Insights on blastomere nuclearity

Mónica Gil · Gustavo D’Ommar · Maria E. Póo · Anna Sosa · Marta Piras · Romano Piras · Francisco Rísquez

Received: 26 July 2006 / Accepted: 6 September 2006 / Published online: 16 December 2006
© Springer Science+Business Media, LLC 2006

Abstract *Purpose:* To analyze the results of our transferred embryos, especially those that “changed” their blastomere nuclearity from Multinucleated (MN) to Mono-nucleated during development.

Methods: Pregnancies where at least one MN embryo was transferred were retrospectively evaluated and categorized in order to record and follow-up on the ones that were implanted. Embryos were classified as normal (when all blastomeres were mono-nucleated on day one and two of development), corrected (multinucleated embryos on day one that became mono-nucleated on day two) and non-corrected (multinucleated either on day one, on day two or both days).

Results: There were 633 transfer cycles analyzed. Thirty-three percent (206) had at least one embryo with a MN blastomere at a given stage of development. Pregnancy and implantation rates were 29.0% and 19.0% for the group of exclusively mono-nucleated embryo transfers, and 28.6% and 15.8% for the group with at least one MN embryo transferred. The pregnancy outcome for “corrected” and “non-corrected” embryos could be corroborated unequivocally in only 9 cases, with an outcome of 8 and 4 normal babies, respectively.

Conclusions: Because the amount of data analyzed is not satisfactorily large, differences were not significantly different; however, a trend may exist showing that normal at term pregnancies obtained from corrected embryos are more likely to occur than those from non-corrected embryos. Nuclear observation on a daily basis should be one of the strate-

gies used to select the best embryos for transferring, to improve implantation rates and avoid multiple pregnancies.

Keywords Embryo development · Embryo selection · Embryo transfer · Implantation rate · Multinucleated blastomeres

Introduction

Over the years, people who work with assisted reproductive technologies have made many efforts to select the best embryos for transfer. Several selection strategies have been used for this purpose. The first and most widely practiced is selection by embryo morphology. This method calls for revising cell number, symmetry, embryo fragmentation and blastomere nuclearity. Other selection strategies are early zygote evaluation, early division and embryo auto selection, through culturing in sequential media with transfers performed at the blastocyst stage. An additional selection strategy consists in using preimplantation genetic diagnosis (PGD), which allows identification of embryos with a correct chromosome complement through fluorescence in-situ hybridization (FISH).

Since the final goal is to select the best embryos for transferring, we have to monitor embryos very carefully during development. Our study focuses in one of the embryo selection parameters: BLASTOMERE NUCLEARITY; particularly, on the outcome of the transferred embryos that showed blastomeres multinucleation (which is defined as two or more nuclei present in one cell at a given time) and changed its nuclearity during development.

The presence of multinucleated (MN) blastomeres in embryos arising through IVF procedures has been described

M. Gil (✉) · G. D’Ommar · M. E. Póo · A. Sosa · M. Piras · R. Piras · F. Rísquez
Clínica de Fertilidad, Centro Médico Docente La Trinidad,
Caracas, Venezuela
e-mail: mgilco@cantv.net

to range from 14.3% to 74% [1–6] and 43% to 87% of the patients to have MN embryos [3, 4, 7].

The fact that embryos with MN blastomeres present poor implantation potential has been well documented [2, 8–10]. Moreover, there are reports that show that transferring of only mono-nucleated embryos yields better pregnancy rates than transferring of mixed mono-nucleated and MN embryos [9–11].

Multinucleation has been associated with developmental arrest [1–4, 7, 12–15], poor embryo morphology [1, 2, 7], elevated fragmentation [2, 3], repeated cycles [3], blastomere asymmetry [16, 17] and higher multinucleation rate on 2-cells when compared with more developed embryos [1, 3, 5, 7, 13, 15, 18]. Multinucleation has also been associated with higher blastomere diameter [1, 6], decrease in blastocyst formation rate [5, 7, 15, 19], reduced capacity of development on siblings of MN embryos [2, 5], high amount of oocytes retrieved and elevated E₂ levels [2, 3, 20], follicles hypoxia [21], culture media [13, 22], the use of GnRH antagonist [23] and apoptosis [6].

