Coarse Granulation in the Perivitelline Space and IVF-ICSI Outcome

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Purpose: To study the effect of the presence of coarse granules in the perivitelline space (PVS) of oocytes on embryonic development, and on implantation and pregnancy rates in IVF.

Methods: The study population included 24 patients treated during the period 1995–2000. The majority or all of their oocytes exhibited repeatedly coarse granulation in the PVS. Clinical and laboratory cycle characteristics of their 65 IVF-ICSI cycles and the resulting implantation and pregnancy rates were compared to a matched control group of 65 IVF-ICSI cycles without granulation in the PVS.

Results: A total of 623 oocytes were retrieved, 418 oocytes fertilized, and 246 embryos were transferred in the study group. No difference was detected between the study and control group with regard to patients' clinical data, IVF cycle characteristics, mean number of oocytes retrieved and fertilized, and mean number of embryos transferred. Only seven pregnancies were achieved in the study group, leading to pregnancy and implantation rates of 10.7 and 5.7%, respectively. Pregnancy and implantation rates were significantly higher in the control group of matched IVF-ICSI cycles without granulation in the PVS (32.5 and 11.5%, respectively).

Conclusions: The presence of coarse granules in the PVS correlates with low implantation and pregnancy rates in IVF-ICSI cycles and might be regarded as a distinct entity, part of the yet poorly defined condition of "egg factor infertility."

KEY WORDS: Egg factor; granulation; implantation and pregnancy rate; IVF; perivitelline space.

INTRODUCTION

Oocyte quality and organization is instrumental in the patterning of early mammalian embryos and, in part, account for variation in rates of fertilization, cleavage, implantation, and miscarriage (1,2). Since the introduction of intracytoplasmic sperm injection (ICSI) as a routine procedure in IVF, stripping of oocytes facilitates precise determination of the meiotic progression

stage, as well as assessment of morphological features of the cytoplasm, perivitelline space (PVS), and zona pellucida (ZP). It is generally accepted that good-quality, human-metaphase-II oocytes should contain the following morphological features: clear, moderately-granular cytoplasm, a small PVS, and a clear colorless ZP (3). However, many oocytes retrieved during IVF exhibit, among others, a number of variations from the normal aforementioned type with regard to colour, granularity and homogeneity of the cytoplasm, size and content of the PVS, cytoplasmic incorporations, and shape of oocyte or ZP (4–6).

Several abnormal morphological features of the oocyte have been previously studied. These include

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dark cytoplasmic incorporations, dark ZP, large perivitelline space, cytoplasmic vacuoles, refractile bodies, cytoplasmic granularity, areas of necrosis, and irregular shape (7–9). Low embryo quality and reduced fertilization rates were associated with each of these abnormalities.

In most cases, oocytes with normal morphology are harvested together with morphologically abnormal oocytes, and thus the resulting embryos arise from oocytes with heterogeneous morphological features. As the routine embryo transfer strategy is to transfer more than one embryo, particular association between a specific embryo and its implantation potential is not feasible. This, in turn, may account for the contradicting data in the literature on the effect of abnormal morphological features of oocytes on implantation (7–9).

Coarse granulation in the PVS (Fig. 1) is a morphological abnormality occasionally seen after stripping of the oocyte in preparation for ICSI. In some patients we observed this phenomenon repeatedly in the majority of retrieved oocytes (>80%). In this specific group of patients, all transferred embryos arise from oocytes with coarse granules in the PVS, and therefore the impact of coarse granules can be assessed not just in terms of their effect on early stages of embryonic development in the IVF laboratory, but also on implantation.

Fig. 1. Oocyte with coarse granulation in the perivitelline space (PVS).

