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Research Article

Developmental Status of Human Oocytes that Fail to Cleave After Intracytoplasmic Sperm Injection

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Abstract

Background: Oocyte maturity is crucial for the success of intracytoplasmic sperm injection (ICSI). However, the oocyte appearance under a light microscope is the only indicator of maturity. Examining the chromatin status of the oocytes that fail to fertilize after ICSI may enrich the knowledge about maturity and possible causes of fertilization failure. ***Objectives***: To investigate the developmental progress of human oocytes that fail to cleave after ICSI, and the stages at which the progress has been arrested. ***Methods***: An observational study of human oocytes from women having ICSI cycles. Oocytes that failed to cleave after ICSI were collected and immunostained to visualize the meiotic spindles and chromatin using immunofluorescence microscopy. ***Results***: 100 failed oocytes were successfully immunostained. Oocytes showed the following developmental stages: anaphase-I (4%), telophase-I (9%), prometaphase-II (14%), metaphase-II (24%), anaphase-II (33%), one pronucleus (6%), two pronuclei (9%), and syngamy (1%). The meiotic spindle was seen in most of the prometaphase-II and metaphase-II oocytes. Sperm chromatin showed different levels of decondensation in the oocytes. Meiotic spindles were absent in MII oocytes that showed condensed sperm chromatin. At least one polar body was identifiable in all of the oocytes studied. Many apparently MII oocytes haven't reached the MII stage at ICSI time, despite having a visible first polar body. ***Conclusions***: The appearance of a polar body in the oocyte doesn't guarantee that the oocyte has reached the MII stage. Oocyte cleavage may be arrested at any developmental stage from sperm injection to syngamy.

Keywords: Chromatin, Cleavage failure, Fertilization failure, ICSI, Oocyte, Meiotic spindle.

حالة التطور للبويضات البشرية التي تفشل في الانقسام بعد حقن الحيامن داخل الهيولى

الخلاصة

الخلفية: نضج البويضات أمر بالغ الأهمية لنجاح حقن الحيامن داخل الهيولى (ICSI). ومع ذلك، فإن مظهر البويضة تحت المجهر الضوئي هو المؤشر الوحيد للنضج. قد يؤدي فحص حالة الكروماتين للبويضات التي تفشل في الإخصاب بعد الحقن المجهري إلى إثراء المعرفة حول النضج والأسباب المحتملة لفشل الإخصاب. **الأهداف**: التحقيق في تقدم تطور البويضات البشرية التي تفشل في الانقسام بعد الحقن المجهري، والمراحل التي توقف فيها التقدم. **الطرائق**: دراسة قائمة على الملاحظة للبويضات البشرية من النساء اللواتي لديهن دورات الحقن المجهري. تم جمع البويضات التي فشلت في الانقسام بعد الحقن المجهري وتلوينها مناعيا لتصور المغازل الانتصافية والكروماتين باستخدام الفحص المجهري المناعي. **النتائج**: تم صبغ 100 بويضة فاشلة بنجاح في المناعة. أظهرت البويضات مراحل النمو التالية: طور ألأنافيس (4٪)، تيلوفيس-1 (9٪)، بروميتافيس-II (14٪)، ميتافيس-II (24٪)، أنافيس-II (33٪)، نواة واحدة (6٪)، نواتين(9٪) ، والزواج (1٪). شوهد المغزل الانتصافي في معظم بويضات الطور الأول الثاني والطور الثاني. أظهر كروماتين الحيامن مستويات مختلفة من إزالة التكثيف في البويضات. كانت المغازل الانتصافية غائبة في بويضات MII التي أظهرت كروماتين الحيامن المكثفة. كان من الممكن التعرف على جسم قطبي واحد على الأقل في جميع البويضات التي تمت دراستها. العديد من بويضات MII على ما يبدو لم تصل إلى مرحلة MII في وقت الحقن المجهري، على الرغم من وجود جسم قطبي أول مرئي. **الاستنتاجات**: لا يضمن ظهور جسم قطبي في البويضة أن البويضة قد وصلت إلى مرحلة MII. يمكن إيقاف انقسام البويضات في أي مرحلة من مراحل النمو من حقن الحيامن إلى الزواج الزاهري.