Studies on the chromosomal status of MN embryos indicate that most of them resulted in chaotic or abnormal complement numbers [17, 24]. Kligman et al. [8] found 74.5% of chromosomal abnormalities in MN embryos versus 32.3% on mono-nucleated embryos using five FISH probes.

Based on all of the above observations, consensus has been reached: embryos with MN blastomeres are highly unsuitable for replacement and should be excluded for transferring unless non-MN embryos are available [7, 8, 12].

Reports on MN cells that evolved to mono-nucleated blastomeres [13, 25, 26], eventually giving rise to normal pregnancies [2, 3, 7, 9, 27], led us to investigate the outcome of our transferred MN embryos, specifically those that “changed” from MN to mono-nucleated blastomeres (those, we named “Corrected Embryos”).

Materials and methods

Between February 2003 and January 2005, 704 patients underwent IVF treatment at our Fertility Clinic, Centro Médico Docente La Trinidad, Caracas, Venezuela. These are the cases included in our study. All transfers done during this period were retrospectively analyzed and categorized to determine the fate of the transferred embryos, especially those with MN blastomeres.

Ovarian stimulation

Ovarian stimulation protocols have been previously described by Rísquez [28]. Briefly, pituitary down-regulation was achieved by treatment with the gonadotrophin-releasing hormone (GnRH) analogue leuprolide acetate (Lupron; Ab-

bott Laboratories) initiated in the preceding midluteal phase; or antagonist (Cetrotide, Serono) when follicles reached 14 mm. Ovarian stimulation was induced with recombinant FSH (rFSH; Gonal-F; Serono), and recombinant LH (rLH; Luveris; Serono). Follicular development was monitored by vaginal ultrasound and serum E₂ measurements. For ovulation induction 10,000 IU of human chorionic gonadotrophin (HCG; Profasi; Organon or Ovidrel; Serono) was administered. Oocyte retrieval was performed 36 h after HCG injection by transvaginal ultrasound-guided puncture.

Laboratory routine

Standard laboratory procedures were used [29]. Briefly, oocytes were inseminated or injected 5–6 h post-egg retrieval by standard procedures. Fertilization was confirmed by the presence of two pronucleus 18–20 h after insemination/injection. Pronuclei per zygote were scored based on characteristics described by Scott et al. [30]. Embryos were cultured individually in 20 μ l droplets of P1 (Irvine Scientific) or Global media (Life Global) supplemented with 10% of synthetic serum substitute (SSS; Irvine Scientific) under mineral oil (Lite oil, Life Global) on 5% CO₂ in air at 37°C for three days. Evaluation, score and record of each embryo were performed on a daily basis using an X40 objective on an inverted Nikon Diaphot 200 microscope using Hoffman modulation contrast optics on a heated stage (Tokai Hit Co., LTD, Japan). On day three, between one and three embryos were routinely transferred using Frydman Classic Catheter 4,5-5,5 or Short Frydman Set (Laboratoire C.C.D.). Luteal phase was supported by micronized vaginal progesterone (Crinone, Serono), given once daily starting the day after egg retrieval. Clinical pregnancies were determined by a positive HCG test and the presence of fetal heartbeat by ultrasound at 28 days post transfer. Upon delivery, the babies weight, length, and Apgar score were recorded, as well as the eventual presence of any abnormality detected upon clinical examination.

Embryo evaluation and data collection

Development of each normally fertilized embryo was monitored on daily basis. Early division was observed 26–28 h post insemination/injection. A second observation was performed four hours later on the same day, expecting that most of the embryos should be already divided into two cells. Although this observation is not our common practice, it was performed in order to have records of blastomere nuclearity. From day-1 up to day-3, cell number, symmetry, fragmentation and blastomere nuclearity were carefully observed and recorded. Blastomeres were classified as “OK” when only one nucleus was present, and as “MN” when two or more nuclei were observed. When nuclearity could not be easily

Fig. 1

determined by use of a standard Hoffman 40x objective, we used the objective provided for laser hatching (Fertilase, by Medical Technologies, Montreux, Switzerland) and could identify, most of the times, the nucleus status. According to the blastomere nuclearity, embryos were classified as: a) “Normal,” when all blastomeres were “OK” on day-1 and -2 of development. b) “Corrected,” when a MN blastomere on day-1 became mono-nucleated on day-2 (Figs. 1 and 2), and c) “Non-Corrected,” when at least one MN blastomere was present on both day-1 and -2, or multinucleation appeared on day-2. Those embryos that showed 2 pronuclei on day-1 were classified as either Normal or Non-Corrected depending on their appearance on day-2. All those embryos that failed to exhibit nuclei due to the fact that they were in metaphase when being examined were excluded from the study group.

