Polar body fragmentation in IVM oocytes is associated with impaired fertilization and embryo development

B. I. Rose · D. Laky

Received: 12 February 2013 / Accepted: 12 March 2013 / Published online: 27 March 2013
© Springer Science+Business Media New York 2013

Abstract

Purpose The significance of finding a fragmented first polar body in an oocyte prepared for ICSI is controversial with most recent publications suggesting that it is not prognostic for oocyte fertilization or embryo development. Our purpose was to look at this question in the context of oocytes not stimulated for conventional IVF.

Methods Oocytes obtained for IVM and obtained from follicles at most 12 mm in diameter were evaluated for their polar body morphology soon after they entered metaphase II when they were denuded in preparation for ICSI. Records were evaluated retrospectively for the fertilization rate and the embryo growth rate (cell number) on each day of development for embryos with normal appearing polar bodies or fragmented polar bodies, but no other cytoplasmic dysmorphisms.

Results Oocytes with fragmented polar bodies were significantly less likely to fertilize than oocytes with normal appearing polar bodies ($p<0.0001$). Embryos which developed from oocytes with fragmented polar bodies had significantly impaired growth compared to embryos that developed from oocytes with normal appearing polar bodies ($p=0.0328$).

Conclusions Fragmented polar bodies likely reflect cytoplasmic incompetence.

Keywords Fragmented polar body · Oocyte dysmorphism · Cytoplasmic competence · IVM · In vitro maturation · Side-effects of gonadotrophin stimulation

Introduction

Oocyte morphology presents the embryologist with the first opportunity to potentially predict oocyte fertilization and embryo development. Trying to understand the significance of specific dysmorphisms has been the subject of a number of research studies of oocytes obtained after gonadotrophin stimulation for conventional IVF. Some of the dysmorphisms that have been studied include abnormal first polar body morphology, presence of vacuoles or refractile bodies, and abnormalities of the perivitelline space. Although there have been a number of papers which found a negative impact of some or all of these dysmorphisms [4, 5, 9, 12, 13], there are also a number of studies that find no impact for most of these dysmorphisms on fertilization or embryo development [1, 2, 7]. Thus, as noted in the meta analysis recently published by Rienzi, et al., there is little consensus in the literature about the impact of any of these dysmorphisms on oocyte fertilization or embryo development [10]. Some of these studies have examined a number of dysmorphisms in the same paper and others have focused on individual dysmorphisms.

Since the first polar body is easy to evaluate with basic microscopy and is clearly a reflection of a functional component of cytoplasmic competence, morphological abnormalities of the first polar body have been one of the more commonly studied dysmorphisms. Recent research has shown that some polar body abnormalities may be an artifact of oocyte handling or ageing. Verlinsky et. al., noted that after evaluating oocyte morphology at the time of denudation of granulosa cells and also 16 h later, all polar bodies that had a rough appearance became fragmented and many of those polar bodies which initially had a smooth

Capsule In clinical IVM, oocytes with fragmented polar bodies are less likely to fertilize and are less likely to develop into an embryo with a high potential to implant than embryos developing from oocytes with normal appearing polar bodies.

B. I. Rose · D. Laky
Infertility Solutions, P. C., 1275 South Cedar Crest Boulevard,
Allentown, PA 18103, USA

B. I. Rose (✉)
Department of Obstetrics and Gynecology,
St. Luke's University Health Network, 801 Ostrum Street,
Bethlehem, PA 18015, USA
e-mail: brucerose50@gmail.com

appearing surface developed a rough appearing surface or became fragmented [11]. Ciotti et. al. more directly looked at this by evaluating polar bodies in 180 oocytes at the time of granulosa cell denudation and 3 h later. Initially, 11.1 % of oocytes had a fragmented polar body, but after 3 h, 22.8 % did [2].

Oocytes with fragmented polar bodies (FPB) at the time of granulosa cell denudation may still have impaired cytoplasmic competence. For example, ageing may not explain the 11 % of oocytes having fragmented polar bodies in the Ciotti study. However, these results make some papers in the literature suspect for relevance, if the reader cannot determine or infer when oocyte morphology was assessed. As pointed out by Chamayou et. al., another shortcoming of the literature on polar body morphology is that none of the studies isolate polar body abnormalities from other dysmorphisms that may also have an impact on pregnancy rates if transferred [1]. A third concern is the impact of high dose gonadotropins on the granulosa cells and the oocyte. Some dysmorphisms may be related to the ovulation induction process that commonly precedes IVF. The granulosa cells surrounding the oocyte, under conventional IVF stimulation, are producing an abnormal quantity of products that clearly alters the molecular environment in the oocyte cytoplasm. High dose gonadotropins may thus obscure our ability to fully understand the significance of a given dysmorphism.