MATERIAL AND METHODS

Patients

All records of IVF-ICSI cycles performed in our IVF unit during the years 1995–2000 were reviewed. In 24 patients who underwent a total of 65 IVF-ICSI cycles, coarse granulation in the PVS was recorded in at least 80% of oocytes retrieved in all their IVF cycles. There was no other sign of abnormal oocyte morphology, such as pigmentation of the zona pellucida, cytoplasmic vacuoles, cytoplasmic granulation, areas of necrosis, or irregular oocyte shape. These patients formed the study group and their background clinical and laboratory data, as well as their IVF cycle characteristics and results, were recorded.

A control group consisting of 65 IVF-ICSI cycles was formed by selecting a matching control cycle for each cycle in the study group. Selection of the control cycles was based on the principle of first follow-up technique, i.e., for each cycle in the study group, the first follow-up IVF-ICSI cycle performed in our IVF unit matched for age of the women and number of oocytes retrieved was selected as a control cycle.

Stimulation Protocol

Based on our patients' clinical profile and previous response to ovulation induction or controlled ovarian hyperstimulation (COH), either the long or short gonadotrophin-releasing hormone agonist (GnRH-a) protocols were used. The long protocol consisted of pituitary down-regulation with a depot preparation of the GnRH analogue triptorelin (Decapeptyl CR, 3.75 mg, Ferring, Malmo, Sweden), administered on the first day of the menstrual cycle. Once down-regulation was ascertained ($E_2 < 50$ pg/mL; $P < 0.2$ ng/mL; no follicular activity on ultrasound), ovarian stimulation was commenced using urinary FSH (Metrodin, Teva, Nethanya, Israel) followed by human menopausal gonadotrophin (hMG, Pergonal, Teva or Menogon, Ferring, Kiel, Germany). Three ampoules of FSH per day were given for 5 days, followed by three ampoules of hMG daily. The dose was individually adjusted according to ovarian response monitored by follicular development on ultrasound and serum E_2 levels. The short protocol consisted of daily s.c. injection of triptorelin (Decapeptyl 0.1 mg, Ferring), starting from the first day of the menstrual cycle, followed by ovarian stimulation using the same regimen as described above and starting on the third day of the cycle. Human chorionic gonadotrophin (Chorion, 10000 IU, Teva, Israel) was

administered when at least three follicles of 18 mm in diameter were seen, with serum E₂ levels within the acceptable range for the number of mature follicles present. Oocytes were aspirated 34–36 h after hCG administration using an ultrasound-guided transvaginal approach.

Gamete Preparation for ICSI and Assessment of Oocyte and Embryo Morphology

Immediately after retrieval, all oocytes were incubated in IVF medium (P1, Irvine, Santa Ana, CA). In all patients cumulus and corona cells were removed 1–3 h after oocyte retrieval, first by exposure to 60 IU hyaluronidase/mL (Sigma, St. Louis, MO), then by repeated pipetting for 15 s using fine pulled glass pipettes. Thereafter, oocytes were adequately rinsed several times and incubated in modified human tubal fluid (HTF) (Irvine, Santa Ana, CA). Assessment of oocyte morphology was performed immediately before sperm injection, using inverted light microscopy at a magnification of 200 $\times$, and recorded separately for each oocyte. Sperm preparation for ICSI was performed by two-layer Percoll (40%/80%) gradient centrifugation. The precipitate was washed twice with modified HTF. Injected oocytes were washed in fresh medium and incubated in a standard fashion. Embryos were culture in IVF medium (P1, Irvine, Santa Ana, CA).

Embryo quality was graded according to morphological criteria as suggested by Staessen *et al.*, (10). Grade 1—even and homogeneous blastomeres without fragmentation; Grade 2—even and homogeneous blastomeres with <20% fragmentation; Grade 3—uneven and nonhomogeneous blastomeres with 20–50% fragmentation; Grade 4—uneven and nonhomogeneous blastomeres with >50% fragmentation. The best morphologically assessed embryos were transferred using a Wallace catheter (H.G.Wallace Ltd., Colchester, Essex, United Kingdom). Supernumerary embryos of Grade 1 or 2 were cryopreserved. Our policy is to transfer 2–3 embryos when high quality embryos are available. The number of transferred embryos may be increased up to four in case where only low quality embryos are available for transfer and/or in case of repeated implantation failure (more than three failed IVF cycles).

