Article

Donor oocyte dysmorphisms and their influence on fertilization and embryo quality

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Abstract

The aim of this study was to identify predictors of fertilization rate (FR) and embryo quality (EQ) in 126 donor women who underwent 160 intracytoplasmic sperm injection (ICSI) cycles. Main outcome measures were oocyte dysmorphisms (OD) [perivitelline space (normal/large), perivitelline debris (no/yes), oocyte shape (spherical/non-spherical), zona pellucida (normal/abnormal), first polar body morphology (normal/fragmented or irregular), cytoplasmic granularity (normal/excessive), cytoplasmatic vacuoles (no/yes) and colour of cytoplasm (normal/dark)], semen source (ejaculated or testicular), donor age, number of days of stimulation, plasma oestradiol concentration (pg/ml) on the day of human chorionic gonadotrophin administration, FR and day 2 EQ. No significant predictors of fertilization were identified. However, the presence of a dark cytoplasm decreased by 83% the likelihood of obtaining good quality embryos (OR = 0.17; 95% CI: 0.04–0.74). Oocytes with a larger than normal perivitelline space were associated with 1.8 times higher chance of having good quality embryos (OR = 1.80; 95% CI: 1.15–2.80). Patients and ovarian stimulation characteristics evaluated here did not affect FR and EQ. ICSI technique may guarantee good FR in spite of OD; however, certain cytoplasmic anomalies at the oocyte level, such as a dark cytoplasm, could compromise subsequent embryo development.

Keywords: embryo quality, fertilization, ICSI, oocyte donors, oocyte dysmorphisms

Introduction

Specific anomalies or dysmorphisms prior to microinjection may be detected by examination with light microscopy of oocytes following cumulus cell removal after retrieval. These anomalies or dysmorphisms have been observed in a high proportion (63%) of MII oocytes obtained after retrieval (De Sutter *et al.*, 1996; Balaban *et al.*, 1998).

It has been suggested that dysmorphic phenotypes which arise early in meiotic maturation may be associated with failed fertilization and aneuploidy, while those occurring later in

maturation may cause a higher incidence of developmental failure (Van Blerkom and Henry 1992; Ebner, 2006).

However, the data published in the literature give rise to contradictory results (for review, see Balaban and Urman, 2006). In fact, decreased fertilization rates (FR) with respect to some oocyte dysmorphisms (OD) have been reported by Xia (1997) and Ebner *et al.* (2000), while others have failed to observe that association (Alikani *et al.*, 1995; De Sutter *et al.*, 1996; Serhal *et al.*, 1997; Balaban *et al.*, 1998; Hassan-Ali *et al.*, 1998; Ebner *et al.*, 1999, 2002; Loutradis *et al.*, 1999; Kahraman *et al.*, 2000; Meriano *et al.*, 2001; Mikkelsen and Lindenberg, 2001; Ciotti *et al.*, 2004; Otsuki *et al.*, 2004;

Suzuki *et al.*, 2004; De Santis *et al.*, 2005).

The relationship between embryo quality (EQ) and oocyte morphological characteristics is equally controversial. Some groups reported a clear effect of some dysmorphisms on EQ (Xia, 1997; Loutradis *et al.*, 1999; Ebner *et al.*, 2000, 2002; Mikkelsen and Lindenberg, 2001; Otsuki *et al.*, 2004; Suzuki *et al.*, 2004), while others did not find that association (Alikani *et al.*, 1995; De Sutter *et al.*, 1996; Serhal *et al.*, 1997; Balaban *et al.*, 1998; Hassan-Ali *et al.*, 1998; Ebner *et al.*, 1999; Kahraman *et al.*, 2000; Meriano *et al.*, 2001; Ciotti *et al.*, 2004; De Santis *et al.*, 2005).

In most reports, OD were evaluated in oocytes coming from infertile patients´ cycles. As an exception, the article from Suzuki *et al.* (2004) evaluated chimpanzee OD, finding the same types of cytoplasmic alterations as seen in humans. No article on OD on fertile women relating to their subsequent influence on reproductive outcomes has been found. The aim of this work was to evaluate oocyte morphology in ovarian stimulation donor cycles and its influence in FR and EQ. Other factors with possible influence on the final outcome [semen source, donor age, number of days of ovarian stimulation and plasma oestradiol concentration on the day of human chorionic gonadotrophin (HCG) administration] were also evaluated using logistic regression analysis.