Statistical analysis was performed with the Student’s *t*-test. Significance was set at 0.05.

Fig. 2

Results

Seven hundred and twenty five cycles were performed from which 633 resulted in embryo transfer (87.31%).

Embryo multinucleation was present in 33% of the cycles studied. The extent and type of multinucleation (number of MN blastomeres per embryo, number of MN embryos per case, time at which blastomere multinucleation became evident and/or disappeared) varied widely and was unrelated to the procedure performed, IVF or ICSI (31% and 69% for the mono-nucleated group, and 28% and 72% for the MN group).

Of the 633 cycles transferred between February 2003 and January 2005, we transferred exclusively mono-nucleated embryos in 427 cycles (968 transferred embryos), resulting in 124 clinical pregnancies (PR = 29.0%) with 184 implanted embryos (IR = 19.0%). The remaining 206 cycles (500 transferred embryos), which had at least one embryo transferred that was MN at a given stage of development, resulted in 59 pregnancies (PR = 28.6%) with 79 implanted embryos (IR = 15.8%). The women’s average age was very similar for the two groups (33.2 and 34.5, respectively), as well as the number of embryos transferred per cycle (2.27 and 2.43, respectively). Akin results were also found for the embryo score at the time of transfer (91.9 for the mono-nucleated group and 88.5 for the MN group) and the culture media used: Global (59.3% and 58.6%) or P1 (40.6% and 41.2%) both respectively for the mono- and multi-nucleated groups. No significant differences were found regarding the procedure performed, pregnancy and implantation rates, woman’s age, number of embryos transferred, embryo score and the culture media used.

Even though the IR for the MN group is slightly lower than the one for the mono-nucleated group (15.8% versus 19.0%), the difference is not statistically significant and far smaller than expected, based on transfers where at least one embryo, out of the 2.43 transferred, was MN, with a supposed lower implantation potential. The relatively similar IR found for both groups suggests that an important number of MN embryos can change to “corrected” and/or that even some apparently “non-corrected” embryos could actually give rise to implantation. Cycles with transfers of exclusively “corrected” or “non-corrected” embryos occur very seldom; therefore, we decided to look into our cohort of transferred cycles with at least one MN embryo where the transfer of “corrected” embryos could be unequivocally correlated to the pregnancy outcome, as well as cases where transfer of “non-corrected” embryos could be also correlated to the pregnancy outcome. Table 1 shows that nine such cases were detected; five cases were from “corrected” embryos (cases # 1–5), three cases were from “non-corrected” embryos (cases # 7–9), and one case (# 6) was from both “corrected” and “non-corrected” embryos.

Table 1 Follow up of implanted embryos

No. of case	Embryos transferred	Embryo development		Embryo classification			Delivered babies
		Day 1	Day 2	Normal	Corrected	Non-Corrected	
1	2	2C 2MN	4C 4OK	0	2	0	2
2	2	2C 2MN	4C 4OK	1	1	0	2
		2C 1MN	4C 4OK				
3	2	2C 2OK	4C 4OK	0	2	0	1
		2C 1MN	4C 4OK				
4	3	2C 2MN	4C 4OK	0	3	0	2
		2C 2MN	4C 4OK				
		2C 1MN	4C 4OK				
5	3	2C 2OK	4C 4OK	1	2	0	2
		2C 1MN	4C 4OK				
		2 PN	4C 4MN				
6	3	2C 1MN	4C 4OK	1	1	1	3
		2C 2OK	4C 4OK				
7	2	2 PN	4C 4OK	1	0	1	2
		2 PN	4C 1MN				
8	2	2C 2OK	4C 4OK	1	0	1	2
		2C 1MN	4C 1MN				
		2C 1MN	6C 2MN				
9	3	2C 2OK	5C 2MN	0	0	3	1
		2C	4C 1MN				

Note. PN: Pronucleus; C: Cell; MN: Multinucleated blastomere; OK: Mononucleated blastomere.