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INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is accepted as a reliable and effective way to overcome human infertility. In the majority of cases, ICSI can lead to successful fertilization, pregnancy, and healthy offspring. Fertilization rate after ICSI is at about 70 to 80% [1]. However, this suggests that despite injecting sperm into the oocytes, fertilization failure still commonly occurs. On the other hand, some ICSI oocytes succeed in fertilization (i.e., form 2 pronuclei) but fail in proceeding to the cleavage stage (mitosis). Normally, sperm entry into the oocyte results in fertilization, which is defined by the formation of two "pronuclei" that contain the male and female chromosomes in the now-called "zygote." Fertilization

failure may occur even when a sufficient number of apparently normal sperm and oocytes are used. The causes of failure could be related to sperm (e.g., sperm head decondensation defects and sperm aster defects) [2,3], or the oocyte (e.g., spindle defects, nuclear and/or cytoplasmic immaturity) [4,5]. However, the most common cause of fertilization failure after ICSI is agreed to be oocyte activation failure, attributed either to sperm- or oocyte-related factors [6,7]. The developmental competence of the oocyte depends on many aspects; among them, nuclear and cytoplasmic maturities are crucial. Oocyte nuclear maturity is achieved when the oocyte reaches the metaphase-II stage, while cytoplasmic maturity is achieved when specific molecular mechanisms and events are established in the ooplasm to achieve the oocyte's developmental competence and support embryogenesis (e.g., the accumulation of mRNA, specific proteins, cellular substrates, and nutrients) [8]. Physiologically, nuclear and cytoplasmic maturations of the oocyte are synchronized; however, this is not necessarily the case in oocytes obtained after controlled ovarian hyperstimulation done prior to ICSI, where nonsynchronous maturation of the oocyte is often noticed [9]. Practically, direct examination of the denuded retrieved oocyte under light microscopy is the only tool to evaluate oocyte maturity in almost all IVF laboratories. Unfortunately, cytoplasmic maturation cannot be evaluated by this method, and nuclear maturation can only be assumed by 2 features: disappearance of the oocyte germinal vesicle (i.e., disintegration of the nuclear envelope) and the presence of the first polar body (PBI) in the perivitelline space. Many studies challenge the accuracy of these morphological criteria in deciding oocyte nuclear maturity. Over the last three decades since the first successful live birth following ICSI, a great number of studies have been performed to investigate fertilization failure and understand the mechanisms behind it. However, major challenges facing these studies were represented by the preciousness of the human oocytes, extreme sensitivity of human gametes, and the difficulty of studying the intracellular structure and function of the gametes without losing their viability. Studies on chromatin behavior in the oocyte after ICSI are relatively few, especially in the Middle East region. Based on the above, we aimed in this study to investigate the maturity state and developmental progress achieved by human oocytes that fail to cleave after intracytoplasmic sperm injection (ICSI) and the stages at which the progress has been arrested. This was done by visualizing the chromatin material and meiotic spindles (MS) in the studied oocytes by immunofluorescence technique.

METHODS

Patient selection

The study was approved by the ethical committee of Mustansiriyah University, College of Medicine. The study included IVF cases attended to Kamal Al-Sameraie Hospital for Infertility Management (Baghdad) from March to December 2022. On the ICSI day, consent was taken from the female patient, in addition to a short history to confirm trigger injection time. We collected apparently metaphase-II (MII) oocytes that failed to cleave after ICSI from cycles with no apparent reason for fertilization failure.

Inclusion criteria

Female less than 38 years, oocyte injected with motile sperm, sperm morphological abnormality ≤96% (WHO 2021 semen parameters), and freshly provided semen.

Exclusion criteria

Female age 38 years or more, oocyte injected with immotile sperm, sperm morphological abnormality >96% (WHO 2021 semen parameters), sperm taken from frozen/thawed semen, or fresh or frozen/thawed testicular aspiration or biopsy (Figure 1).

