB of both PN are scattered. NPB are classified as polarized when all NPB are aligned in a row at the PN junction.

In the second classification system zygotes were allocated to six different classes depending on the number and the distribution of NPB (Tesarik and Greco, 1999). In pattern 0 zygotes, NPB in both PN are either polarized and the number of NPB <7 or unpolarized and the number of NPB $\geqslant 7$. Pattern 1 zygotes have a marked difference (>3) in the number of NPB. Pattern 2 zygotes have <7 NPB without polarization in at least one of the PN. Pattern 3 zygotes have $\geqslant 7$ NPB with polarization in at least one of the PN. Pattern 4 zygotes have <3 NPB in at least one of the PN. Pattern 5 zygotes have polarized distribution in one pronucleus and unpolarized in the other. Evaluation of fertilized oocytes according to the first scoring system by Scott and Smith (1998) was carried out only in Clinic A, whereas the second classification system was used in both clinics. Multinucleated embryos were recorded only in Clinic B.

Embryo quality was evaluated 40–42 h (day 2 transfer) or 65–67 h (day 3 transfer) after fertilization and embryo selection was based solely on embryo quality. Embryos were scored according to the commonly used morphological criteria: grade 1: no fragments and equal blastomeres, grade 2: $<20\%$ fragmentation, grade 3: unequal blastomeres and/or 20–50% fragmentation, grade 4: $>50\%$ fragmentation. The implantation rate (clinical pregnancy rate), pregnancy rate (deliveries and on-going pregnancies), percentage of morphologically good embryos (grade 1 and 2 embryos), embryos with at least three blastomeres 40–42 h after the fertilization and embryos with multinucleated blastomeres (MNB) were calculated for each class of zygotes and for halo-positive and halo-negative embryos. For the calculation of good quality embryos, only the quality of embryos on day 2 was considered whereas for implantation and pregnancy rates both the second and third day embryo transfers were used. IVF and ICSI embryos were combined in this study.

Comparison of the implantation rate, pregnancy rate, the percentage of morphologically good embryos, embryos with MNB and embryos containing three or more blastomeres 40–42 h after the fertilization between the different classes of zygotes was performed using χ^2 test.

Results

A total of 764 fertilized oocytes were evaluated according to the first classification system. Of the zygotes 44% ($n = 337$) belonged to class I, 36% ($n = 278$) to class II and 20% ($n = 149$) to class III. The implantation rates and pregnancy rate after single embryo transfers, the percentage of morphologically good embryos and embryos with at least three blastomeres at 40–42 h after the fertilization are shown in Table I. The only significant difference found between these groups was the cleavage rate. The number of embryos having three or more blastomeres 40–42 h after fertilization in class III (38.9%) was significantly ($P < 0.005$) lower than either of the first two classes (57.3 and 54% respectively). However, the percentage of morphologically good embryos in all three classes was similar. In Clinic A 105 single embryo transfers were performed during the study period. A total of 48 of the transferred embryos (46%) belonged to class I, 41 embryos (39%) to class II and 16 embryos (15%) to class III. There was a tendency towards higher implantation for embryos belonging to the first two classes (implantation rate 29.2% for class I and 31.7% for class II) compared with embryos belonging to class III (18.8%), however the difference was not significant.

Table I. The percentage of embryos with three or more blastomeres 40–42 h after fertilization, embryo quality (i.e. those with less than 20% fragmentation), implantation rates and pregnancy rates after 105 single embryo transfers in different classes of zygotes ($n = 764$), evaluated according to the first classification system

Type of zygotes	*n*	⩾3 blastomeres (%)	Good morphology (%)	Implantation rate (%) (preg./ET)	Deliveries and ongoing pregnancies (%) (preg./ET)
I	337	57.3	58.6	29.2 (14/48)	22.9 (11/48)
II	278	54	58.2	31.7 (13/41)	29.3 (12/41)
III	149	38.9[a]	56.7	18.8 (3/16)	12.5 (2/16)

[a]$P < 0.005$ when compared with the first and the second classes.
ET = embryo transfer; preg. = pregnancies.

