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ORIGINAL ARTICLE

Oocyte maturation-index as measure of oocyte cohort quality; a retrospective analysis of 3135 ICSI cycles

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KEYWORDS

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Abstract *Objective:* To investigate the use of an oocyte M-Index as a measure of the reproductive competence of oocyte cohorts collected following COS for ICSI.

Design: A retrospective analysis of 3135 autologous ICSI cycles.

Setting: A private IVF clinic.

Materials and methods: Oocytes were denuded immediately after oocyte collection and the in vivo oocyte M-Index was calculated for the oocyte cohort collected (number of normal metaphase II oocytes per total number of normal oocytes collected). The measured outcomes were analyzed according to the M-Index (0–20%, 21–40%, 41–60%, 61–80%, and 81–100%) and female age (20–30, 31–40 years).

Abbreviations: COS, controlled ovarian stimulation; OPU, oocyte pickup; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; M-Index, oocyte maturation index; MII, metaphase II; MI, metaphase I; GV, germinal vesicle; PB, polar body; GnRH, gonadotropin releasing hormone; rFSH, recombinant follicle stimulating hormone; hMG, human menopausal gonadotropin; hCG, human chorionic gonadotropin; E2, estrogen; P4, progesterone; 2PN, 2 pronuclei; SET, single embryo transfer; DET, double embryo transfer.

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Main outcomes: Clinical pregnancy.

Results: 60,955 oocytes were collected from the 3135 ICSI cycles, 57,214 (93.9%) were normal and 39,364 (68.8%) of these were metaphase II oocytes. 71.6% of metaphase I oocytes reached nuclear maturity by the time of the ICSI procedure. Trend analyses of fertilization and clinical pregnancy to M-Index showed that fertilization increased significantly ($p < 0.0001$) with an increasing M-Index, from 64.0% (M-Index 0–20%) to 78.1% (M-Index 81–100%) as well as clinical pregnancy ($p < 0.001$) from 23.3% to 48.1%. No predictive threshold value could be determined from the data using ROC analysis. Analyzing the data across a 40% M-Index cut-point, both embryology and clinical pregnancy outcomes were significantly higher for cycles with an M-Index of $>40\%$.

Conclusion: Our analysis shows that a simple maturation index calculated at the time of oocyte collection in a given ICSI cycle provides important prognostic information with regard to potential pregnancy outcomes and may reflect the importance of cytoplasmic maturation in oocyte competence.

1. Introduction

The inefficiencies inherent in human reproduction in the production of competent gametes have seen the continued use of technologies that produce and use multiple entities – follicles, oocytes, and embryos – to ensure the highest probability of a successful pregnancy. COS and induction of final oocyte maturation are the leading limiting steps in managing female fertility, in human IVF. Female etiology and the selected COS parameters determine not only the number oocytes collected but also the number of mature oocytes and the number of competent oocytes. Approximately 20% of collected oocytes obtained from COS maybe nuclear immature; either at the MI or GV (1). Routinely in the case of insemination by ICSI all MII ooctyes are injected with a sperm. The number inseminated from a cohort of oocytes collected from COS includes those oocytes that were mature at the time of oocyte collection (in vivo matured) and those immature oocytes (MIs and GVs) that progress to maturity (MII, in vitro matured) at the time of insemination, 3–4 h post-oocyte-collection. Up to 60% of immature MI oocytes will continue to progress in vitro and reach maturity within 6 h of oocyte collection (2). İnsemination therefore occurs regardless of the inherent competence of the oocytes, only on macroscopic morphological features including signs of nuclear maturity (3).

The morphological features for a healthy oocyte were short listed by Swain and Pool (4), as simply; a single PB, a 'normal-looking' cytoplasm, an appropriate zona thickness, and proper perivitelline space and by the Istanbul consensus workshop on embryo assessment as (5); a spherical structure, enclosed by a uniform zona pellucida with a uniform translucent cytoplasm free of inclusions and a size appropriate PB. However, according to these authors and others, all these features often fail to be predictive of fertilization and developmental competence and therefore have little or no bearing on the intrinsic quality of the oocyte (3). Studies, such as by Swain and Pool (4) and Rienzi et al. (3), have shown and discussed the fact that even 'healthy' looking oocytes have the potential to be incompetent. Ultimately oocyte competence is dependent on the completion of two major interdependent largely microscopic molecular processes, one nuclear and the other cytoplasmic. While the extrusion of the 1st PB indicates the completion of nuclear maturation it may bear no reflection of the quality of the genome of the oocyte. Although cytoplasmic maturation is just as critical to the competence of an oocyte there are no macroscopic markers indicating its completion (6,7).

