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Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection

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The fertilization rates and further development of 528 human metaphase II oocytes directly injected by a single spermatozoon were analysed with respect to their morphological features at the light microscopy level at the time of retrieval. The deviations of oocyte morphology which were most frequently observed, after removal of cumulus cells, were dark incorporations, dark zona pellucida, large perivitelline space, spots, vacuoles, refractile bodies and irregular shape. These deviations correlated neither with the fertilization rate nor with the embryo quality score, as compared to 'ideal' oocytes. Since the majority of oocytes displayed deviations from the 'ideal' morphotype but were still fertilized and developed in culture at a normal rate, they were probably as normal as 'ideal' oocytes. Since some of these morphotypes, such as refractile bodies, have been shown to be associated with failure of fertilization, it seems that intracytoplasmic sperm injection may be an appropriate method of treatment for couples in whom repeated failure of in-vitro fertilization is associated with the retrieval of dysmorphic oocytes in the presence of normal semen characteristics.

Key words: intracytoplasmic sperm injection/oocyte morphology

Introduction

It is well known that the overall fertilization rate in in-vitro fertilization (IVF) programmes does not exceed 60–70% (Wittmaack *et al.*, 1994). About one-third of inseminated oocytes do not become fertilized, and this is mostly due to the absence of sperm penetration, as has been shown by the analysis of unfertilized oocytes (Dyban *et al.*, 1992).

When direct intracytoplasmic sperm injection (ICSI) is employed, the need for the spermatozoon to penetrate the oocyte is rendered obsolete, but not all oocytes become fertilized even when the spermatozoon has been successfully placed in the ooplasm (Dozortsev *et al.*, 1994). In some cases, this can be attributed to deficiency of the sperm-associated oocyte activating factor (Dozortsev *et al.*, 1995a). Additionally, we have suggested that some intrinsic oocyte problems can also be held responsible for fertilization failure. This is especially relevant since it is known that some oocyte anomalies, which can be assessed at the light microscopy level, such as refractile bodies, are associated with failure of fertilization in the presence of apparently normal spermatozoa. Furthermore, Van Blerkom and Henry (1992) have reported that 13% of unfertilized oocytes after IVF show morphological anomalies which are probably incompatible with normal fertilization. These anomalies are gross and account for only a fraction of the fertilization failures after IVF. It could well be that there are less obvious morphological anomalies in retrieved oocytes which can influence the fertilization outcome, but these minute anomalies are usually not detected in IVF oocytes because these oocytes are not cleaned free of their cumulus cells, except in cases of preimplantation genetic diagnosis on the first polar body (Verlinsky *et al.*, 1992), and therefore fertilization failure may be ascribed to ageing or handling.

Since ICSI requires cleaning of the oocytes from their surrounding cumulus cells within a few hours after retrieval, this creates an opportunity to study in detail some morphological deviations from what is expected to be an 'ideal' human oocyte. It is generally accepted that good-quality human metaphase II oocytes should have a clear, moderately granular cytoplasm, a small perivitelline space and a clear, colourless zona pellucida (Veeck, 1988). A lot of oocytes, however, exhibit an infinite number of variations from the type described above with regard to colour, granularity and homogeneity of the cytoplasm, size of the perivitelline space, colour of the zona pellucida, cytoplasmic incorporations, shape of the oocyte, etc. (Van Blerkom, 1990). The present paper reports the results of a retrospective study in which a possible relationship between morphological variations in injected oocytes and the outcome after ICSI in terms of fertilization rates and embryo quality was assessed.

Materials and methods

Data from 56 consecutive patients entering our ICSI programme during a period of 3 months were reviewed for this study. All patients presented with severe sperm deficiencies or had at least one previous cycle of failed IVF. The stimulation was performed in a standard way, using the combination of a long-acting gonadotrophin-releasing hormone agonist (goserelin, Zoladex®; ICI, Gent, Belgium) with daily administration of human menopausal gonadotrophin (Humegon®; Organon, Oss, The Netherlands). Oocyte retrieval was performed by transvaginal aspiration under ultrasound guidance 34–37 h after the injection of human chorionic gonadotrophin (Pregnyl®; Organon).

