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# Oocyte morphology predicts outcome of intracytoplasmic sperm injection

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**To examine the influence of cytoplasmic morphology on the success rate of intracytoplasmic sperm injection (ICSI), the morphology of 837 metaphase II oocytes was assessed after cumulus stripping. The main abnormalities detected were excessive granularity, cytoplasmic inclusions such as vacuoles, smooth endoplasmic reticulum clustering and refractile bodies. Microinjection was performed in 538 oocytes with normal cytoplasm, 142 out of 161 with excessive granularity and 112 out of 138 with cytoplasmic inclusions. Very poor oocytes were not injected. No difference was found in fertilization rate. The embryos achieved cleaved normally and a similar number of good quality embryos among the three groups was noted. The outcome of transfer of embryos derived solely from normal oocytes (group A: 72 patients, 183 embryos) was compared with those from oocytes with cytoplasmic abnormalities (group B: 34 patients, 85 embryos). In group A, 17 clinical pregnancies (24% per patient, implantation rate 10%) were established. In group B, only one clinical pregnancy (3% per patient, implantation rate 1%) was established, from the transfer of embryos derived from oocytes with homogeneous granularity of the cytoplasm. No pregnancy resulted following the transfer of embryos from eggs with cytoplasmic inclusions. The difference was statistically significant. The outcome of ICSI is dependent on the quality of the oocytes retrieved. Normal fertilization and early embryo development were achieved in oocytes with abnormal cytoplasm morphology, but the resulting embryos failed to demonstrate the same implantation potential as those derived from oocytes with normal cytoplasm.**

*Key words:* implantation rate/intracytoplasmic sperm injection/oocyte morphology

## Introduction

Intracytoplasmic sperm injection (ICSI) is now a well-established technique which can achieve substantial fertilization and pregnancy rates in couples with intractable male infertility (Van Steirteghem *et al.*, 1993a,b). In contrast with conventional in-vitro fertilization (IVF), no correlation is found between fertilization and pregnancy rates and semen parameters in patients undergoing ICSI (Mansour *et al.*, 1995). Other prognostic factors may be more relevant: for example, the age of the female partner has been shown to have the same effect on the outcome of ICSI as on the outcome of conventional IVF (Abdelmassih *et al.*, 1996).

Little attention has been focused on oocyte morphology in standard IVF techniques, because it is often difficult to assess the cytoplasmic morphology of the oocyte and the exact stage of maturation as the oocytes are always surrounded by cumulus or corona cells at the time of collection. Since the progression of the oocyte through meiosis can continue *in vitro*, and fertilization is likely to occur when the oocytes are mature, the precise assessment of the nuclear maturity of the oocyte is not as critical for conventional IVF as for ICSI. As only mature (metaphase II) oocytes are suitable for ICSI, the cumulus cells are routinely removed for accurate assessment of the nuclear maturity and to facilitate handling during the microinjection procedure. A more precise assessment of the oocytes and the cytoplasmic morphology is therefore possible.

Since oocyte quality may be an important prognostic factor, a retrospective study was undertaken to assess the influence of cytoplasm morphology of the oocyte on the success rate of ICSI.

## Materials and methods

Our study included 106 patients undergoing ICSI treatment between January 1995 and February 1996. All women were <37 years of age, with a mean age of 31.8 ± 4.2 years. The range of duration of infertility was 2–3 years. All couples enrolled in the study had solely male factor infertility. Ovarian stimulation was induced in all patients using the same drug regime. The long down-regulation with gonadotrophin releasing hormone (GnRH) analogue (Suprefact nasal spray, Hoechst UK, Hounslow, Middlesex, UK) and gonadotrophin was used, as previously described (Ranieri *et al.*, 1995) and oocyte retrieval was performed transvaginally under ultrasound control, 36 h after human chorionic gonadotrophin (HCG) administration. A minimum of five eggs was retrieved per patient.