With the existence of no single observable factor that truly reflects oocyte competence, routine IVF programs, must include all the macroscopic features that have been shown to be associated with oocyte quality in their oocyte assessment schemes. In our IVF program, because oocyte denudation was performed immediately after oocyte retrieval, the M-Index determined accurately represents the in vivo oocyte maturity rate from a particular COS cycle. In this study we investigated whether such an index could possibly be used to predict the reproductive potential of a collected cohort of oocytes.

2. Materials and methods

2.1. Study design and scope

In this retrospective study the results of 3135 autologous fresh only ICSI cycles performed at Antalya IVF during the period 2004–2010 were analyzed. The major indications for ICSI treatment were; diminished ovarian reserve (3.9%), endometriosis (1.1%), male (46.6%), other (5.2%), ovulation (8.9%), tubal (9.4%) and unexplained (24.9%). Cycles excluded from analysis were all cycles where less than 5 oocytes were collected, where the female age was greater than 40 years and where there were no embryos available for transfer. The removal of cumulus cells from all collected oocytes was performed immediately after the OPU procedure. The oocytes were assessed after denuding and grouped according to their nuclear maturity status (MII, MI, GV). A MI oocyte was defined as an oocyte with no GV and no PB, a MII oocyte was defined as having a spherical shape, enclosed with a uniform zona pellucida, a uniform translucent cytoplasm and an extruded size appropriate 1st PB. Abnormal or degenerate oocytes collected were not inseminated by ICSI and were not included in the analysis. These were defined as oocytes that were exceptionally large, or oocytes with exceptionally large PBs, multiple large vacuoles, clusterings of smooth endoplasmic reticulum in the cytoplasm, or a diffused, darkened or highly irregular cytoplasm. For each cycle an M-Index was calculated, which was the ratio of normal in vivo only matured MII oocytes (MII) to the total number of normal oocytes collected.

2.2. COS and oocyte collection

Oocytes for all the ICSI cycles were obtained after a standard COS using a GnRH antagonist (Cetrotide 0.25 mg; Merck Serono) protocol with a combination of rFSH (Gonal-F; Merck Serono) and hMG (Menopur; Ferring pharmaceuticals). The total dose of FSH was primarily determined from the day 2 antral follicle count (150–450 IU). Final oocyte maturation was induced with an hCG (Ovitrelle 250 μg/0.05 ml; Merck Serono) trigger, when at least three follicles reached 17 mm in diameter. Transvaginal ultrasound-guided oocyte (Ovum aspiration needle, Cook Medical) retrieval was performed 36 h after ovulation induction. All oocytes were retrieved from their follicular fluids and rinsed in preparation of denuding.

2.3. ICSI and embryo culture

The Sydney IVF media suite (Cook Medical) in conjunction with a 37 °C humidified atmosphere of 5% O_2, 5% CO_2 culture system was used for the culture of oocytes, zygotes and embryos. Oocytes and embryos were cultured in 80 μL media droplets under light mineral oil. Immediately following the oocyte collection all oocytes were denuded using a standard denuding procedure using hyaluronidase (80 IU/mL; Irvine Scientific) and denuding pipettes (Cook Flexipet Pipettes; Cook Medical). Following a preincubation period of 3–4 h a standard ICSI procedure was performed where all mature (metaphase II) oocytes with a normal morphological appearance and no significant cytoplasmic inclusions were fertilized by injection, including any in vitro matured oocytes. Fertilization checks were performed 16–18 h after the ICSI procedure and the zygotes with the same fertilization status were cultured in groups. Embryo developmental assessments were performed daily thereafter until the decision to transfer was made. The embryo assessments included blastomere number, blastomere size and regularity, and the percentage of fragmentation. Good quality embryos were those with equal sized, spheric blastomeres with $<10\%$ fragmentation and scored as 1 on a scale of 1–5.

2.4. Embryo transfer

Ultrasound guided embryo transfer was performed on day 2 or 3 by standard procedure, with a maximum of 2 embryos being transferred, using a Labotect embryo transfer catheter (Labotect GmbH).

2.5. Luteal phase support

Luteal phase support consisted of the daily self-administering of E2 (Estrofem 2 mg BD; Novo Nordisk) and P4 (Crinone 8% BD; Merck Serono). The support continued for at least 9 weeks of gestation if pregnant. A clinical pregnancy was defined as an ultrasound confirmed fetal sac 7 weeks after embryo transfer.