Following retrieval, the oocytes were exposed briefly to 80 IU/ml hyaluronidase (type VIII; Sigma Chemical Co., St Louis, MO, USA) and mechanically cleaned from their surrounding cumulus cells by aspiration through a glass pipette of ~200 μm inner diameter. Next, all oocytes were examined under an inverted microscope (Zeiss Axiovert 135) at a magnification of ×320 and those with a first polar

Table I. Outcome of metaphase II oocytes submitted to intracytoplasmic sperm injection, according to their morphological appearance

	Total injected	Damaged	No PN	1 PN	2 PN (%)	3 PN	Embryo score
'Ideal'	194	6	44	2	135 (72)	7	3.08 ± 1.07
One anomaly	206	10	45	13	129 (66)	9	3.12 ± 1.17
Two or more anomalies	128	4	37	5	78 (63)	4	3.25 ± 1.09
Type of anomaly							
Dark cytoplasm	3	–	1	–	2 (67)	–	3.30 ± 0.30
Dark zona pellucida	136	10	24	6	94 (75)	2	3.31 ± 1.05
Large perivitelline space	66	1	21	3	39 (60)	2	3.52 ± 1.02
Refractile body	98	1	26	10	57 (59)	4	3.24 ± 1.14
Dark incorporations	105	5	25	2	68 (68)	5	3.07 ± 1.13
Spots	24	–	6	–	17 (71)	1	2.96 ± 1.26
Irregular shape	28	1	8	–	18 (67)	1	2.89 ± 1.15
Vacuole	24	1	9	2	10 (43)	2	3.00 ± 1.04
Multiple vacuoles	6	2	2	–	2 (50)	–	2.10 ± 1.50
Total	528	20	126	20	342 (67)	20	3.14 ± 1.12

PN = pronucleus.

body present were selected for micromanipulation. At this time, all oocytes were scored by two independent observers for the presence or absence of any of the following traits: dark incorporations in the cytoplasm, vacuoles, spots, refractile bodies, darkness and/or increased granularity of the cytoplasm, dark zona pellucida, large perivitelline space and irregular shape. Oocyte incubation prior to and following injection took place in Earle's medium supplemented with 10% fetal cord serum under mineral oil (Squibb, Princeton, NJ, USA) at 37°C in an atmosphere of 5% CO_2 in air. All semen samples were obtained by masturbation and in all cases sufficient motile spermatozoa could be recovered for injection. Sperm injection was performed as described elsewhere (Dozortsev *et al.*, 1994). Assessment of fertilization and further embryonic development took place after 18 and 42 h respectively. Embryonic quality was graded using a grading system modified after the system described by Puissant *et al.* (1987). Differences in fertilization rates between different groups of oocytes were analysed using a χ^2 test, and differences in embryo quality scores were analysed using a Mann–Whitney *U* test.

Results

A total of 528 oocytes were at second metaphase at the time of retrieval and could be injected. Of these, 20 (3.8%) were damaged following the procedure and were excluded from further analysis. Table I shows the relationship between individual morphological abnormalities and fertilization rate. Since no statistically significant differences were found between any of the morphological categories separately, all oocytes were divided into three groups: oocytes with 'ideal' morphology (n = 194) and oocytes exhibiting one (n = 206) or two or more morphological deviations (n = 128). Following assessment of the presence of two pronuclei (PN), no statistically significant differences were found between the rates of normal fertilization in the three groups (respectively 72, 66 and 63%, Table I). Also, the rates of 1PN and 3PN formation were not significantly different. Furthermore, there was no significant difference in the quality scores of embryos at the cleavage stage between oocytes displaying abnormal features and oocytes without abnormalities, as assessed by the degree of fragmentation at the time of transfer (Table I). Since most embryo transfers were mixed, i.e. embryos originating from all three groups of oocytes were transferred together, it was not possible to study the relationship between oocyte morphology at the time of retrieval and pregnancy rates or outcome.