Three to four hours after egg collection, following incubation of <30 s in culture medium containing 80 IU/ml hyaluronidase (Type VIII, H-3757 Sigma), oocytes were individually treated by gentle pipetting to remove the cumulus and corona radiata cells. Oocyte cytoplasm morphology was assessed at ×100 and ×200 magnification and the eggs were classified as having (i) normal morphology, (ii) excessive cytoplasmic granularity, (iii) cytoplasmic inclusions (Veek, 1991). (i) Normal oocytes showed clear cytoplasm with uniform texture and homogeneous fine granularity (Figure 1). (ii) Granular oocytes were dark, with granularity either homogeneous affecting the whole cytoplasm (Figure 2), or concentrated as a dark mass in the central portion of the oocyte with a clear peripheral ring (Figure 3) (Van Blerkom and Henry, 1992). (iii) Cytoplasmic inclusions comprised vacuoles presumed to be of endocytotic origin (Figure 4), accumula-

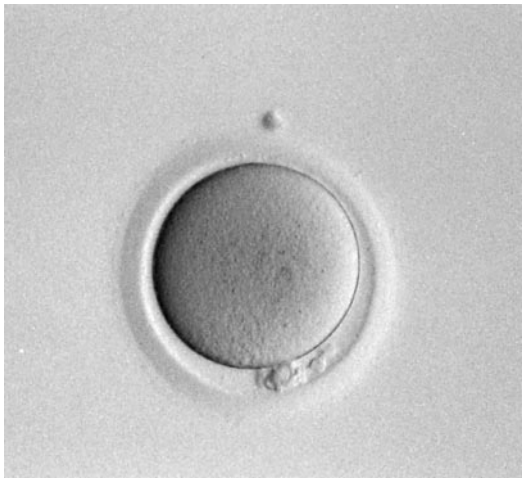

**Figure 1.** Oocyte with normal cytoplasm.

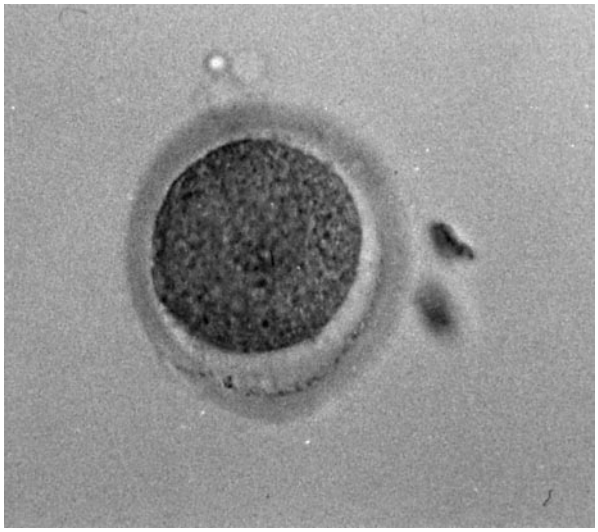