Table II. The percentage of embryos with three or more blastomeres 40–42 h after fertilization, embryo quality (i.e. those with less than 20% fragmentation), implantation rates and pregnancy rates after 105 single embryo transfers for embryos developed from halo-positive or halo-negative fertilized oocytes ($n = 764$)

Existence of halo	*n*	⩾3 blastomeres (%)	Good morphology (%)	Implantation rate (%) (preg./ET)	Deliveries and ongoing pregnancies (%) (preg./ET)
Positive	517	53.2	60.9	29.9 (23/77)	24.7 (19/77)
Negative	247	51	52.2[a]	25 (7/28)	21.4 (6/28)

[a]$P < 0.05$ when compared with the halo positive zygotes.
See Table I for abbreviations.

The presence of halo was recorded only in Clinic A. From 764 checked zygotes clear cortical cytoplasm existed in 517 (67.7%) zygotes. In the cohort of halo positive zygotes, some of the zygotes had symmetrical halo located homogeneously along the cortex of cytoplasm whereas some zygotes exhibited asymmetrical halo located predominantly on one side of the PN. The percentage of morphologically good embryos, embryos having at least three blastomeres 40–42 h after fertilization, implantation rate and pregnancy rate after single embryo transfers for embryos developed from halo positive and halo negative zygotes are shown in Table II. During the study period 77 (73%) cleavage stage embryos derived from halo-positive zygotes and 28 (27%) from halo-negative zygotes were transferred. The only difference between these two groups was the percentage of good quality embryos (equal blastomere size and <20% fragmentation) which was statistically higher ($P < 0.05$) in the halo positive zygotes (60.9%) compared with the halo negative zygotes (52.2%). The implantation or pregnancy rate did not differ significantly.

A total of 1520 zygotes was scored according to the second classification system (Tesarik and Greco, 1999). For 1178 zygotes (77%) single NPB pattern was attributed whereas for 342 zygotes (23%) two or more patterns were simultaneously described. For calculations only those embryos classified to a single NPB pattern were used. The percentage of zygotes developing into morphologically good cleaved embryos, those with three or more blastomeres 40–42 h after the fertilization, implantation rate and pregnancy rate after single embryo transfers for pattern 0 and non-pattern 0 groups, are shown in Table III. The implantation rate and ongoing pregnancy rate were 33.5% (64/191) and 27.7% (53/191) respectively after 191 single embryo transfers. Out of the 191 single embryo transfers 144 were performed with embryos characterized by single NPB pattern whereas 47 single embryo transfers were performed with embryos characterized simultaneously by two or more NPB patterns. From 144 single embryo transfers performed with embryos belonging only to single NPB class, 56 (39%) were performed with embryos derived from the pattern 0 zygotes and 88 (61%) derived from the non-pattern 0 (combined patterns 1–5). After the 144 single embryo transfers 47 (32.6%) clinical pregnancies and 39 (27.1%) deliveries or ongoing pregnancies were achieved. The implantation rate and pregnancy rate did not differ significantly between the two groups, being 33.9% (19/56) and 28.6% (16/56) for the pattern 0 and 31.8% (28/88) and 26.1% (23/88) for non-pattern 0 groups. Similarly, no difference in the proportions of embryos with three or more blastomeres, good morphology or embryos containing MNB (18.6 versus 18.8%) at 40–42 h after the fertilization between pattern 0 and non-pattern 0 groups was observed. Some differences were seen within the zygote classes (1–5) in terms of cleavage rate and embryo morphology, but these differences could be caused by the small sample size.