Clinical in vitro maturation (IVM) involves harvesting oocytes prior to their being stimulated by gonadotropins (generally with follicles at most 10–12 mm in diameter). Oocytes obtained in this manner may be a better (or a complementary) context in which to study factors related to cytoplasmic competence than oocytes obtained from conventional IVF. IVM also presents the opportunity to evaluate oocyte competence in greater detail than many studies that have often used embryo grading on day 2. With IVM, other variables such as the time required for oocyte maturity (extrude a polar body) or an embryo's cell number growth pattern, may be assessed.

If IVM oocytes with fragmented polar bodies have decreased cytoplasmic competence, it may simplify the embryologist's task in oocyte selection when either an individual patient or her society requests that excess embryos not be created. It could also simplify the IVM process whenever a reasonable number of normal oocytes have been fertilized and other oocytes become mature at later times with fragmented polar bodies.

Methods

For this study, patients were candidates for IVM if they met routine criteria for IVF, were less than 38 years old, and had a total antral follicle count of 20 or more. Patients self-selected IVM as an alternative to conventional IVF. All

patients were pre-treated with oral contraceptives for cycle timing. Most IVM patients received low doses (at most 300 U FSH) of gonadotropins for priming. All patients received hCG for priming. Patients with a thin endometrial lining thickness were managed with supplemental estrogen. HCG was given 38 h prior to oocyte harvesting when the lead follicle averaged 10–12 mm and the endometrial lining was at least 6 mm in diameter. All embryo transfers were on the third day after the first fertilization was observed.

This was a retrospective study utilizing oocytes obtained for clinical IVM. Oocytes from the IVM cases in patients under age 38 performed at Infertility Solutions, P. C. in 2011 and 2012 were candidates for this study. Two cases were excluded. (In one case, two oocytes were stripped 6 h before assessed as mature and in the other an early transfer was done to avoid a winter storm emergency.) The study included 38 IVM cases with 447 oocytes retrieved. Oocytes thought to be mature 2 to 4 h after retrieval were denuded with hyaluronidase and a denuding pipette and morphology was assessed at that time. Oocytes were re-evaluated at the end of the day, the morning after retrieval, the end of the day after retrieval and the morning of the second day after retrieval. If thought to be mature at one of these times, oocytes were then denuded with a denuding pipette, evaluated for morphology, and injected with sperm. The embryologist recorded first polar body morphology, presence of vacuoles, presence of refractile bodies, enlarged polar bodies and any other findings that were atypical.

Embryos were assessed primarily by their cell numbers on each day since fertilization. On the day of transfer, embryo morphology had minimal impact on embryo selection since poor embryo morphology was uncommon. Depending on when fertilization took place, embryos were assigned a 3-tuple consisting of their cell number on the morning of each day that they were evaluated prior to transfer. For example, an embryo which fertilized normally the day after ICSI, was 4 cells 2 days after ICSI, and was 8 cells 3 days after ICSI on the day of transfer, was assigned the 3-tuple (1, 4, 8). Table 1 ranks these growth tuples into 4 categories based on the likelihood of their being chosen for transfer. Embryos would first be selected from category A for transfer and if not available, then from category B, etc.

Data was analyzed using the Fisher exact test, the Chi-squared test, and the *t*-test utilizing the software Prism for Windows (GraphPad Software, Inc., www.graphpad.com). All *p* values quoted are two-sided. A probability greater than 0.05 was considered not significant.

Results

Of the 447 oocytes collected, 288 became mature. Of these, 219 did not have vacuoles, refractile bodies, enlarged polar

Table 1 Embryo transfer ranking characterization

Category A	(1, 2, 7), (1, 2, 8), (1, 3, 7), (1, 3, 8), (1, 4, 7), (1, 4, 8)
Category B	(1, 2, 5), (1, 2, 6), (1, 2, 9), (1, 3, 5), (1, 3, 6), (1, 3, 9), (1, 4, 6), (1, 4, 9), (1, 5, 7), (1, 5, 8), (1, 5, 9)
Category C	(1, -, -), (1, 2, -), (1, 3, -), (1, 4, -), (1, 5, -)
Category D	(n, *, *), (*, m, *), (*, *, p) where $n > 1$, $m > 5$, $p > 9$ and * is any number (1, 1, -), (1, 1, 1), (1, 2, 2), (1, 2, 3), (1, 2, 4) (1, 3, 3), (1, 3, 4), (1, 4, 4), (1, 4, 5), (1, 5, 5), (1, 5, 6)