Materials and methods

Patients

A total of 1622 MII oocytes obtained from 160 ovarian stimulation cycles were evaluated, coming from 126 oocyte donors with proven fertility, all under 35 years of age. Treatments were performed in the IVF Unit between January 2005 and January 2006. ICSI was performed for moderate to severe male factor in 144 cycles and spermatozoa from ejaculated semen were used. In 16 cases of non-obstructive azoospermia, spermatozoa were recovered by open testicular biopsy. Donors had a mean age of 26.3 ± 4.3 years (range 18–34).

Ovarian stimulation and oocyte retrieval

Anonymous donors were stimulated with a short standard protocol with leuprolide acetate agonist (Procrin; Abbott, Madrid, Spain) starting on day 3 cycle concomitant with variable doses of recombinant human FSH (Puregon; Organon, The Netherlands or Gonal F; Serono, London, UK). Ovarian response was monitored by transvaginal ultrasound and plasma oestradiol concentrations. Ovulation was induced with 250 μg of recombinant HCG (Ovitrelle; Serono, London, UK). Oocytes were aspirated 36 h after recombinant HCG administration by transvaginal ultrasound-guided needle aspiration under sedation.

Sperm and oocyte preparation

Semen analyses were carried out according to the recommendations of the World Health Organization (WHO, 1999). The sperm samples were treated with a discontinuous gradient method using PureSperm (Nidacom International AB, Goteborg, Sweden). After centrifugation at 300 *g* for 15–20 min, the sperm pellet was washed with sperm medium (G-Sperm plus; Vitrolife, Kungsbacka, Sweden) and then resuspended with culture medium (G-fert plus; Vitrolife). For severe oligoasthenozoospermia, a mini swim-up technique was used to obtain the motile spermatozoa. In cases of azoospermia, open testicular biopsies were performed on the day of oocyte retrieval. Seminiferous tubules were thoroughly cut under dissecting microscope in a Petri dish (Falcon; Becton Dickinson, France) using hypodermic needles attached to insulin syringes or scalpels. The contents of the Petri dish were transferred to a conical tube (Nunc, Denmark) and centrifuged at 200 *g* for 5 min. The pellet was diluted and disseminated on an ICSI dish (Falcon) to use for microinjection.

Following retrieval, all oocytes were washed in a buffer (G-Mops plus; Vitrolife) and then cultured for at least 1 h at 37ºC in an atmosphere of 6% CO_2 in G-fert plus medium. Surrounding oocyte cumulus and corona radiata cells were removed by a brief exposure to 80 IU/ml hyaluronidase (Hyase; Vitrolife) followed by gentle pipetting. Prior to ICSI, all oocytes were examined under an inverted microscope (Nikon Eclipse TE2000S, Japan) at a magnification of ×400 with Hoffman modulation optics and those with a first polar body (MII) were selected for evaluation and microinjection.

ICSI procedure

ICSI was carried out between 4 and 5 h after oocyte retrieval on a heated stage (Tokai Hit Thermoplate, Model MATS-U505R30, Japan) at 37ºC, mounted on an inverted microscope (Nikon Eclipse TE2000S, Japan) equipped with Hoffmann modulation optics and Narishige (Narishige, Japan) micromanipulation system. Conventional microinjections were performed according to Palermo *et al.* (1992) and Van Steirteghem *et al.* (1993). All oocytes were microinjected by the same embryologist. Immediately after ICSI, and were cultured in G1.3 plus medium (Vitrolife) individually in microdrops 6 per dish covered with 3 ml of sterile equilibrated mineral oil (Ovoil; Vitrolife) at 37ºC in an atmosphere of 6% CO_2.

Oocyte dysmorphisms

Just before ICSI (40–41 h post-HCG administration or 4–5 h after retrieval), all oocytes were evaluated by two observers for the presence or absence of any OD. The microscope was equipped with a colour video camera (Sony, Model No. SSC-DC50AP, Sony Corporation, Japan) connected to a computer and the Cronus 2000 software (Cronus, Version 2.8.4; Research Instruments Ltd, Cornwall, UK) was employed for this purpose.