2.6. Outcomes and analysis

The M-Index, fertilization rate and clinical pregnancy rate, were calculated for each of the 2 female age groups. Category matrixes were drawn using M-Index groups; 0–20%, 21–40%, 41–60%, 61–80% and 81–100% and female age groups; 20–30 years and 31–40 years to examine the affect on oocyte maturity, fertilization rate and ongoing pregnancy rate. Spearman's correlation test was applied to evaluate the correlation between oocyte maturity and increasing age. For the categorical variables of fertilization, M-Index, and pregnancy, $\chi 2$ and $\chi 2$ for trend analyses were used to test statistical significance. Differences were considered significant if the p-value reached <0.05.

3. Results

In total, 60,955 oocytes were collected from the 3135 ICSI cycles performed during the study period. Fifty-seven thousand two hundred and fourteen oocytes were assessed as normal, non-degenerate oocytes (93.9%). The oocyte nuclear maturity distribution of the normal oocytes at the time of oocyte collection was; 39,364 (68.8%) MII oocytes, 11,102 (19.4%) MI oocytes and 6748 (11.8%) GV oocytes. The overall clinical pregnancy rate for the 3135 embryo transfers was 45.2% ($n = 1417$).

All MII oocytes ($n = 47{,}315$) with a normal morphological appearance, both in vivo and in vitro matured, were inseminated by ICSI 3–4 h after egg collections. The total number of oocytes injected represented a 20.2% increase in the number of mature oocytes. Seventy-one point six percent of the metaphase I oocytes collected completed their nuclear maturation in vitro, in the time from oocyte collection to the time of the ICSI procedure. A trend analysis of fertilization to M-Index showed that there was a significant ($p < 0.001$) increase in fertilization rate with an increasing M-Index, from 64.0% for the lowest M-Index (0–20%) to 78.0% for the highest M-Index (81–100%) group (Table 1).

The pregnancy rates from all the ICSI cycles grouped according to the different M-Index categories followed a normal growth curve trend (Fig. 1). The pregnancy rate was rapidly increasing from the 0–20% (23.3%) to the 41–60% (44.7%) M-Index point and then slowdown in growth to the 81–100% (48.1%) M-Index point. The same trend in pregnancy rates relative to M-Index was observed for the two female age groups. Although no predictive value for pregnancy outcome could be extracted from the data using ROC analysis for the M-Index, the graphical representation of the results (Fig. 1) shows that patients with a $\leqslant 40\%$ M-Index may have a suboptimal reproductive potential. Re-categorizing the data across a 40% M-Index cut-point (Table 2) showed that in both female age groups there was a significant reduction in reproductive potential from ICSI cycles with an M-Index of $\leqslant 40\%$. In the $\leqslant 40\%$ group 47.3% had male factor infertility and in the $>40\%$ group 46.5% (z-test, $p = 0.822$). The pregnancy rate in the $\leqslant 30$ year female age group was 36.3% if the M-Index was $\leqslant 40\%$ and 50.5% if $>40\%$, and in the >30 year age group, 27.7% and 43.7% respectively (Table 2). In the $\leqslant 30$ year female age group 12.3% of COS cycles had an M-Index of $\leqslant 40\%$, while in the >30 year age group 18.0% had an M-Index of $\leqslant 40\%$.

The embryology outcomes analyzed across the 40% M-Index cut-point also showed a significant improvement from ICSI cycles with an M-Index of $>40\%$ (Table 3). Significantly more ($p < 0.001$) oocytes were collected from COS

Table 1 Fertilization rates according to M-Index levels.

M-Index 0–20	M-Index 21–40	M-Index 41–60	M-Index 61–80	M-Index 81–100	*p*-Value
64% (1060/1662)	68% (2520/3720)	71% (6824/9658)	75% (12,595/16,762)	78% (12,135/15,513)	<0.001

Chi square test for trend analysis.

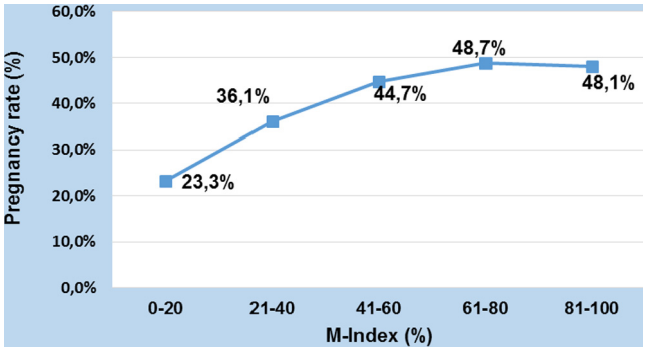

Figure 1 Trend analysis of pregnancy outcome according to M-Index.