Eight normal, healthy babies (or may be even 9) were born from “corrected” embryos (2 babies in case # 1, 1 in case # 2, 1 in case # 3, 2 in case # 4, at least 1 of the 2 born in case # 5, and 1 in case # 6). As to the “non-corrected” embryos, four healthy babies were born (1 baby each in cases # 6, # 7, # 8, and # 9).

Discussion

Many studies associate embryo MN with many different factors; thus, there is not a unique explanation. However, several explanations have been proposed: karyokinesis in the absence of cytokinesis [1], partial fragmentation of nuclei [31], changes in temperatures and suboptimal culture conditions [22], abnormal human sperm centrosome [32], cytoskeletal and spindle malfunction, and defective migration of chromosomes at mitotic anaphase [12, 13, 31, 33].

The occurrence and timing of multinucleation is quite variable. Additionally, correction of abnormal blastomere nuclearity might occur, as shown by Pickering et al. [13], who disaggregated human embryos and found that some multinucleated cells produced mono-nucleated blastomeres. Staessen and Van Steirteghem [25], using 3 probes FISH, also found that 30.4% of embryos that had at the 2-cell stage both blastomeres bi- or multi-nucleated contained, upon further cleavage, only mononuclear diploid blastomeres, concluding that the genetic constitution of bi- or multi-nuclear

blastomeres, especially those that further divide, is not always abnormal.

According to other authors, no correlation is found between multinucleation and maternal age or the procedure performed, IVF or ICSI [3, 7, 8, 20, 25]. In contrast to general assumption, we found no difference when comparing results of pregnancies where at least one MN embryo was transferred with results on pregnancies with no MN embryos transferred.

Accurate blastomere nuclearity detection and characterization is not easily attained on a single observation. Detection can be achieved by time studies [5], but these might compromise embryo quality. Conciliating the number and timing of embryo visualizations whilst avoiding disturbing them is critical. It should also be noted that, besides timing, there are other limiting factors for proper nuclear visualization, like the presence of granular cytoplasm, cytoplasmic fragments and overlapping blastomeres, all of them making difficult to properly assess embryo nuclearity beyond the 4-cell stage. Hence, it is possible that some of the embryos classified as “non-corrected” might have evolved into “corrected” at the time of transferring, and undoubtedly afterwards. We believe that a careful daily observation gives us a proper idea of embryo nuclearity and eventual MN correction.

Only those cases where exclusively MN embryos are transferred (because of no good, mono-nucleated embryos were available) are helpful for determining their implantation

rate, but these cases occur very seldom. When both normal and MN embryos are transferred, depending on the pregnancy outcome, one can eventually deduce the fate of the MN embryo(s). Using this approach, Balakier and Cadesky [7] reported 19 MN embryos transferred (6%) in a cohort of 338 patients: four of them implanted (21%), two led to spontaneous abortion and two healthy infants were born. Joris et al. [27] found one case, within 24 pregnancies, that evidenced the implantation of a MN 2-cell embryo. Pelink et al. [9] reported eight cases with only MN embryos transferred from which one pregnancy was established; in this particular case, two embryos were transferred, both with one mono-nucleated and one MN blastomere. Van Royen et al. [3] also reported one MN embryo that implanted in a single embryo transfer, and five infants born from 44 double MN embryo transfers. Jackson et al. [2] described 25 cycles in which all transferred embryos had at least one MN blastomere; three implantations were established in this group, which resulted in one spontaneous abortion and two healthy twins. According to these reports, between 10 and 12 babies were apparently born from embryos that were MN at a given stage; it is not known, however, whether these embryos experienced a correction before transfer. In our series we detected 12 infants born unequivocally from embryos that were MN at a given stage: 8 from corrected embryos and 4 from non-corrected. This is obviously a minimum figure, since more, undetectable cases might exist.

Different groups have tried to explain how can we have good outcomes from such “bad embryos.” They proposed ideas such as cytokinesis being resumed on later cell divisions [8], that multinucleation could be a precursory step of nuclear membrane breakdown during cell division, as a temporary and reversible phenomenon [25], the presence of pseudonuclei with a possible fusion between them afterwards [8, 31] and that, even though the blastomeres are totipotent, there is a preference for the chromosomally abnormal cells to allocate in the trophectoderm [34].