The 3-tuple (a, b, c) represents the observed cell number of an embryo on the morning after ICSI was performed (a), the following day (b), and the day following that (c). The symbol “-” means that no observation was recorded for that day because the embryos had already been transferred to the patient

bodies or enlarged perivitelline spaces and were not enlarged oocytes. For these oocytes, 85 had FPB and 134 did not. Oocytes without FPB were significantly more likely to fertilize than oocytes with FPB (91 % versus 56.5 %, $p < 0.0001$) (Table 2). The results did not change if the oocytes with FPB and other dysmorphisms were added to the above analysis. There were 18 mature oocytes with a FPB and another dysmorphism. Six of these oocytes fertilized and 12 did not (33.3 % fertilization rate).

The likelihood of selection for transfer (Table 1) characterization was applied to our study group and showed that there was impaired growth/development of fertilized embryos formed from oocytes with the isolated dysmorphism of FPB compared to embryos arising from oocytes with normal appearing polar bodies (Chi-squared test: $p = 0.0328$) (Table 3).

Discussion

FPB were shown to be a reflection of diminished cytoplasmic competence in the setting of this study. Oocytes with fragmented polar bodies had both impaired fertilization and impaired development in terms of their potential for being selected for transfer compared to oocytes with normal

Table 2 Impact of fragmented polar bodies

	Normal polar bodies	Fragmented polar bodies
MII oocytes	134	85
Fertilized oocytes (%)	120 (91) ²	35 (36.5) ²
Good embryos ¹ (%)	36 (30) ³	6 (7.1) ³

¹ Category A or B embryos

² $p < 0.0001$ Fisher exact test

³ $p = 0.067$ Fisher exact test

Table 3 Embryo development

Embryo category at the time of transfer	Normal appearing polar body as an oocyte ^a	Fragmented polar body as an oocyte ^a
A (best)	20	5
B	16	1
C	38	25
D (worst)	44	21

^a Embryo development is significantly different by the Chi-squared test ($p = 0.0382$)

appearing polar bodies. Since oocytes in this study were acquired without endogenous or conventional IVF gonadotropin stimulation, one would have expected that these oocytes would be less competent overall. Our results suggest that FPB are a reflection of this decreased competence on an individual oocyte basis. If gonadotrophin stimulation (as in conventional IVF) could overcome this possibly intrinsic lack of cytoplasmic competence, one might expect that FPB would be seen less frequently in mature oocytes produced in conventional IVF. We looked at all conventional IVF with ICSI cases performed in the same time period as this study in women under age 38 with presumed normal ovarian reserve to see if the incidence of FPB was different. This was not an optimal comparison group since 26 % of conventional IVF cases were in patients greater than age 34 compared to only 13 % in the IVM group (mean age of patients for the IVF group was 32.1 and the mean age for the IVM group was 30.9; $p = 0.08$ by the t -test). There were 584 oocytes from 58 cases. In spite of being a sub-optimal comparison group, FPB were more frequently observed in oocytes matured after IVM than in the conventional IVF cohort. The percentage of oocytes with FPB (including other dysmorphisms) in oocytes matured for IVM was 35.8 %. Whereas, with conventional IVF, the percentage of mature oocytes with FPB was 28.9 % ($p = 0.049$).

Since fragmented polar bodies are associated with IVM oocytes having decreased fertilization and impaired development and since the oocytes studied were from patients who ranged in age from 24 to 37, we also compared the incidence of FPB for women below age 35 or greater than age 34 and found no difference ($p = 0.506$). In our population, age did not appear to be an important variable for the occurrence of FPB.

The only morphological abnormality of the first polar body evaluated in this study was FPB. Prior investigators have generally placed polar bodies into one of three or four morphological categories: intact polar bodies with smooth surfaces or rough appearing surfaces (this may be one or two categories), polar bodies that have fragmented, or polar bodies that are enlarged. We did not look at enlarged polar bodies, in part because we do not transfer embryos arising from oocytes having them although their significance is not

completely understood. The primary problem in understanding the enlarged polar body group may be the infrequency of seeing large polar bodies, which have an incidence of about 3 % [3, 4]. Several studies have found that enlarged polar bodies have a decreased potential for fertilization and have impaired development [5, 8] and Ebner et al. recommends against transferring them [4]. Others have not found an impact of very large polar bodies, which may be related to their infrequent incidence ([3] with 25 out of 875 oocytes, [1] with 5 out of 967 oocytes). The morphological categories of oocytes having polar bodies with a smooth surface or a rough surface was also not evaluated, because a rough appearing polar body is likely to reflect ageing such as could be induced by prolonging the time period between the denuding of granulosa cells from the oocyte and performing ICSI [11].