Statistical Analysis

The statistical package “Sigmastat” (Jandel Corporation, San Rafael, CA) was used for data analysis.

Table I. Clinical Data, Hormonal Profile, and Previous Number of IVF Cycles of Patients in the Study and Control Groups

	Study group	Control group
No. of patients	24	65
No. of cycles	65	65
Age (years)	30.5 $\pm$ 5.0	31.4 $\pm$ 5.2
Primary/secondary infertility	14/10	27/38
Duration of infertility (years)	4.7 $\pm$ 2.5	4.6 $\pm$ 3.1
Coexisting etiology of female infertility		
Anovulation	2	2
Tubal	3	5
Basal LH level (IU/l)	4.6 $\pm$ 2.0	5.0 $\pm$ 3.0
Basal FSH level (IU/l)	6.2 $\pm$ 2.3	6.6 $\pm$ 2.1
Mean no. of previous IVF cycles	3.0 $\pm$ 3.2	3.0 $\pm$ 3.8

Note. No statistical difference was detected between study and control groups.

Clinical characteristics were analyzed using unpaired Student's 2-tailed *t*-test. All other analyses were performed using chi-square analysis. A *P* value of <0.05 was considered significant. Values are reported as mean $\pm$ SD.

RESULTS

Clinical data, hormonal profiles, and the number of previous IVF cycles in the study and control groups were comparable and are presented in Table I. The IVF cycle characteristics of all patients are depicted in Table II. No difference was observed between the study and control groups in terms of mean number of oocytes retrieved, E₂ level on hCG day, fertilization rate, mean number of embryos transferred, or percent of high quality embryos transferred. Semen quality on OPU day was similar in the study and control cycles (mean sperm concentration of $7 \times 10^6 \pm 12 \times 10^6$ (range 0.0001×10^6 – 43×10^6) and mean percent of motility of $16.5\% \pm 16.8\%$ (range 0–52%) in the study group vs. mean sperm concentration of $10 \times 10^6 \pm 12.8 \times 10^6$ (range 0.0001×10^6 – 38×10^6) and mean percent of motility of $18.6\% \pm 15.2\%$ (range 4–50%) in the control group. In both groups spermatozoa with normal morphology were available for injection for all oocytes.

Significantly lower implantation and clinical pregnancy rates of 5.7 and 10.7%, respectively, were achieved in the study group compared to 11.6 and 32.5%, respectively, in the control group (*P* < 0.01, 0.05, respectively, Table II).

Table II. IVF Cycle Characteristics of All Cycles of Patients in the Study Group and the Control Cycles

	Study group	Control group	P value
No. of cycles	65	65	
Ovulation induction protocol			
Long	42	49	
Short	23	16	
No. of hMG ampules per cycle	40.6 ± 18.4	41.8 ± 14.7	
E ₂ level on hCG day (pg/mL)	2020 ± 1014	1925 ± 776	
No. of oocytes retrieved	9.5 ± 4.9	9.4 ± 4.5	
No. of oocytes fertilized	6.4 ± 3.7	6.6 ± 3.7	
Fertilization rate (%)	69 ± 22	71 ± 20	
No. of embryos transferred	3.3 ± 0.9	3.3 ± 0.8	
Percent of Grades 1–2 embryos in each ET	94% ± 17	92% ± 17	
Pregnancy rate per cycle	10.7% (7/65)	32.3% (21/65)	0.01
Implantation rate per embryo	5.7% (14/246)	11.5% (29/253)	0.05

DISCUSSION

Previous studies have assessed the impact of different abnormalities in oocyte morphology on IVF outcome in terms of fertilization, cleavage rate, and embryo quality. Oocyte abnormalities, such as degenerative appearance, vacuolated ooplasm, cytoplasmic inclusion bodies, fractured first polar body, or possession of refractile bodies, were found to correlate with impaired fertilization and cleavage rates (11–15). Chromosomal disorders, defects in cytoplasmic factors, and abnormal mitochondria-cell respiratory function were also associated with failure of fertilization (16,17). These disorders and abnormalities are often referred to as “egg factor infertility.”