Normal oocytes showed a clear cytoplasm with homogeneous fine granularity, a round or ovoid first polar body with smooth surface and size within a small perivitelline space, and a colourless zona pellucida with regular shape (**Figure 1**). Extracytoplasmic OD evaluated before ICSI were: large perivitelline space, presence of perivitelline debris, non-spherical oocyte shape, abnormal zona pellucida and fragmented/irregular first polar body (**Figure 2a–e**). Cytoplasmic OD

Figure 1. Normal oocyte.

included excessive cytoplasmic granularity centrally located or affecting the whole gamete, presence of vacuoles and dark cytoplasm (**Figure 2f–h**). Vacuoles are considered fluid-filled membrane-bound cytoplasmic inclusions (Van Blerkom, 1990), and can be clearly distinguished morphologically from smooth endoplasmic reticulum clusters (sERC) under the inverted microscope (Otsuki *et al*., 2004). These clusters are not separated from the rest of the cytoplasm by a membrane (Ebner, 2006) and are implicated in implantation failure (Otsuki *et al*., 2004). Therefore, in this study, the presence of sERC was not evaluated.

Assessment of fertilization

Normal fertilized oocytes were recognized by the presence of 2 pronuclei and the extrusion of the second polar body 16–18 h after microinjection. Fertilized oocytes were cultured in fresh 50 μl of G1.3 plus medium in individual microdroplets covered with mineral oil at 37ºC in an atmosphere of 6% CO_2. Pronuclear scoring (Scott, 2003) was not evaluated in this study.

Day 2 embryo quality

EQ was assessed 44–47 h after ICSI. Embryos that showed 4 cells, less than 25% of cytoplasmic fragments, without blastomere multinucleation (MNB) and equal or similar (<20% difference) cells were considered as good quality embryos, and the remainder as poor quality ones.

Statistical analysis

In addition to OD, other independent factors with possible influence on the final outcome were also evaluated, including semen source (ejaculated or testicular); donor age; number of days of ovarian stimulation and plasma oestradiol concentration (pg/ml) on the day of HCG administration.

The associations between FR and EQ (dependent variables) and the independent variables were assessed using odds ratios

(OR) calculated by unconditional logistic regression analysis. OR refer to the number of times that fertilization or good quality embryo events occurred in oocytes with and without the characteristic of interest. Comparisons were also made in variables with more than one category using the first category as reference (OR = 1). Continuous confounding variables such as donor age, number of days of ovarian stimulation and plasma oestradiol concentration (pg/ml) on the day of HCG administration were not categorized, and were introduced as continuous variables in the model (years of age ranged from 18 to 34, stimulation days from 7 to 17, and oestradiol concentration (pg/ml) from 1230 to 9916).

All analyses, including OR and 95% confidence intervals (95% CI), were performed using the STATA statistical program. The likelihood ratio statistic was used to evaluate the overall significance for each variable, and *P*-values were estimated to assess linear trends. Tests for trends were performed for each ordinal variable including all confounding potential variables.

Results

The general characteristics of the cycles are shown in **Table 1**. The mean age of donors was 26.3 ± 4.3, producing 10.1 oocytes per cycle, to give a total of 160 embryo transfers. Sixteen cycles involving 156 oocytes required male testicular biopsy due to non-obstructive azoospermia. **Table 2** shows the distribution of oocyte morphological alterations. Frequency of alterations ranged from 3.4% for oocytes with dark cytoplasm to 82.4% for first polar body fragmented or irregular. In total, 94.5% of all oocytes showed at least one dysmorphism (*n* = 1533).

Global FR was 66.7% (1082/1622) and was not affected by OD (**Table 3**). Other general variables did not have a predictive effect on FR (**Table 4**). Nevertheless, a large perivitelline space and the use of testicular spermatozoa for ICSI decreased the fertilization rate in 28 and 30% respectively [OR = 0.70 (95% CI = 0.50–1.02) and OR = 0.70 (95% CI = 0.47–1.05)], although in the logistic regression analysis these variables were not quite significant (*P* = 0.07 and *P* = 0.09 respectively).