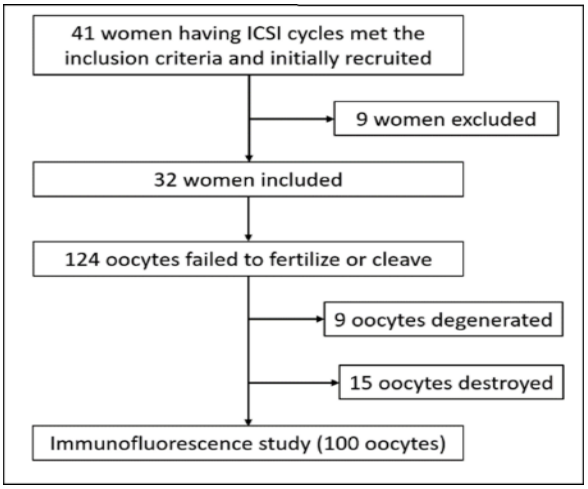

Figure 1: Flowchart of the study.

Sample selection

Cumulus-oocyte-complexes (COCs) were collected from 41 patients in the morning, kept in culture medium in the incubator at 37ºC and 6% CO_2 concentration for a while, then the oocytes were denuded by mechanical pipetting in hyaluronidase to remove cumulus cells, and then the oocytes were inseminated by intracytoplasmic sperm injection (ICSI). After ICSI, inseminated oocytes were put in culture medium covered with mineral oil and left in the incubator. All injected oocytes were checked daily to follow up their progress. Oocytes that failed (124) to cleave (i.e., no mitotic division by day-2 post injection) were collected to be included in the study.

Immunofluorescence assay

The oocytes were immunostained to visualize meiotic spindles and chromatin material. Meiotic spindles were

visualized by beta-tubulin monoclonal antibody that detects beta-tubulin protein subunits forming the microtubules of the meiotic spindles. Chromatin material was visualized by the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI) that stains nuclear DNA in the cells. At 37 ^{O}C, the oocytes were fixed in 4% paraformaldehyde in phosphate buffered solution (PBS) for 10 minutes. Then, oocytes were transferred into phosphate buffered solution with tween-20 (PBST) & stored in the refrigerator (at 4^{O}C) until the time of immunostaining [10]. For immunostaining, the oocytes were allowed to equilibrate with the room temperature, passed through 0.25% Triton X-100 in PBS for 15 minutes for permeabilization, transferred into a blocking solution containing Normal Goat Blocking Buffer, and incubated for 30 minutes. The oocytes were then incubated in anti-beta tubulin monoclonal antibody as the primary antibody (Elabscience®, 1:100 dilution) for 1 hour. After three washes in PBST (5 minutes each), the oocytes were incubated in the secondary antibody Goat Anti-Mouse IgG (FITC conjugated) (Elabscience®, 1:50 dilution) for 1 hour to label the sites where the primary antibody was attached. Washed in PBST three times, the oocytes were then counterstained for chromatin by DAPI (0.1 mg/mL) for 20 minutes and washed in PBST. Finally, each oocyte was mounted on positively charged glass slides containing anti-fade mounting medium (Anti-Fluorescence Quenching Agent, Elabscience®) and covered with a cover slip. The slides were specially prepared to preserve the oocytes' 3D structure.

Data analysis

The immunostained slides were examined and photographed using an immunofluorescent microscope (Zeiss Axio Imager 22) with a camera (Cool Cube 1) connected to a PC with immunofluorescence imaging software (MetaSystems Isis).