Discussion

Recent studies have suggested that embryo quality could be estimated based on zygote morphology (Scott and Smith, 1998; Tesarik and Greco, 1999; Scott *et al.*, 2000). In the classification systems used in the literature the localization and the number of NPB in PN as well as the appearance of a cytoplasmic halo are said to be predictive of embryo quality. In this study, we evaluated the predictive value of two different 2PN classifica-

Table III. The percentage of embryos with three or more blastomeres 40–42 h after fertilization, embryo quality (i.e. those with <20% fragmentation), implantation rates and pregnancy rates after 144 single embryo transfers for different patterns of zygotes ($n = 1178$), classified according to the second classification system

Pattern of the zygotes	*n*	⩾3 blastomeres (%)	Good morphology (%)	Implantation rate (%) (preg./ET)	Deliveries and ongoing pregnancies (%) (preg./ET)
0	440	65	63.9	33.9 (19/56)	28.6 (16/56)
1	87	62.1	83.3[c]		
2	135	53.3[a]	62.1		
3	112	69.6	68.8	31.8 (28/88)	26.1 (23/88)
4	36	52.8[b]	65.6		
5	368	69	71.5[d]		

[a]$P < 0.05$ when compared with the 0, 3 and 5 patterns.
[b]$P < 0.05$ when compared with the 5 pattern.
[c]$P < 0.05$ when compared with the 0, 2, 3, 4 and 5 patterns.
[d]$P < 0.05$ when compared with the 0 and 2 patterns.
See Table I for abbreviations.

tion systems on human cleavage stage embryo morphology and implantation rates in single embryo transfers.

The nucleolus is a distinct nuclear construct where ribosomal RNA are produced. In most mitotic cells the formation of an active nucleolus occurs through the fast fusion of separate small precursor nucleoli originally formed at the chromosomal locations of ribosomal genes. The development of a nucleolus in newly formed PN includes some specific features not found in normal mitotic cells (Tesarik *et al*., 1987; Tesarik and Kopecny, 1989a, 1989b, 1990). Several nucleolar precursor bodies are formed in both PN but they do not usually fuse to a single construct (Payne *et al*., 1997). Electron microscopy studies have shown that NPB in human zygotes consist of densely packed 3 nm thick protein filaments, for which the exact composition is unknown (Tesarik *et al*., 1987). The nucleoli in human embryos are thought to be inactive in the sense of ribosomal RNA synthesis before the third blastomere division, but chromatin infiltration may occur at the time of NPB formation and for the proper assembly of NPB also early nucleic acid (RNA) synthesis takes place (Tesarik and Kopecny, 1989b, 1990). It has been suggested that DNA encoding ribosomal genes of early human embryo is incapable of initiating the transcription unless it is associated with protein matrix of nucleolar precursor bodies (Tesarik *et al*., 1987). The pattern of embryonic nucleologenesis and the molecular composition of NPB seems to vary in a species-specific manner (reviewed by Flechon and Kopecny, 1998).

The number of NPB in PN varies from one to roughly ten, characteristically fewer NPB are seen in female than in male PN (4.2 versus 7.0) (Payne *et al*., 1997). During PN development the NPB are mobile and their distribution may change from random to aligned at the junction of the two PN (Scott and Smith, 1998). It has been reported that the random distribution of NPB diminishes during the PN migration (Wright *et al*., 1990). On the contrary, others failed to show any correlation between the time lapse of injection of oocytes and the number and polarization of NPB (Nagy *et al*., 1998). Although the activity of the NPB during PN development seems to be minimal, yet their polarization may be related to the embryonic development. It has been demonstrated that the distribution of chromatin in both maternal and paternal PN is polarized (Van Blerkom *et al*., 1995). It has also been suggested that the polarization of chromatin and NPB represents an early step of the formation of embryonic axis, which determines subsequent cell fate in preimplantation embryos (Edwards and Beard, 1997). Although the importance of NPB polarization has been explained by several hypotheses, their direct function, if any, has not been determined.

One part of the 2PN scoring system published by Scott and Smith (1998) is the evaluation of NPB polarization. The polarized NPB pattern is considered as a good sign for further development whereas scattered NPB pattern is not. When our data was analysed according to this classification a clear difference in embryo cleavage rate between combined classes I/II and class III was seen. The embryos with both or at least one pronucleus polarized at 16–18 h after insemination/injection had faster cleavage rate than those with scattered NPB. Although the implantation rate and pregnancy rate of the class III embryos was lower than those of the classes I and II, the difference was not significant. However, these results support the idea that the NPB polarization might be connected to successful embryo development. Our finding that zygotes with scattered NPB distribution seem to have less potential to produce pregnancies is in contrast with Tesarik's observation (Tesarik and Greco, 1999). This discrepancy could be explained by different times of fertilization assessments. In this study, as well as in that by Scott and Smith, fertilization was assessed 16–18 h after insemination/injection, whereas in Tesarik's studies the zygotes were checked at 12–20 h.