Overall, 37% (400/1082) of all embryos showed good quality (4 cells, <25% of cytoplasmic fragmentation, no MNB and equal or similar blastomeres) 44–47 h after ICSI. In the remaining embryos (63%), 46 zygotes presented an embryo cleavage blockage (4.3%). **Table 5** shows the effect of OD on EQ. Centrally or extended excessive cytoplasmic granularity and the presence of vacuoles decreased by 32 and 49% respectively the possibility of obtaining good embryos, although these reductions were not statistically significant. The presence of dark cytoplasm increased the risk of obtaining poor quality embryos in 83% [OR = 0.17 (95% CI = 0.04–0.74)]. An opposite effect was seen with a large perivitelline space, which had a beneficial effect on embryo quality, increasing the chance of obtaining good embryos [OR = 1.80 (95% CI = 1.15–2.80)] (**Table 5**). On the other hand, semen source (ejaculated or testicular), donor age, number of days of ovarian stimulation and concentration of plasma oestradiol concentration (pg/ml) on the day of HCG administration had no effect on EQ (**Table 6**).
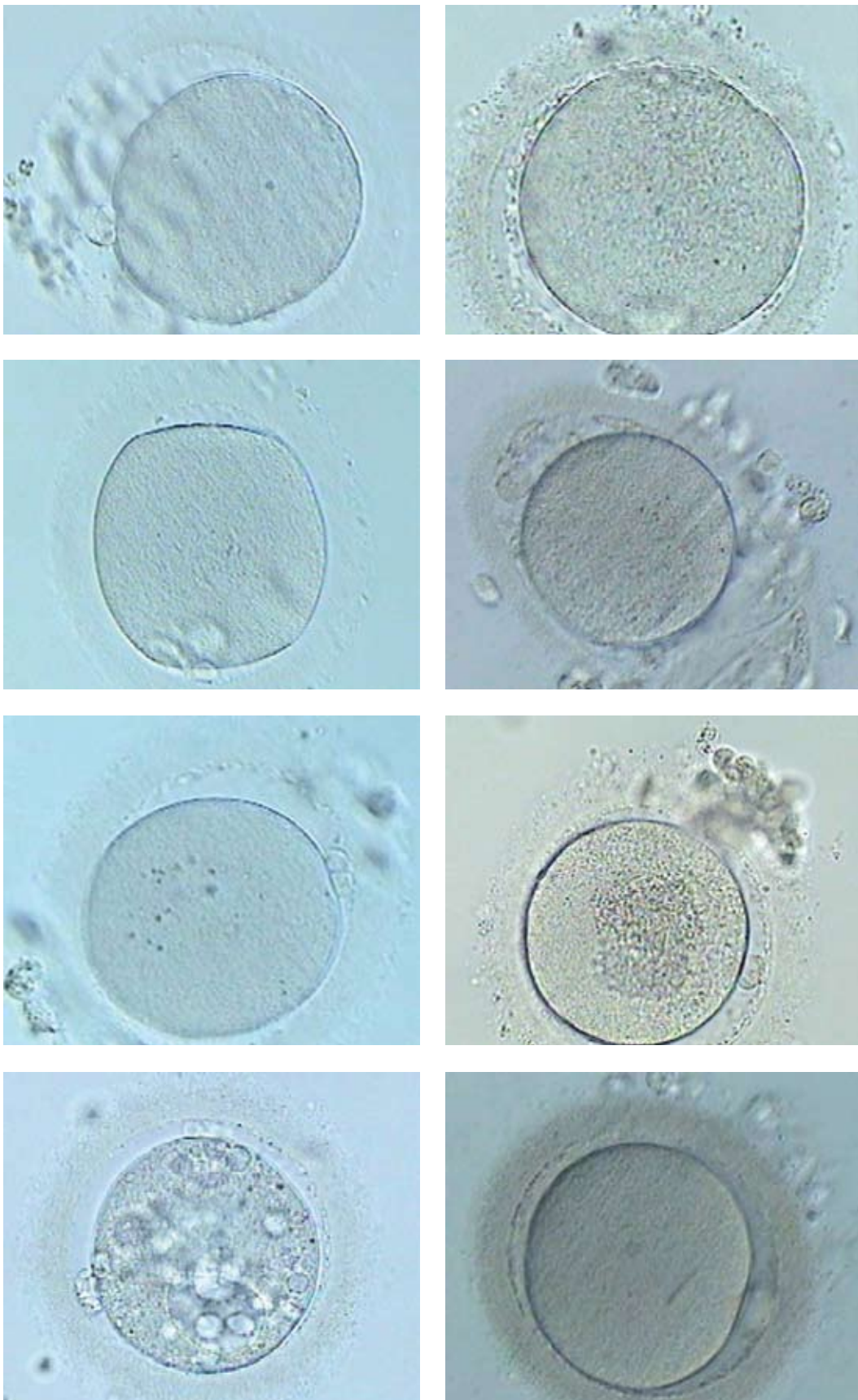
Figure 2. Oocyte dysmorphisms: (**a**) large perivitelline space, (**b**) perivitelline debris, (**c**) non-spherical oocyte, (**d**) abnormal zona pellucida, (**e**) fragmented first polar body, (**f**) excessive centrally located granularity, (**g**) presence of vacuoles, and (**h**) dark cytoplasm.