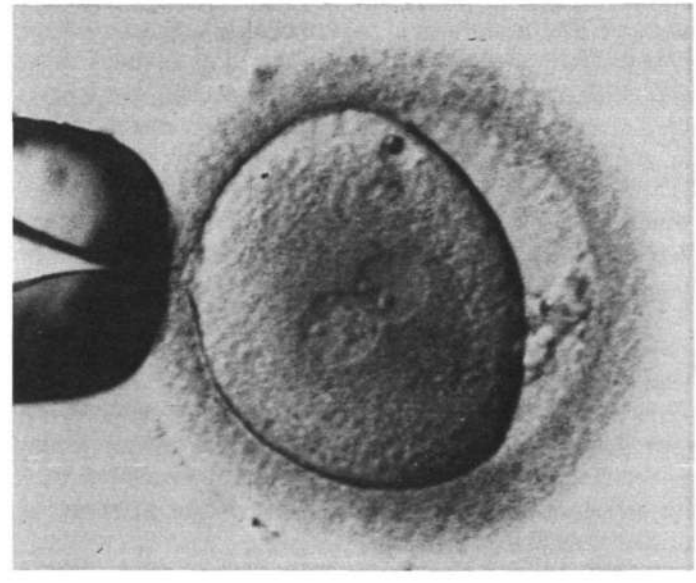

Figure 1. Zygote obtained by intracytoplasmic sperm injection of an oocyte displaying a bull's eye. Both pronuclei and the bull's eye are clearly visible. Original magnification: ×320.

Discussion

The present study is the first study on a large number of human oocytes cleaned from their cumulus cells prior to fertilization which investigates the correlation between minor morphological deviations from the 'ideal' morphotype and fertilization rates and subsequent development in culture after ICSI. Our data show that there is no correlation between the deviations most frequently observed at the light microscopy level and fertilization rates after ICSI. The only exception was a group of oocytes displaying vacuoles, which are a sign of severe degeneration (Van Blerkom, 1990). Of special practical interest is the finding of the same fertilization rate for 'ideal'

oocytes and oocytes containing a refractile body (Alikani *et al.*, 1992), a defect which in routine IVF is usually associated with failure of fertilization (Veeck, 1988). This would imply that the persistent retrieval of such oocytes from cycle to cycle in a particular patient is an indication to perform ICSI. Moreover, this study suggests that eventually all oocytes can be fertilized by ICSI, independently of their morphological peculiarities at the light microscopy level, since the fertilization rate did not differ significantly between any groups of oocytes.

We also did not find any deviation from the normal distribution of embryo quality scores for any of the groups of oocytes. This suggests that there is no correlation between oocyte morphology and the quality score of the resulting embryo, although this of course does not necessarily imply that those embryos will have the same developmental potential as those resulting from 'ideal' oocytes. Indeed, other studies have shown that aged or unfertilized oocytes also retain the ability to become fertilized after ICSI, but that their developmental potential is impaired (Dozortsev *et al.*, 1995b). It is interesting to note that so-called 'ideal' oocytes represent only 34% of all oocytes retrieved. It may be that some of the deviations observed are the result of the hormonal stimulation and others may be induced by handling procedures immediately after aspiration, but the origin of most of them is largely unknown. Since fertilization rates in IVF in the presence of normal semen characteristics are up to 70%, at least half of all fertilized oocytes come from a group displaying one or more deviations from what we would consider to be the 'ideal' morphotype. Since oocytes are not cleaned immediately after retrieval in routine IVF, it is not known which of the deviations allow normal fertilization. On the other hand, it is definitely known which deviations (such as refractile bodies and bull's eyes) do impair fertilization. Therefore, it is interesting to note that after ICSI oocytes displaying a refractile body or a bull's eye (Figure 1) are fertilized in the same proportion as 'ideal' oocytes.

Larger studies will be needed to investigate the relationship between patient-related factors and oocyte morphology on one hand, and the correlation between the size and shape of certain of the observed anomalies in some oocytes and their behaviour after ICSI on the other hand.

In conclusion, oocytes showing deviations from the 'ideal' morphotype are probably as normal as 'ideal' oocytes, at least in terms of capacity for fertilization and embryo quality following ICSI. Moreover, even oocytes displaying morphological anomalies associated with low fertilization rates in routine IVF, such as refractile bodies, can be fertilized at a normal rate by ICSI. It therefore seems possible that some couples with unexplained failure of IVF, all of whose oocytes are dysmorphic, may benefit from ICSI.

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