Our choice to exclude enlarged polar bodies from analysis had no impact on the results. Oocyte maturation produced only 4 enlarged polar bodies (1.4 %). All of these had various additional dysmorphisms. The single oocyte with an enlarged and fragmented polar body did not fertilize. Four enlarged mature oocytes were also excluded. None of these had the dysmorphisms focused on in this paper. Although three of the four oocytes fertilized, only one produced a “transferable” embryo in terms of embryo cell number.

Another issue may be that we did not use a traditional embryo morphology assessment in our classification of embryos suitable for transfer (Table 1). The incidence of low-grade embryos in terms of morphology in this study group was only 5.4 % (11 embryos). Three of these embryos had a normal first polar body and eight had a fragmented polar body. On the day of transfer, five of these embryos were category D and five were category C. The category C embryos were all day two embryos. The eleventh embryo came from an oocyte with a fragmented polar body and was category A (1, 2, 7). Any bias created by not using traditional embryo morphology in the classification scheme of Table 1 would have worked against the results of this study.

The decreased medication use and the related decreased requirement for patient monitoring are major cost saving benefits of IVM compared to IVF [6]. However, an obvious concern about IVM is that it appears to be more labor intensive for the laboratory [6]. The opportunity to individualize the amount of effort placed into the laboratory portion of IVM by eliminating laboratory efforts directed at oocytes known to have limited value (when a reasonable number of

higher value oocytes exist for a given patient) will make IVM even more cost efficient and likely promote its expanded use. It is therefore important that other programs look at FPB in a prospective manner to try to arrive at a consensus about the significance of FPB in an IVM setting.

References

1. Chamayou S, Ragolia C, Alecci C, Storaci G, Maglia E, Russo E, et al. Meiotic spindle presence and oocyte morphology do not predict clinical ICSI outcomes: a study of 967 transferred embryos. *Reprod Biomed Online*. 2006;13:661–7.
2. Ciotti PM, Notarangelo L, Morselli-Labate AM, Felletti V, Porcu E, Venturoli S. First polar body morphology before ICSI is not related to embryo quality or pregnancy rate. *Hum Reprod*. 2004;19:2334–9.
3. De Santis L, Cino I, Rabelotti E, Calzi F, Persico P, Borini A, et al. Polar body morphology and spindle imaging a predictors of oocyte quality. *Reprod Biomed Online*. 2005;11:36–42.
4. 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.
5. Fancosovits P, Zsuzsa, Tothne G, Murber A, Takacs FZ, Papp Z, et al. Correlation between first polar body and further embryo development. *Acta Biol Hung*. 2006;57:331–8.
6. Jurema MW, Nogueira D. In vitro maturation of human oocytes for assisted reproduction. *Fertil Steril*. 2006;86:1277–91.
7. Loutradis D, Drakakis P, Kallianidis K, Milingos S, Dendrinis S, Michalas S. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. *Fertil Steril*. 1999;72:240–4.
8. Navarro PA, de Araújo MM, de Araújo CM, Rocha M, dos Reis R, Martins W. Relationship between first polar body morphology before intracytoplasmic sperm injection and fertilization rate, cleavage rate, and embryo quality. *Int J Gynaecol Obstet*. 2009;104:226–9.
9. Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, et al. Significance of metaphase II human oocyte morphology on ICSI outcome. *Fertil Steril*. 2008;90:1692–700.
10. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. *Hum Reprod Update*. 2011;17:34–45.
11. Verlinsky Y, Lerner S, Illkevitch N, Kuznetsov V, Kuznetsov I, Cieslak J, et al. Is there any predictive value of first polar body morphology for embryo genotype or developmental potential? *Reprod Biomed Online*. 2003;3:336–41.
12. Xia P. Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality. *Hum Reprod*. 1997;12:1750–5.
13. Yakin K, Balaban B, Isiklar A, Urman B. Oocyte dysmorphism is not associated with aneuploidy in the developing embryo. *Fertil Steril*. 2007;88:811–6.