Table 1. General characteristics of stimulation cycles.

Parameter	Number[a]
Patients	126
Cycles	160
Mean age ± SD (years)	26.29 ± 4.28
Oocytes/patient (mean)	12.87
Oocytes/cycle (mean)	10.14
Cycles with ejaculated spermatozoa	144
Cycles with testicular spermatozoa	16
Mean no. (± SD) of stimulation days	10.73 ± 1.93
Mean oestradiol concentration on HCG day (pg/ml)	3880.97 ± 1590.99

HCG = human chorionic gonadotrophin.
[a]Unless otherwise stated.

Table 2. Distribution of oocyte dysmorphisms. Values are percentages.

Parameter	Donated oocytes, n = 1622
Large perivitelline space	10.9
Perivitelline debris	40.9
Non-spherical oocyte shape	37.7
Abnormal zona pellucida	27.3
Fragmented/irregular first polar body	82.4
Excessive cytoplasm granularity	31.8
Presence of vacuoles	4.2
Dark cytoplasm	3.4

Table 3. Influence of dysmorphisms on fertilization rate (FR) after intracytoplasmic sperm injection in donor oocyte patients.

	No. of oocytes	FR (%)	Odds ratio[a]	95% CI
Perivitelline space				
Normal	1446	67.4	1.00	
Large	176	61.4	0.72	0.50–1.02
Perivitelline debris				
No	958	66.2	1.00	
Yes	664	67.5	1.05	0.84–1.32
Oocyte shape				
Spherical	1010	66.8	1.00	
Non-spherical	612	66.5	0.98	0.77–1.24
Zona pellucida integrity				
Normal	1180	66.6	1.00	
Abnormal	442	67.0	1.01	0.78–1.31
First polar body				
Normal	285	67.4	1.00	
Fragmented/irregular	1337	66.6	0.92	0.72–1.18
Cytoplasmic granularity				
Normal	1106	67.6	1.00	
Excessive	516	64.7	1.17	0.90–1.50
Presence of vacuoles				
No	1554	66.9	1.00	
Yes	68	61.8	0.81	0.51–1.29
Cytoplasm colour				
Normal	1567	66,8	1.00	
Dark	55	65.5	1.04	0.58–1.85

All comparisons were not significant
[a]Odds ratio adjusted for all variables of the table

Table 4. Influence of general variables on fertilization rates (FR) after intracytoplasmic sperm injection in oocyte donor patients.

	No. of oocytes	FR (%)	Odds ratio[a]	95% CI
Semen source				
Ejaculated	1466	67.4	1.00	
Testicular	156	60.3	0.70	0.47–1.05
Donor age	1622	–[b]	1.02	0.99–1.06
Number of ovarian stimulation days	1622	–[b]	1.00	0.93–1.09
Serum oestradiol concentration on HCG day (pg/ml)	1622	–[b]	0.97	0.90–1.05

All comparisons were not significant
HCG = human chorionic gonadotrophin.
[a]Odds ratio adjusted for all variables of the table.
[b]Continuous variable not categorized.

Table 5. Influence of dysmorphisms on embryo quality after intracytoplasmic sperm injection in donor oocyte patients.

	No. of embryos	Good (%) quality[a]	Odds ratio[b]	95% CI	P-value
Perivitelline space					
Normal	974	35.6	1.00		
Large	108	50.0	1.80	1.15–2.80	0.01
Perivitelline debris					
No	634	37.1	1.00		
Yes	448	36.8	1.02	0.74–1.41	NS
Oocyte shape					
Spherical	675	37.3	1.00		
Non-spherical	407	36.4	0.92	0.67–1.26	NS
Zona pellucida integrity					
Normal	786	36.1	1.00		
Abnormal	296	39.2	1.11	0.81–1.53	NS
First polar body					
Normal	192	32.8	1.00		
Fragmented/irregular	890	37.9	1.33	0.85–2.09	NS
Cytoplasmic granularity					
Normal	748	42.5	1.00		
Excessive	334	34.5	0.68	0.46–1.01	NS
Presence of vacuoles					
No	1040	37.6	1.00		
Yes	42	21.4	0.51	0.24–1.09	NS
Cytoplasmic colour					
Normal	1046	37.9	1.00		
Dark	36	8.3	0.17	0.04–0.74	0.02

[a]4-cell embryos, <25% of cytoplasmic fragmentation, no blastomere multinucleation, equal or similar blastomeres.
[b]Odds ratio adjusted for all variables of the table.