In our study, we focus on coarse granulation in the PVS as an isolated morphological abnormality of oocytes. In some patients, we repeatedly observed this phenomenon in all or the vast majority of oocytes retrieved. In this group of patients, all transferred embryos were derived from oocytes with granulation in the PVS. Therefore, we were able to study the cause/effect relationship between this specific morphological abnormality and implantation. We found that in the ICSI setup, fertilization rate and embryo quality were not affected by this extracytoplasmic picture. However, compared with a matched, control group of IVF-ICSI cycles in which all embryos were derived from morphologically normal oocytes, significantly reduced implantation and pregnancy rates were observed. Therefore, a relationship between coarse granulation in the PVS and reduced embryonic implantation potential is suggested.

Perivitelline space granularity has been also studied by Hassan-Ali *et al.* (18). In their study the presence of coarse granules in the PVS correlated with the dose of

hMG used for ovarian stimulation. The incidence of oocytes with PVS granulation ranged between 17% in the low dose hMG group and up to 45% in the group that required high doses of hMG for ovarian stimulation. They concluded that granulation in the PVS is probably a physiological phenomenon related to the maturational events in oocytes, which is enhanced by exposure to high dosage of hMG. Our study does not support this theory. There was no difference in the number of ampules used for stimulation, E₂ level on hCG day, number of oocytes retrieved, or number of embryos transferred between the study group and the control group. In agreement with our study results, Hassan Ali *et al.* found that fertilization rate, cleavage rate, and embryo quality were not affected by the presence of granulation in the PVS. The added value of our study is the assessment of the effect of this morphological phenomenon on implantation and pregnancy rates. Significantly lower implantation and pregnancy rates were achieved following transfer of embryos resulting from oocytes with coarse granulation in their PVS.

The difference between these two studies on coarse granulation in the PVS may lie in the study population. We chose to observe the IVF-ICSI results in patients where all or almost all oocytes were affected with granulation in the PVS and in whom this phenomenon repeatedly appeared in consecutive IVF-ICSI cycles. This population with a specific type of oocyte abnormality differs from the general IVF-ICSI population in whom coarse granulation in the PVS appears occasionally and sporadically. Sporadic appearance of granulation in the PVS may be related to different etiologies and the one suggested by Hassan-Ali *et al.* can be considered among them. Patients in whom granulation in the PVS repeatedly

appears in the majority or all oocytes in consecutive IVF cycles represent a distinct patient population who probably have an inherent oocyte defect.

Information gathered recently suggests that coarse granulation in the PVS may result from entrapment of cumulus cells in the PVS due to abnormal development of the Z.P. This theory is mainly supported by animal studies, where total lack of ZP protein 1 led to loosely organized ZP composed of only ZP proteins 2 and 3 (19). In the presence of this abnormal ZP, granulosa cells accumulate in the PVS as coarse granular material (19). Moreover, a reduced implantation rate was recorded in the presence of the abnormal ZP lacking ZP protein 1. The reduced implantation that we observed in our study could have resulted from the presence of coarse granulation in the PVS or from a possible ZP abnormality or from both. Another interesting option would be to relate granulation in the PVS to the embryo 2PN score as suggested by Scott (20), in order to differentiate between the contribution of oocyte and ZP to the etiology of increased granularity in the PVS. More studies are needed for better understanding the nature of coarse granulation in the PVS, its relation to ZP abnormalities, and its impact on fertilization, early embryonic development, and implantation.