Embryos with multinucleated blastomeres may not be the best choice for transferring but, given the fact that sometimes are the only option, it is reassuring to know that they do have a chance to implant and give rise to healthy babies. We do not know whether corrected embryos behave like normal embryos and should not even be included in the MN group. Also, it is very likely that the embryos we call non-corrected underwent correction, thus actually belonging to the corrected group. Regardless of these speculations, it is important to emphasize that careful nuclear observation on a daily basis should be part of the strategies used for embryo selection. This observation allows optimization on the decision that has to be made, on each patient, for the number and type of embryos to transfer

in order to improve implantation rates and avoid multiple pregnancies.

Acknowledgments The authors thank Dhamellis P. de De Visnyey for her helpful suggestions and Luis R. Gil for his kind collaboration in the study.

References

- Hardy K, Winston RM, Handyside AH. Binucleate blastomeres in preimplantation human embryos in vitro: Failure of cytokinesis during early cleavage. *J Reprod Fertil* 1993;98(2):549–58
- Jackson KV, Ginsburg ES, Hornstein MD, Rein MS, Clarke RN. Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles. *Fertil Steril* 1998;70(1):60–6
- Van Royen E, Mangelschots K, Vercruyssen M, De Neubourg D, Valkenburg M, Ryckaert G, Gerris J. Multinucleation in cleavage stage embryos. *Hum Reprod* 2003;18(5):1062–9
- Moriwaki T, Suganuma N, Hayakawa M, Hibi H, Katsumata Y, Oguchi H, Furuhashi M. Embryo evaluation by analysing blastomere nuclei. *Hum Reprod* 2004;19(1):152–6
- Meriano J, Clark C, Cadesky K, Laskin CA. Binucleated and micronucleated blastomeres in embryos derived from human assisted reproduction cycles. *Reprod Biomed Online* 2004;9(5):511–20
- Hnida C, Engenheiro E, Ziebe S. Computer-controlled, multilevel, morphometric analysis of blastomere size as biomarker of fragmentation and multinuclearity in human embryos. *Hum Reprod* 2004;19(2):288–93
- Balakier H, Cadesky K. The frequency and developmental capability of human embryos containing multinucleated blastomeres. *Hum Reprod* 1997;12(4):800–4
- Kligman I, Benadiva C, Alikani M, Munne S. The presence of multinucleated blastomeres in human embryos is correlated with chromosomal abnormalities. *Hum Reprod* 1996;11(7):1492–8
- Pelink MJ, De Vos M, Dekens M, Van Der Elst J, De Sutter P, Dhont M. Embryos cultured *in vitro* with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection. *Hum Reprod* 1998;13(4):960–3
- Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W, Gerris J. Characterization of a top quality embryo, a step towards single-embryo transfer. *Hum Reprod* 1999;14(9):2345–9
- Duke M, Basile N, Johnson M, Copperman A, Sandler B, Barritt J. Transfer of Embryos with Even a Single Multinucleated Blastomere Significantly Reduces Pregnancy and Implantation Rates. *Fertil Steril* 2003;80(Suppl 3):O270:S104
- Munné S, Cohen J. Unsuitability of multinucleated human blastomeres for preimplantation genetic diagnosis. *Hum Reprod* 1993;8(7):1120–5
- Pickering SJ, Taylor A, Johnson MH, Braude PR. An analysis of multinucleated blastomere formation in human embryos. *Hum Reprod* 1995;10(7):1912–22
- Levy R, Benchaib M, Cordonier H, Souchier C, Guerin JF. Laser scanning confocal imaging of abnormal or arrested human preimplantation embryos. *J Assist Reprod Genet* 1998;15(8):485–95
- Yakin K, Balaban B, Urman B. Impact of the presence of one or more multinucleated blastomeres on the developmental potential of the embryo to the blastocyst stage. *Fertil Steril* 2005;83(1):243–5