**Figure 2.** Oocyte with homogeneous granularity affecting the whole cytoplasm.

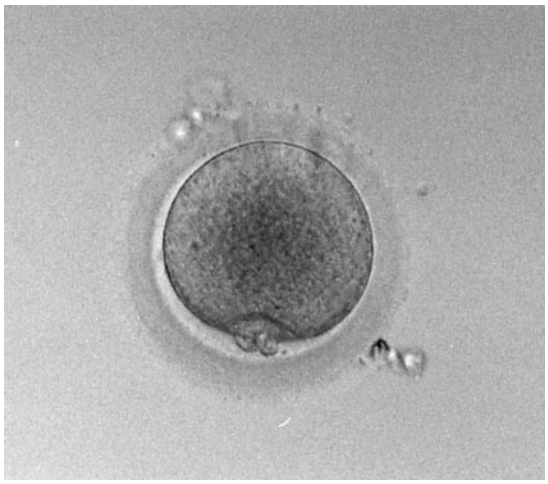

**Figure 3.** Oocyte with excessive granularity concentrated in the central part of the cytoplasm.

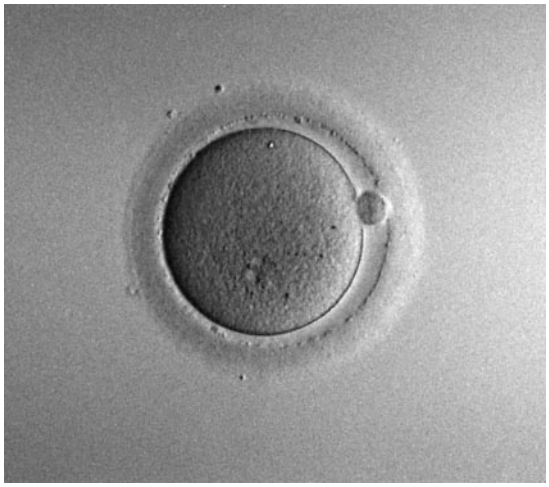

**Figure 4.** Oocyte with vacuoles.

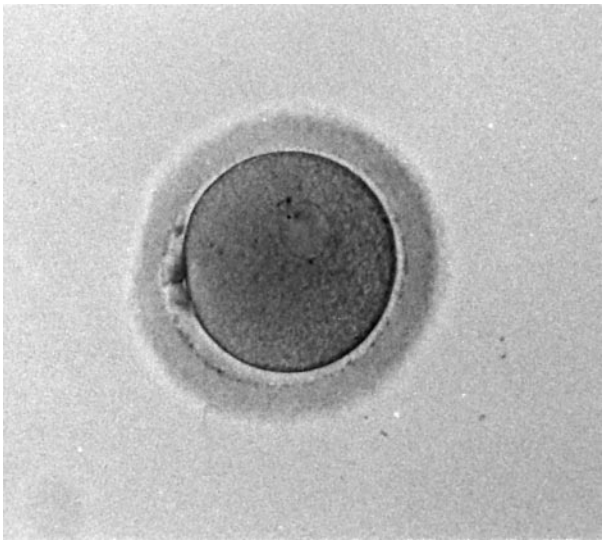

**Figure 5.** Oocyte with cluster of saccules in the smooth endoplasmic reticulum.

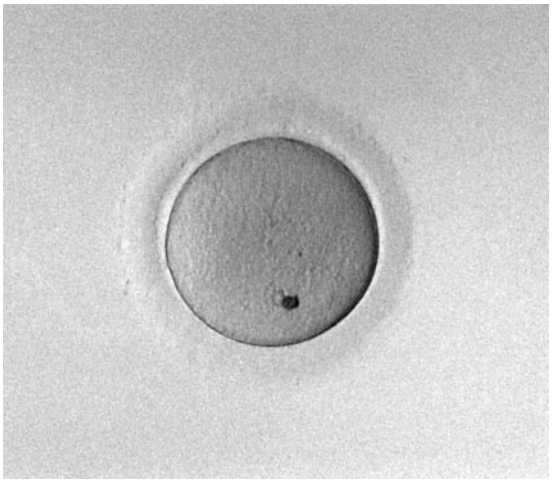

**Figure 6.** Oocyte with refractile body.

**Table I.** Biological performance of oocytes undergoing intracytoplasmic sperm injection according to cytoplasmic morphology

| | Normal cytoplasm | | Granular cytoplasm | | Cytoplasmic inclusions | |
|---|---|---|---|---|---|---|
| | *n* | Rate (%) | *n* | Rate (%) | *n* | Rate (%) |
| Retrieved metaphase II oocytes | 538 | 64 | 161 | 19 | 138 | 17 |
| Injected oocytes | 536 | 99 | 142 | 88 | 112 | 81 |
| Fertilized oocytes | 336 | 63 | 81 | 57 | 68 | 61 |
| Cleaved embryos | 290 | 86 | 66 | 81 | 65 | 95 |
| Grade I | 173 | 60 | 37 | 56 | 40 | 62 |
| Grade II | 95 | 33 | 25 | 38 | 19 | 29 |
| Grade III | 22 | 7 | 4 | 6 | 6 | 9 |