cycles with an M-Index of >40%, and from these ICSI cycles significantly more ($p < 0.001$) grade 1 embryos and embryos suitable for freezing were obtained. Not only were the mean values significantly different but a greater proportion of the oocytes collected in cycles with a >40% M-Index were grade 1 embryos (good quality) and were frozen. In the ICSI cycles with an M-Index of >40% (1.73 ± 0.446) significantly more ($p < 0.001$) embryos were transferred compared to cycles with an M-Index of ⩽40% (1.45 ± 0.499). Approximately 55% of the cycles with an M-Index of ⩽40% had SET, as compared to only 27% with an M-Index of >40%. The SET pregnancy rate for all the ICSI cycles with an M-Index of >40% was 43.9% compared to 32.4% for the cycles with an M-Index of ⩽40% ($p = 0.186$).

4. Discussion

Denuding the oocytes immediately after oocyte retrieval provides an accurate maturational profile of the collected oocyte cohort. This profile converted to an index may provide valuable information on the reproductive competence of the oocyte cohort retrieved from a particular COS cycle. Although all oocytes are exposed to the LH surge from the trigger to initiate final oocyte maturation at the same point in time, oocytes retrieved are not only at different stages of nuclear maturity, but probably also cytoplasmic maturity. Oocyte maturation has been defined as the period taken by an oocyte to progress from the first to the second meiotic arrest, which involves coordinated, but asynchronous nuclear and cytoplasmic maturational modifications (7,8). The end point of both processes may not necessarily be reached by the time of oocyte collection, but may under certain conditions be completed in vitro. Oocytes that reach nuclear maturity in vitro at a known time point have been shown to only reach full competence 2–3 h after the extrusion of the 1st polar body (meiosis II arrest), presumably the time at which cytoplasmic maturity has also been reached (9). Not all metaphase II oocytes at the time of ICSI, 3–4 h after oocyte collection, may therefore have reached full developmental competence.

In two studies where oocytes were also denuded shortly after oocyte retrieval the percentage of oocytes that were mature at the time of denudation was 73% (2) and 68% (6), which was very similar to the 68.8% obtained in our study. These oocytes plus the group of metaphase I oocytes that reach nuclear maturity within the 1st hour after oocyte retrieval will presumably represent the fully competent oocyte cohort from a COS cycle at the time of ICSI, 3–4 h after oocyte collection. This proportion will invariably vary from one COS cycle to another, inter- and intra-patient. The M-index may be predictive of the proportion of developmentally competent oocytes from a COS cycle.

In routine IVF practice all mature oocytes, excluding only those nuclear mature oocytes with gross abnormal features, are inseminated by ICSI, which will include both oocytes that reached nuclear maturity in vivo and in vitro. It has been shown that the proportion of normally activated oocytes that

Table 2 Clinical pregnancy outcomes according to female age and M-Index.

Age-group	M-Index ⩽ 40% $N = 465$	M-Index > 40% $N = 2670$	*p*-Value
20–30 yrs	36.3% (77/212)	50.5% (766/1516)	0.0001
30–40 yrs	27.7% (70/253)	43.7% (504/1154)	<0.001

Chi square analysis.

Table 3 Embryology outcomes according to M-Index categories.

Parameters mean (std)	M-Index ⩽ 40% $N = 465$	M-Index > 40% $N = 2670$	*p*-Value
No of oocytes collected	14.5 (7.86)	17.2 (8.84)	<0.001
No of grade 1 embryos	2.0 (2.64)	4.7 (4.28)	<0.001
No of embryos frozen	1.2 (2.53)	3.9 (4.32)	<0.001
No of embryos transferred	1.45 (0.499)	1.73 (0.446)	<0.001

t-test analysis.

contained 2PN at fertilization check increases with increasing time post 1st polar body extrusion, from 43% at 2 h to 61% at 3–6 h (4) Only 25% of oocytes injected shortly after the extrusion of the 1st polar body showed normal fertilization (6). In a study by Yu et al. (9) they showed that allowing for maturation completion benefited the development of competence of in vitro matured oocytes, as it allowed normal spindle assembly. In our study fertilization increased significantly ($p < 0.001$) from 64% for the 0–20% M-index group to 78% for the 81–100% M-index group. A higher M-Index in our study translates into a greater number of in vivo matured metaphase II oocytes at the time of oocyte collection and therefore more oocytes would potentially have reached full competence by the time of ICSI insemination. The potentially greater number of fully competent oocytes at the time of insemination may therefore be the reason for the differences in fertilization rates seen.