RESULTS

Figure 1 shows the number of patients included and oocytes that have met the inclusion criteria of this study. Ova pickup (OPU) took place in the morning, with a mean time interval between trigger injection and OPU of 35.68 hours. Denudation of oocytes was performed at a mean time interval of 1.08 hours after OPU. Intracytoplasmic sperm injection was performed after denudation of oocytes, this took place at a time interval ranging from 1.05 to 3.5 hours and a mean time of 2.14 hours after OPU. Beta-tubulin protein molecules were visualized as green-colored structures under the FITC filter of the immunofluorescence microscope. This has identified meiotic spindles (when present), in addition to the peripheral zones of the oocyte cytoplasm (and sometimes the polar body) and the extracellular fragments in the perivitelline space (PVS) of the oocyte. DAPI stain visualized the chromatin material as blue structures under the immunofluorescence microscope. This includes the chromatin of the oocyte, the injected sperm, the polar bodies, and—sometimes—the nuclei of occasional corona radiata cells still attached to the outer surface of the zona pellucida. According to the presence and the arrangement of the meiotic spindle, polar body(s), and male and female chromatin material, the oocytes that failed to cleave after ICSI showed 8 different developmental stages in which they were arrested: anaphase-I, telophase-I, prometaphase-II, metaphase-II, anaphase-II, one pronucleus (1PN), two pronuclei (2PN), and syngamy stage. Four oocytes (4%) were found to be at the anaphase stage of the first meiotic division and had not reached the metaphase-II stage at the time of ICSI. This was evident as the oocyte showed—near its plasma membrane—2 sets of chromosomes connected to each other by spindle microtubules, and the first polar body is starting to form with only a restriction separating it from the oocyte cytoplasm (Figure 2).

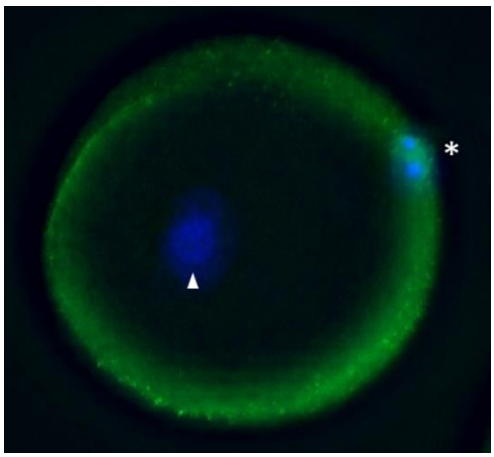

Figure 2: Anaphase-I oocyte, note the separating 2 sets of chromosomes (asterisk) and the sperm partially decondensed chromatin (arrowhead).

A second polar body was not seen. The male chromatin is seen with a moderate degree of decondensation. Nine oocytes (9%) were found to be at the telophase-I stage, where the first polar body boundaries are clearly demarcated, and the 2 sets of chromosomes (of the oocyte and PBI) are more separated but still connected to each other by the spindle microtubules. Male chromatin was seen either condensed or partially decondensed (Figure 3). A second polar body was not seen in those oocytes. Fourteen oocytes (14%) were at the prometaphase stage of the second meiotic division. In this stage, the first polar body is completely separated from the oocyte with no microtubules attached to its chromosomes. The oocyte chromosomes are condensed and aggregated but still not arranged at the metaphase equatorial plate of the meiotic spindle. The meiotic spindle was seen in most of these oocytes starting to form with its fibers intermingling with scattered oocyte chromosomes (Figure 4).

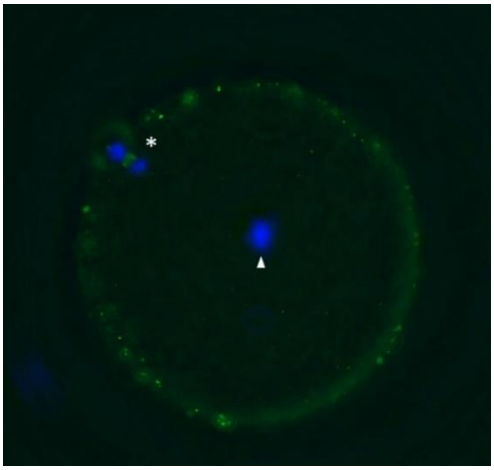

Figure 3: Telophase-I oocyte, note the separating 2 sets of chromosomes and the forming first polar body (asterisk), and the sperm chromatin (arrowhead).