REFERENCES

- Gardner RL: Can developmentally significant spatial patterning of the egg be discounted in mammals? *Hum Reprod Update* 1996;2:3–27
- Gabrielsen A, Petersen K, Mikkelsen AL, Lindenberg S: Intracytoplasmic sperm injection does not overcome an oocyte defect in previous fertilization failure with conventional in-vitro fertilization and normal spermatozoa. *Hum Reprod* 1996;11:1963–1965
- Veeck LL: Oocyte assessment and biological performance. *Ann NY Acad Sci* 1988;541:259–274
- Van Blerkom J: Occurrence and development consequences of aberrant cellular organization in meiotically mature human oocytes after exogenous ovarian hyperstimulation. *J Electron Microscop Tech* 1990;16:324–346
- Kahraman S, Yakin K, Donmez E, Samli H, Bahce M, Cengiz G, Sertyel S, Samli M, Imirzalioglu N: Relation between granular cytoplasm of oocyte and pregnancy outcome following intracytoplasmic sperm injection. *Hum Reprod* 2000;15:2390–2393
- Talevi R, Gualtieri R, Tartaglione G, Fortunato A: Heterogeneity of the zona pellucida carbohydrate distribution in the human oocyte failing to fertilize in vitro. *Hum Reprod* 1997;12:2773–2780
- Alikani M, Palermo G, Adler A, Bertoli M, Blake M, Cohen J: Intracytoplasmic sperm injection in dysmorphic oocyte. *Zygote* 1995;3:283–288
- De Sutter P, Dozortsev D, Qian C, Dhont M: Oocyte morphology does not correlate with fertilization rate and embryo quality after intracytoplasmic sperm injection. *Hum Reprod* 1996;11:595–597
- Serhal PF, Ranieri DM, Kinis A, Marchant S, Davies M, Khadum IM: Oocyte morphology predicts outcome of intracytoplasmic sperm injection. *Hum Reprod* 1997;12:1267–1270
- Staessen C, Camus M, Bollen N, Devroey P, Van-Steirteghem AC: The relationship between embryo quality and the occurrence of multiple pregnancies. *Fertil Steril* 1992;3:626–630
- Veeck LL: The morphological assessment of the human oocytes and early concept. In *Handbook of the Laboratory Diagnosis and Treatment of Infertility*, BA Keel, BW Webster (eds), Boca Raton, FL, CRC Press, 1990, pp 353–369
- Veeck LL: Preembryo grading. In *Atlas of the Human Oocyte and Early Conceptus*, Vol 2, Baltimore, Williams and Wilkins, 1991, pp 121–149
- Balaban B, Urman B, Sertac A, Alatas C, Senai A, Mercan R: Oocyte morphology does not affect fertilization rate, embryo quality and implantation rate after intracytoplasmic sperm injection. *Hum Reprod* 1998;12:3431–3433
- Oehninger S, Acosta A, Kruger T, Veeck L, Jones HW: Failure of fertilization in IVF: The “occult” male factor. *J In Vitro Fert Embryo Transf* 1988;5:181–187
- 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;12:1750–1755
- Suzuki S, Kitai H: Ovum quality: Role in human fertility. In *Gamete and Embryo Quality*, L Mastroianni, Jr, HJT Coelingh Bennink, S Suzuki, HM Verner (eds), London, Parthenon Publishing, 1993, pp 63–68
- Angell RR: Cytogenetic analysis of unfertilized human oocytes. In *Gamete and Embryo Quality*, L Mastroianni, Jr, HJT Coelingh Bennink, S Suzuki, HM Verner (eds), London, Parthenon Publishing, 1993, pp 47–62
- Hassan-Ali H, Hisham-Saleh A, El-Gezeiry D, Baghdady I, Ismaeil I, Mandelbaum J: Perivitelline space granularity: A sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. *Hum Reprod* 1998;12:3425–3430
- Rankin T, Talbot P, Lee E, Dean J: Abnormal zona pellucida in mice lacking ZP1 results in early embryonic loss. *Development* 1999;126:3847–3855
- 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