The translocation of organelles (mostly mitochondria) from the cortex of cytoplasm (i.e. the appearance of cytoplasmic halo) after the extrusion of the second polar body in fertilized human oocytes has been elegantly documented (Payne *et al*., 1997). The functional significance of this phenomenon has not been determined, but similar withdrawal of organelles occurs also in oocytes of other species undergoing fertilization (Bavister and Squirrel, 2000). The classification system of Scott and Smith included the evaluation of the appearance of cytoplasmic halo. Their results indicated that the presence of halo could be used as a positive marker for good quality embryos. Our results are partially in agreement, since the proportion of the morphologically good embryos was higher in halo-positive embryos than halo-negative embryos. However, after single embryo transfers no difference in implantation rate and pregnancy rate was found between these groups. We believe that the reason we did not find the differences in implantation rate and pregnancy rate between halo-positive and halo-negative embryos that were shown in the work of Scott and Smith (1998) is that these authors included the early cleavage in their scoring system. It has been documented (Sakkas *et al*., 1998) that the early cleavage of blastomeres, usually occurring 27 h after fertilization, is positively correlated with pregnancy rate.

The zygote scoring system published by Tesarik and Greco (1999) is based on the combination of the number and distribution of NPB within PN. The rationale of Tesarik's classification system is that for proper embryonic development interPN synchrony is more important than the actual polarization of NPB within individual PN. Therefore, zygotes having either polarized or unpolarized NPB in both PN at the time of evaluation are regarded as normal (pattern 0). After two independent studies their results indicated a strong association between pattern 0 zygotes and pregnancy rates. Also, pattern 0 embryos have less arrested and multinucleated embryos and more good-morphology embryos than non-pattern 0 embryos (Tesarik and Greco, 1999). In the present study we could neither find an association between embryo quality nor developmental competence and zygote morphology. In addition, implantation rate and pregnancy rate did not differ between pattern 0 and other groups. The idea that pregnancy rates of pattern 0 and non-pattern 0 groups are comparable is also supported by the similar pregnancy rate (29.8%, 14/47) of embryos belonging simultaneously to two or even more zygote classes (data not included in Table III). Interestingly, the differences observed in cleavage rates between the groups seem to indicate that a low number of NPB is associated with slow cleavage rate. However, new studies are required to establish the association because the number of embryos within these classes was small.

In the present study, the selection of embryos for transfer was based on the cleavage stage morphology. The implantation rate were similar for good quality (grade 1 or 2) embryos in both pattern and non-pattern 0 groups. The number of moderate quality (grade 3) embryos transferred was too small for statistical analysis, but it is unlikely that grade 3 embryos would produce better implantation rate than grade 1 or 2 embryos. Although the selection of embryos for transfer was not based on zygote morphology we postulate that the outcome of the study would have been similar even if a prospective randomized protocol, i.e. if the embryos transferred were selected based on their zygote classification, had been used.

In conclusion, we were unable to show that any of the proposed zygote classes would produce consistently better quality embryos or more pregnancies. More detailed studies are needed to resolve whether more defined subclasses with predictive value in embryo development could be found within the proposed classification systems or if new markers with higher potency could be found.

References

Bavister, B.D. and Squirrell, J.M. (2000) Mitochondrial distribution and function in oocytes and early embryos. *Hum. Reprod.,*, **15** (Suppl. 2), 189–198.

Cohen, J., Inge, K.L., Suzman, M. *et al*. (1989) Videocinematography of fresh and cryopreserved embryos: a retrospective analysis of embryonic morphology and implantation. *Fertil. Steril.*, **51**, 820.

Edwards, R.G. and Beard, H.K. (1997) Oocyte polarity and cell determination in early mammalian embryos. *Mol. Hum. Reprod.,* **3**, 863–905.