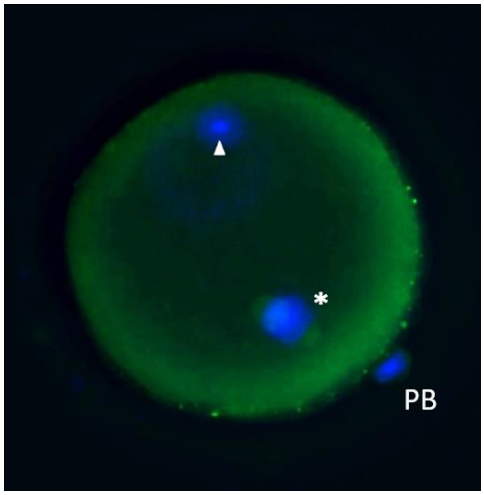

Figure 4: Prometaphase-II oocyte. The oocyte chromosomes are condensed and randomly organized, with meiotic spindles starting to form (asterisk). Sperm chromatin is condensed (arrowhead). The first polar body (PB) is seen here.

Male chromatin was seen condensed or partially decondensed in these oocytes. Twenty-four oocytes (24%) were found to be at the metaphase-II stage of meiosis, where the oocyte showed a single polar body (PBI), chromosomes arranged at a plane near the polar body, and sperm chromatin material. The meiotic spindle was seen in 16 out of the 24 oocytes in this stage, with the chromosomes arranged at its equatorial plate (Figure 5). The male chromatin material was seen condensed in 15 oocytes and partially decondensed in 9 oocytes. Thirty-three oocytes (33%) were found to be at the anaphase-II stage of development. Here, the first polar body (PBI) has already formed, and the oocyte chromosomes are separating into 2 groups connected to each other by the spindle microtubules, with the peripherally positioned group bulging on the oocyte cell membrane in preparation for the formation of the second polar body (PBII). The male chromatin material was seen condensed in 22 oocytes and partially decondensed in 11 oocytes (Figure 6).

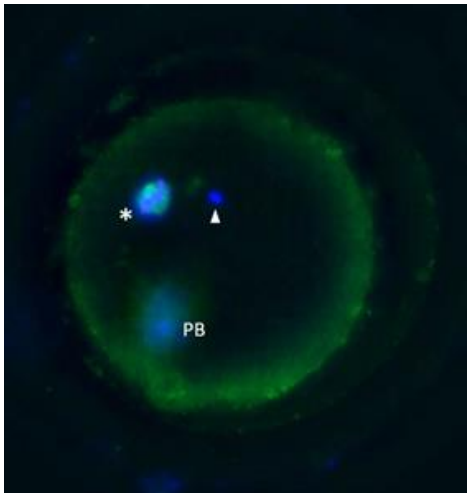

Figure 5: Metaphase-II oocyte. The oocyte chromosomes are condensed and arranged in a flat plain. The meiotic spindle is seen (asterisk). Sperm chromatin is condensed (arrowhead).

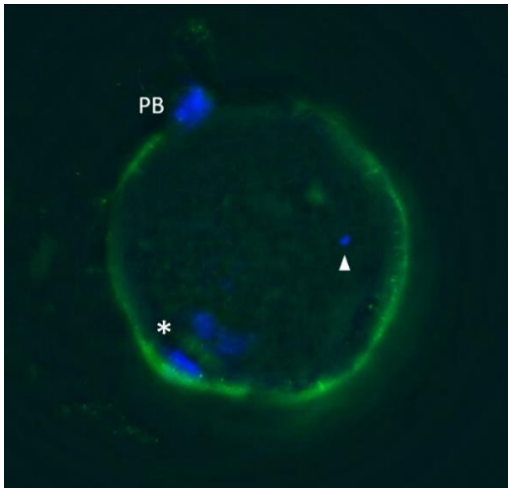

Figure 6: Anaphase-II oocyte. The oocyte 2 sets of chromosomes are separating and connected to each other by spindle microtubules (asterisk), with the first polar body already existing (PB). The sperm chromatin is condensed (arrowhead).