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Table 6. Influence of general variables on embryo quality after intracytoplasmic sperm injection in donor oocyte patients.

	No. of embryos	Good quality[a] (%)	Odds ratio[b]	95% CI
Semen source				
Ejaculated	988	37.9	1.00	
Testicular	94	26.6	0.68	0.28–1.66
Donor age	1082	–[c]	1.00	0.95–1.04
Number of ovarian stimulation days	1082	–[c]	0.99	0.89–1.11
Serum oestradiol concentration on HCG day (pg/ml)	1082	–[c]	0.98	0.88–1.09

All comparisons were not significant
HCG = human chorionic gonadotrophin.
[a]4 cell embryos, <25% of cytoplasmic fragmentation, no blastomere multinucleation, equal blastomeres.
[b]Odds ratio adjusted for all variables of the table.
[c]Continuous variable not categorized.

Discussion

In this work, the presence of extracytoplasmic and cytoplasmic OD, and their influence on FR and EQ in a total of 1622 human MII oocytes, was evaluated. The distinctive feature of this work lies in that the oocytes were from proven fertile donors, being, so far as is known, the first study carried out in this group of patients. Therefore, this was a homogeneous group with limited intervention of additional factors such as those related to female sterility/infertility, maternal age or ovarian stimulation and ovulation induction protocols (use of standard short protocol and constant dose of HCG). The possible effects of confounding factors (semen source, donor age, number of days of stimulation and plasma oestradiol concentration on the day of HCG administration) were considered in the logistic regression model.

OD are a common phenomenon in ovarian stimulation cycles. In this study, it was found that 94.5% of all oocytes (1533/1622) had at least one demonstrable anomaly. It is postulated that this high percentage may be due to three reasons: the stimulation protocol used, the number of evaluated dysmorphisms and morphological criteria, and the characteristics of the patients. These are each discussed separately.

The stimulation protocol was standard short protocol with leuprolide acetate agonist and recombinant human FSH. Ovulation was induced with 250 μg of recombinant HCG. This protocol was used because of its comfort for the patient, since the process of stimulation can be initiated without the need to administer medication in the previous cycle. The hormonal environment in ovarian stimulation regime may be important in oocyte quality (Thibault, 1977; Palermo *et al.*, 1996; Serhal *et al.*, 1997; for review, see Balaban and Urman, 2006). The use of a long protocol with agonists generates greater synchrony of the follicular cohort (Xia, 1997), and can increase the percentage of mature oocytes with respect to a shorter one (Greenblatt *et al.*, 1995). In addition, the use of a short protocol increases the number of oocytes with smooth endoplasmic reticulum

clusters. These clusters have been implicated in implantation failure (Otsuki *et al.*, 2004). In most studies analysing OD, long protocols with gonadotrophin-releasing hormone agonists were used (De Sutter *et al.*, 1996; Serhal *et al.*, 1997; Xia, 1997; Balaban *et al.*, 1998; Hassan-Ali *et al.*, 1998; Ebner *et al.*, 1999, 2000, 2002; Loutradis *et al.*, 1999; Kahraman *et al.*, 2000; Ng *et al.*, 2001; Ciotti *et al.*, 2004). Therefore, that might be one of the reasons why these authors found smaller percentages of oocyte abnormalities than in the study presented here. Moreover, the findings are in agreement with those of Suzuki *et al.* (2004), using oocytes from fertile chimpanzees. Also using a short protocol for ovarian stimulation, these authors found that 89.2% of oocytes showed at least one morphological alteration. Although the evaluation of oocyte morphology in natural cycles (without ovarian stimulation) would contribute excellent data to understanding the appearance of dysmorphisms and the effect of stimulation treatments on oocyte quality, it is rarely feasible in fertile patients.