Flechon, J-E. and Kopecny, V. (1998) The nature of the 'nucleolus precursor body' in early preimplantation embryos: a review of fine-structure cytochemical, immunocytochemical and autoradiographic data related to nucleolar function. *Zygote*, **6**, 183–191.

Ludwig, M., Schöpper, B., Al-Hasani, S. *et al*. (2000) Clinical use of a PN stage score following intracytoplasmic sperm injection: impact on pregnancy rates under the conditions of the German embryo protection law. *Hum. Reprod.*, **15**, 325–329.

Nagy, Z. P., Rienzi, L., Iacobelli, M. *et al*. (1998) Analysis of PN parameters of human zygotes to predict developmental capacity of the derived embryos. *Fertil. Steril.*, **70** (Suppl. 1), 389S–390S.

Payne, D., Flaherty, S.P., Barry, M.F. *et al*. (1997) Preliminary observations on polar body extrusion and PN formation in human oocytes using time-lapse video cinematography. *Hum. Reprod.*, **12**, 532–541.

Pelinck, M.J., Vos, M.D., Dekens, M. *et al*. (1998) Embryos cultured *in vitro* with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection. *Hum. Reprod.*, **13**, 960–963.

Sakkas, D., Shoukir, Y., Chardonnens, D. *et al*. (1998) Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability. *Hum. Reprod.*, **13**, 182–187.

Scott, L., Alvero, R., Leonides, M. and Miller, B. (2000) The morphology of human PN embryos is positively related to blastocyst development and implantation. *Hum. Reprod.*, **15**, 2394–2403.

Scott, L.A. and Smith, S. (1998) The successful use of PN embryo transfers the day following oocyte retrieval. *Hum. Reprod.*, **13**, 1003–1013.

Tesarik, J. and Greco, E. (1999) The probability of abnormal preimplantation development can be predicted by a single static observation on PN stage morphology. *Hum. Reprod.*, **14**, 1318–1323.

Tesarik, J. and Kopecny, V. (1989a) Development of human male pronucleus: ultrastructure and timing. *Gamete Res.*, **24**, 135–149.

Tesarik, J. and Kopecny, V. (1989b) Nucleic acid synthesis and development of human male pronucleus. *J. Reprod. Fertil.*, **86**, 549–558.

Tesarik, J. and Kopecny, V. (1990) Assembly of the nucleolar precursor bodies in human PN is correlated with an early RNA synthethic activity. *Exper. Cell Res.*, **191**, 153–156.

Tesarik, J., Kopecny, V., Plachot, M. *et al*. (1987) High-resolution autoradiographic localization of DNA-containing sites and RNA synthesis in developing nucleoli of human preimplantation embryos: a new concept of embryonic nucleologenesis. *Development*, **101**, 777–791.

Tesarik, J., Junca, A.M., Hazout, A. *et al*. (2000) Embryos with high implantation potential after intracytoplasmic sperm injection can be recognized by a simple, non-invasive examination of PN morphology. *Hum. Reprod.*, **15**, 1396–1399.

Van Blerkom, J., Davis, P., Merriam, J. *et al*. (1995) Nuclear and cytoplasmic dynamics of sperm penetration, PN formation and microtubule organization during fertilization and early preimplantation. *Hum. Reprod. Update*, **1**, 429–461.

Vilska, S., Tiitinen, A., Hyden-Granskog, C. *et al*. (1999) Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. *Hum. Reprod.*, **14**, 2392–2395.

Wittemer, C., Bettahar-Lebugle, K., Ohl, J. *et al*. (2000) Zygote evaluation: an efficient tool for embryo selection. *Hum. Reprod.*, **15**, 2591–2597.

Wright, G., Wiker, S., Elsner, C. *et al*. (1990) Observations on the morphology of PN and nucleoli in human zygotes and implications for cryopreservation. *Hum. Reprod.*, **5**, 109–115.

Ziebe, S., Petersen, K., Lindenberg, S. *et al*. (1997) Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. *Hum. Reprod.*, **12**, 1545–1549.

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