**Table II.** Pregnancy and implantation rates according to oocyte morphology after transfer of embryos obtained with intracytoplasmic sperm injection

| | Group A Normal cytoplasm ($n$ = 72) | Group B ($n$ = 34) | | | $P$ |
|---|---|---|---|---|---|
| | | Granular cytoplasm | Cytoplasmic inclusions | Total | |
| No. of embryos | 183 | 45 | 40 | 85 | >0.05 |
| Grades I + II | 183 | 42 | 38 | 80 | >0.05 |
| Grade III | 0 | 3 | 2 | 5 | |
| Mean no. of embryos transferred | 2.5 ± 0.5 | | 2.5 ± 0.6 | | >0.05 |
| Pregnancy rate per transfer | 17 (24%) | | 1 (3%) | | 0.008 |
| Implanted embryos/ implantation rate | 19 (10%) | | 1 (1%) | | 0.007 |

tions of saccules of the smooth endoplasmic reticulum (SER) giving a pronuclear-like structure (Figure 5) or refractile bodies of ~10 μm in diameter containing lipid material and dense granules (Figure 6) (Veeck, 1991; Van Blerkom and Henry, 1992; Bedford and Kim, 1993).

After preparing the semen sample the ICSI procedure was performed following conventional techniques (Van Steirteghem *et al.*, 1993b). Oocytes with two pronuclei and two polar bodies 14–18 h after ICSI were considered as normally fertilized.

The quality of the embryos was assessed on the day of embryo transfer, ~48 h after egg retrieval. Embryos without fragmentation were categorized as grade I, those with <20% of the volume of the embryo fragmented were categorized as grade II and those with anucleate fragments present in 20–50% of the volume of the embryo were categorized as grade III. Embryos with >50% anucleate fragments were not transferred (Staessen *et al.*, 1992).

To support the luteal phase, 100 mg of progesterone i.m. (Gestone, Paines & Byrne, Greenford, Middlesex, UK) was administered daily for 16 days. A urinary pregnancy test was performed 16 days after embryo transfer and when positive the first ultrasound scan was scheduled for 4 weeks after embryo transfer. Only clinical pregnancies, with a fetal heart confirmed by ultrasound, were recorded.

For statistical analysis Student's *t*-test and the $\chi^2$-test were used as appropriate.

## Results

A total of 982 oocytes were collected. Of these, 837 (85%) were in metaphase II and the remainder in metaphase I or germinal vesicle stage. On assessment of mature oocytes, 538 (64%) were found to have normal cytoplasm, 161 (19%) excessive granularity and 138 (17%) cytoplasmic inclusions such as SER cumulus, vacuoles and/or refractile body.

All the eggs with normal morphology were microinjected. Very poor oocytes were not considered for microinjection, thus 142 (88%) out of 161 oocytes with excessive granularity of the cytoplasm and 112 (81%) out of 138 oocytes with cytoplasmic inclusions were injected (Table I).

There was no difference in biological performance between eggs with normal cytoplasm and eggs with cytoplasmic abnormalities. The fertilization rate was not statistically significantly different ($P > 0.05$) and a normal cleavage rate was observed.

In 72 patients (group A) 183 embryos (mean 2.5 ± 0.5 per embryo transfer) derived entirely from oocytes with normal cytoplasmic morphology were transferred. It was possible to select embryos in this group as only 20% of the oocytes retrieved showed cytoplasmic abnormalities.

In the remaining group of patients (32%), the entire pool of oocytes collected from each patient possessed cytoplasmic abnormalities. Thus, in 34 patients (group B) 85 embryos (mean 2.5 ± 0.5 per embryo transfer) derived entirely from abnormal oocytes with either excessive granularity or cytoplasmic inclusions were transferred. It was not possible to make a selection in this group as all the oocytes showed cytoplasmic abnormalities at collection. The mean number of embryos transferred was the same in both groups ($P > 0.05$). The number of grade I and grade II embryos transferred was not significantly different in the two groups ($P > 0.05$). The distribution of age between groups A and B was not significantly different.

Fifteen singleton and two twin clinical pregnancies (24% per transfer) were established in group A. The implantation rate was 10%. Only one pregnancy (3% per transfer) was established in group B from oocytes with homogeneous granularity of the cytoplasm. No pregnancies were observed from oocytes with cytoplasmic inclusions. The implantation rate was 1%. The difference was statistically significant ($P < 0.01$) (Table II).

## Discussion

Stripping cumulus cells from around the oocytes is an important part of the ICSI procedure. It facilitates handling of the oocytes during microinjection and allows an accurate assessment of cytoplasmic morphology, nuclear maturity and structural abnormalities that may adversely affect the outcome of the treatment. The main morphological abnormalities of the oocyte cytoplasm noted in the patient group, were significant granularity of the cytoplasm (dark centre or homogeneous granularity) and cytoplasmic inclusions (vacuoles of different diameter, SER cumulus, refractile bodies).