A second consideration refers to the number of dysmorphisms evaluated and the morphological criteria used. In the present study, eight morphological alterations (five extracytoplasmic and three cytoplasmic) were analysed, and therefore there is a high probability of finding oocytes with at least one of these dysmorphisms. In fact, a fragmented or irregular first polar body appeared in 82.4% of oocytes. If this dysmorphism is eliminated from the study, the percentage of all oocytes having at least one anomaly falls from 94.5 to 71.7%, which is comparable with other studies (De Sutter *et al.*, 1996; Balaban *et al.*, 1998).

Although the classifications more frequently used are those of Veeck (1986, 1988, 1991, 1999) and Van Blerkom and Henry (1992), other authors have used variations of these (Serhal *et al.*, 1997), or their own classifications (De Sutter *et al.*, 1996; Xia, 1997; Ebner *et al.*, 1999, 2000; Loutradis *et al.*, 1999). In the present study, the authors' own criteria, including some major anomalies, were used.

Differences due to specific patient's characteristics should also be considered. So far as is known, this is the first work in which

OD from proven fertile women have been evaluated. This consideration is important. In fact, intrinsic factors such as age and genetic defects may result in morphological variations of the oocyte (Balaban and Urman, 2006). Sterility causes could be another factor influencing the appearance of morphological alterations in the oocyte. Xia (1997) observed that a female factor increased the number of oocytes with cytoplasmic inclusions, although these findings are not considered consistent by other authors (Mikkelsen and Lindenberg, 2001; Otsuki *et al*. 2004).

No association between OD and FR after ICSI was found, which is in agreement with most published studies. Maternal age, the number of days of ovarian stimulation and the concentration of oestradiol on the day of administration of HCG did not influence the FR. However, the use of spermatozoa coming from the testicle diminished the rate of fertilization from 67.4 to 60.3%, although statistical significance was not reached. This tendency would be in line with Balaban *et al*., (2001) and Goker *et al*., (2002), who observed a significant reduction of the FR when they used testicular spermatozoa of patients with non-obstructive azoospermia, suggesting a low fertilization potential. A larger study may be needed to detect significant changes.

None of the extracytoplasmic dysmorphisms evaluated in this work negatively affected the EQ. This finding is in agreement with the recent review from Balaban and Urman (2006), who concluded that embryo developmental rate is not affected by extracytoplasmic abnormalities of the oocyte. Interestingly, a beneficial effect of increased perivitelline space on EQ was found. It is important to note that oocytes with large perivitelline space in this study had normal sized first polar bodies. In fact, the formation of an atypically large first polar body has been related with poor day 2 embryo quality (Xia, 1997). Notwithstanding, the beneficial effect of an increased perivitelline space found in the present study could be a casual finding and would need confirmation in future works.

This study is also in agreement, although without statistical significance, with findings from other authors, suggesting that severe granularity and the presence of vacuoles increase the risk of having embryos of poor quality. Furthermore, the presence of a dark cytoplasm is another oocyte morphological deviation that may increase the risk of having embryos of poor quality (Loutradis *et al*. 1999; Suzuki *et al*. 2004). The review from Balaban and Urman (2006) confirms that only severe cytoplasmic dysmorphisms such as organelle clustering/centrally located granulation, appearance of smooth endoplasmic reticulum clusters and excessive vacuolization should be considered as oocyte abnormalities, impairing the developmental and implantation potential of the embryo.

The evaluation of oocyte morphology before ICSI is therefore strongly recommended in all scoring systems applied in IVF laboratories, and can be useful as a selection criterion in countries that cannot use other embryo selection tools after the fertilization event due to a restrictive legislation (Balaban and Urman, 2006; Ebner, 2006). Furthermore, one should take into account new studies that may be useful to assess oocyte quality and its developmental potential. In fact, a new non-invasive marker based on the quantitative evaluation of mean retardance of light by the oocyte spindle has recently been developed by Shen *et al*., (2006). Other novel factors that can influence oocyte

growth are found in the follicular fluid, and are largely related to apoptosis (Baka and Malamitsi-Puchner, 2006).

In conclusion, the appearance of OD seems to be a normal phenomenon in ovarian stimulation cycles, independent of the oocyte origin. Dysmorphisms found in oocytes from proven fertile patients did not affect FR after ICSI. However, OD may significantly decrease (or increase) the chance of having good quality embryos. Data on implantation and pregnancy rates of the embryos derived from the dysmorphic oocytes evaluated in this study are currently being collected, although more studies are needed in order to confirm the results presented here. An effort to unify morphological criteria of oocyte evaluation would be a valuable tool to draw further conclusions in the future.

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