16. Munné S, Alikani M, Cohen J. Monospermic polyploidy and atypical embryo morphology. *Hum Reprod* 1994;9(3):506–10
17. Hardarson T, Hanson C, Sjogren A, Lundin K. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. *Hum Reprod* 2001;16(2):313–8
18. Cook CA, Clarke R, Ye Z, Berrios R, Veeck LL, Zaninovic N. Blastomere multinucleation and subsequent preembryo development. *Fertil Steril* 2003;80(Suppl 3):162–3
19. Alikani M, Calderon G, Tomkin G, Garrisi J, Kokot M, Cohen J. Cleavage anomalies in early human embryos and survival after prolonged culture in-vitro. *Hum Reprod* 2000;15(12):2634–3
20. Styne A, Chi L, Krey L, Adler A, Noyes N. Multinucleated embryos in IVF: Associated clinical factors and pregnancy outcome. *Fertil Steril* 2001;76(Suppl 1):S144–5
21. Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: Association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum Reprod* 1997;12(5):1047–55
22. Winston NJ, Braude PR, Pickering SJ, George MA, Cant A, Currie J, Johnson MH. The incidence of abnormal morphology and nucleocytoplasmic ratios in 2-, 3- and 5-day human pre-embryos. *Hum Reprod* 1991;6(1):17–24
23. De Vincentiis S, Nodar F, Lavolpe M, Fiszbañ G, Rawe V, Brugo Olmedo S. Embryo multinucleation (MNC) and clinical outcome: Its relationship with the use of agonists and antagonists of GnRH in ART and the cellular events associated with this nuclear alteration. *Fertil Steril* 2005;84(Suppl 1), P-373:S284
24. Laverge H, De Sutter P, Verschraegen-Spae MR, De Paepe A, Dhont M. Triple colour fluorescent in-situ hybridization for chromosomes X,Y and 1 on spare human embryos. *Hum Reprod* 1997;12(4):809–14
25. Staessen C, Van Steirteghem A. The genetic constitution of multinuclear blastomeres and their derivative daughter blastomeres. *Hum Reprod* 1998;13(6):1625–31
26. Tucker KE, Rijnders PM, Jansen CA. Detection of nuclear and pronuclear abnormalities at the time of early cleavage (EC) assessment on day 1. *Fertil Steril* 2005;84(Suppl 1)O-37:S16
27. Joris H, Nagy Z, De Vos A, Van de Velde H, Staessen C, Van Steirteghem A. Cleavage and morphology of embryos which are multinuclear on day 1 and normal on day 2. *Hum Reprod* 1997;12(Abst book 1),O-191:95
28. Rísquez F. Induction of follicular growth and ovulation with urinary and recombinant gonadotrophins. *Reprod Biomed Online* 2001;3(1):54–72
29. Boiso I, Veiga A, Edwards R. Fundamentals of human embryonic growth in vitro and the selection of high-quality embryos for transfer. In: Edwards R, Rísquez F, editors. *Modern assisted conception*. United Kingdom: Reproductive Healthcare Ltd.; 2003. p. 403–451
30. Scott L, Alvero R, Leondires M, Miller B. The morphology of human pronuclear embryos is positively related to blastocyst development and implantation. *Hum Reprod* 2000;15:2394–2403
31. Tesarik J, Kopečný V, Plachot M, Mandelbaum J. Ultrastructural and autoradiographic observations on multinucleated blastomeres of human cleaving embryos obtained by in-vitro fertilization. *Hum Reprod* 1987;2(2):127–36
32. Palermo G, Munne S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. *Hum Reprod* 1994;9(7):1220–5
33. Munné S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;64(2):382–91
34. Mottla GL, Adelman MR, Hall JL, Gindoff PR, Stillman RJ, Johnson KE. Lineage tracing demonstrates that blastomeres of early cleavage-stage human pre-embryos contribute to both trophoderm and inner cell mass. *Hum Reprod* 1995;10(2):384–91

Mónica Gil completed her Cytology degree at Universidad Central de Venezuela, Caracas, Venezuela on 1991. Her training/work began at Centro Médico Docente La Trinidad, Caracas, Venezuela, in Cytogenetics, Andrology and Embryology. She underwent additional training and assistantship at Institut Dexeus in Barcelona, Spain, and at Obstetrical and Gynecological Associates in Houston, Texas; in 1998 she moved to the United States of America and joined the IVF team in Houston. In 2000 she obtained her post-graduate diploma in Reproductive Biology at University of Queensland in Australia. She was also part of the IVF staff at The Wesley Hospital in Brisbane, Australia. Presently she works as an embryologist with a particular interest in embryo morphology during development at Centro Médico Docente La Trinidad, Caracas, Venezuela.