Cytoplasmic granularity can be homogeneous affecting the whole cytoplasm, or concentrated in the centre with a clear peripheral ring giving a darkened appearance to the cytoplasm. In conventional IVF, where the choice of embryos was restricted to those obtained solely from granular oocytes, term pregnancies have been reported (Veeck, 1988, 1991). However,

oocytes with darkened and granular centres often fail to fertilize and have reduced developmental potential (Veeck, 1988, 1991; Bedford and Kim, 1993). We have demonstrated a normal ICSI fertilization rate with oocytes exhibiting homogeneous cytoplasmic granularity or a granular centre, but the pregnancy rate was very poor and the only pregnancy recorded in the study was observed in a patient whose oocytes had homogeneous granularity.

Cytoplasmic inclusions have been associated with a poor prognosis for fertilization and pregnancy with conventional IVF (Veeck, 1991; Bedford and Kim, 1993). We have shown that patients with vacuoles or SER clusters in the cytoplasm can have normal fertilization with ICSI but no pregnancy was achieved.

The refractile body, so called because of its nature under bright-field microscopy, is a structure 10 μm in diameter that was described by Veeck (1991). The evolution of this structure and its relationship to oocyte maturity and viability are not yet fully understood. When observed in the ooplasm of the human oocyte at collection, it indicates a poor prognosis for fertilization (2%) in conventional IVF (Veeck, 1991). Both mature and immature oocytes have demonstrated refractile bodies and there is a strong tendency for recurrence in the same patient in repetitive treatment cycles (Veeck, 1991). We have demonstrated in this study that eggs with a refractile body can achieve normal fertilization with ICSI but no pregnancies were observed.

The cause of these cytoplasmic morphological abnormalities is probably multifactorial. Firstly, ovarian stimulation is known to have an adverse effect on oocytes. Ovulation induction may result in the maturation of abnormal oocytes that would otherwise become atretic in the absence of stimulation. Human oocytes recovered from stimulated IVF cycles have been shown to have a >40% incidence of numerical chromosomal abnormalities (Wramsby, 1988; Van Blerkom and Henry, 1992).

Secondly, oocyte quality may be directly affected by the hormonal environment. Ovarian steroids, particularly oestrogen and progesterone, are intimately involved in the initiation of cytoplasmic maturation and the final stage of nuclear maturation of the oocytes (Thibault, 1977). The stimulation regimen used for IVF may be important. All patients in our study used the long GnRH analogue regimen, which has been shown to give a significantly higher percentage of mature oocytes than the short regimen in patients undergoing ICSI (Greenblatt *et al*., 1995) and a better pregnancy rate with conventional IVF (Tan *et al.*, 1992).

We did not study the effect of specific gonadotrophin preparation on oocyte morphology; however, a recent study has demonstrated a high degree of morphological and nuclear anomalies in unfertilized eggs in patients undergoing ovarian stimulation with pure follicle stimulating hormone following pituitary desensitization (Wojcik *et al.*, 1995).

Our study demonstrates that the outcome of ICSI is dependent on the quality of the oocytes retrieved. Normal fertilization and early embryo development were achieved after microinjection of oocytes with cytoplasmic abnormalities as previously reported by De Sutter *et al*. (1995). However, this is the first study to report the outcome, following transfer, of embryos derived solely from oocytes with abnormal cytoplasmic morphology. In ideal circumstances these oocytes should not be used for microinjection. Oocytes with abnormal cytoplasm morphology were found to have high frequency of aneuploidy (Van Blerkom and Henry, 1992). Moreover, cytoplasmic abnormalities may be responsible for developmental failure (Van Blerkom *et al.*, 1995). The resulting embryos failed to demonstrate the same implantation potential as those derived from oocytes with normal cytoplasm. These results are now leading us to revise our selection criteria for oocytes destined for microinjection. Selection is now more rigorous than at the commencement of the ICSI programme. This approach is expected to improve the quality of the embryos obtained, and hence the success rates with ICSI.

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