The study of Shu et al. (2) showed that embryos that developed from in vivo matured oocytes had the highest mean number of blastomeres, the highest proportion of good quality embryos on day 3, the highest blastocyst formation rate and the lowest embryo arrest rate. The study of Balakier et al. (6) showed that the proportion of good, fair and poor embryos did not differ, although more embryos from in vitro matured oocytes had arrested development between day 2 and day 3. In our study, we also found that embryology outcomes improved as more oocytes were potentially fully competent at the time of insemination by ICSI. Cycles in which more than 40% of the oocytes collected had reached nuclear maturity in vivo, had significantly more ($p < 0.001$), grade 1 embryos (4.7 vs. 2.0), embryos frozen (3.9 vs. 1.2) and embryos available for transfer (1.73 vs. 1.45).

The improvement in reproductive outcomes according to oocyte maturation at the time of oocyte collection in our study was not only observed in preimplantation embryo parameters, but also in implantation outcomes. Overall, the pregnancy rates increased significantly ($p < 0.001$) with an increasing M-Index, from 23.3% for a 0–20% M-Index to 48.1% for an 81–100% M-Index. Categorizing pregnancy outcome across the 40% M-Index cut-point a significant increase in pregnancy rate was seen in both female age groups for cycles with an M-Index of > 40%. This increase in pregnancy with increasing M-Index could also be related to the level of oocyte developmental competence at the time of insemination by ICSI. It has been suggested that insufficient cytoplasmic maturation in oocytes may lead to anomalies such as multinucleation and aneuploidy both of which will significantly affect the pre- and post-implantation developmental competence of embryos (6). Granted, from the analysis of the data in our study it was shown that more embryos were replaced in the cycles with an M-Index of > 40% and the benefit of a greater number of embryos transferred could be seen as the reason for the observed increased pregnancy rate in this group. However, on analyzing the pregnancy outcomes for SETs across the 40% M-Index cut-point the same trend in pregnancy outcomes was seen (32.4% vs. 43.9%). This reduction in pregnancy outcome related to the affects cytoplasmic immaturity was also seen by the studies of Shu et al. (2) and Balakier et al. (6) where the transfer of embryos exclusively produced from in vitro matured oocytes showed a severely reduced pregnancy outcomes. From these cycles a <10% clinical pregnancy was obtained with none of the pregnancies developing to term. So too, did the study of Raziel et al. (10) showed the benefit of increased oocyte maturity, where a longer interval between hCG trigger and oocyte retrieval was seen to be associated with a significant increase in clinical implantation and ongoing pregnancy.

With the knowledge that the proportion of in vivo matured oocytes may have a significant influence on reproductive outcomes developing COS protocols that will maximize the chances of obtaining a high proportion of in vivo matured oocytes maybe an important strategy to improve the efficiency in assisted conception programs. The trigger type and timing used for final oocyte maturation play a pivotal role in oocyte maturation outcomes. There have been a number of studies that have investigated different aspects of this intervention in relation to oocyte maturity; the timing of trigger administration in relation to follicular development, the time interval from trigger to oocyte collection and the type of trigger. In a timing study, Kolibianakis et al. (11) found that timing the hCG trigger to when 3 follicles of 17 mm was first observed had important consequences on oocyte quality and consequently implantation and pregnancy outcomes. The study of Wang et al. (12) examining the time interval between hCG trigger and oocyte collection showed that oocyte maturation was significantly higher when oocyte collection occurred after 36 h rather than before 36 h from the time of hCG injection. Similarly, Bokal et al. (13), found that increasing the time interval by 4 h from 34 h to 38 h post-hCG improved oocyte quality and embryo developmental competence. In studies looking at the type of trigger, Lamb et al. (14) showed that an additional bolus of FSH administered at the time of hCG trigger improved developmental competence of oocytes and consequently pregnancy outcomes and Humaidan et al. (15) using GnRH agonist as a trigger in antagonist COS speculated that the additional FSH surged induced as a result of the use of an agonist as trigger increased oocyte maturity at oocyte collection. The evidence from these studies clearly illustrates that routine COS can be modified to produce more in vivo matured oocytes which has been shown by our study and other studies to be critical to the development of competent embryos with high implantation potential.

This study has shown that the assessment of oocyte maturation at oocyte collection provides important information on the reproductive potential of an assisted conception cycle, and should therefore be included in the schedule of quality control measures recorded. An M-Index of less 40% in more than 10% of assisted conception cycles could mean a significant reduction in the expected pregnancy rate. The selection of patient-specific COS strategies should take into consideration outcomes affecting oocyte maturation as well as oocyte number.

Conflict of interest and financial support

The authors declare no conflicts of interest and in receipt of no financial support.

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