Six oocytes (6%) exhibited a single pronucleus in its cytoplasm. The pronucleus appeared as a rounded blue structure made of non-condensed peripherally distributed chromatin material. In 3 oocytes out of the 6, the pronucleus was relatively small and eccentric in position, while in the other 3, the pronucleus was large and central, and its outline was well demarcated. These oocytes clearly exhibited both PBI and PBII. No other chromatin material was found in the oocyte cytoplasm other than the pronucleus (Figure 7). Nine oocytes (9%) exhibited 2 pronuclei in their cytoplasm, with no other chromatin material (Figure 8). Among the oocytes, the two pronuclei showed variation in location (centrally *versus* peripherally located), size symmetry (symmetrical versus asymmetrical in size), and spatial orientation (close to each other or apart from each other). One oocyte was found to be at the "syngamy" stage, which is the final stage preceding the first mitotic division (cleavage) of the zygote. Here, the chromatin material was seen as a widely dispersed aggregate near

the central region of the cell. The male and female pronuclei cannot be distinguished, the chromosomes are irregularly scattered, and they show different degrees of coiling. However, mitotic spindles could not be identified in the cytoplasm. PBI and PBII are both present (Figure 9).

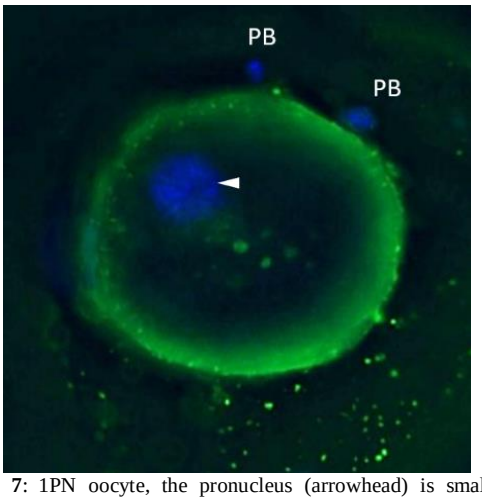

Figure 7: 1PN oocyte, the pronucleus (arrowhead) is small and peripheral in location. The first and second polar bodies (PB) are all visible.

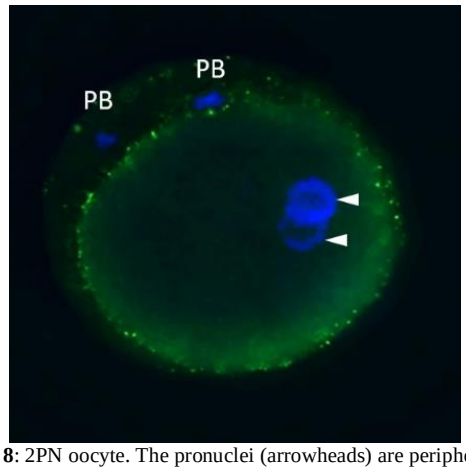

Figure 8: 2PN oocyte. The pronuclei (arrowheads) are peripherally located and contacting each other in abuttal position. The 2 polar bodies (PB) are seen.

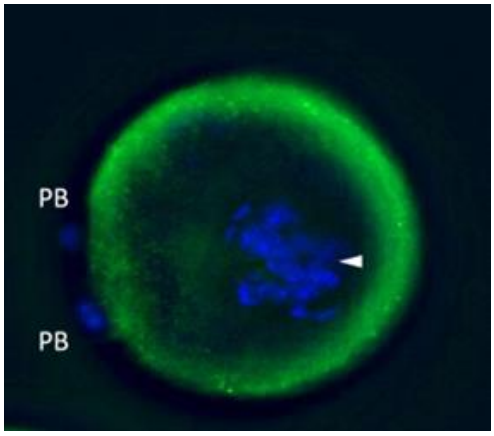

Figure 9: Syngamy stage. The chromatin material is seen as widely dispersed aggregate near the center of the cell. The 2 polar bodies (PB) are seen (arrowhead).

The male (sperm) chromatin was identified in all of the studied oocytes. In one oocyte, the sperm was seen in the perivitelline space (PVS) of the oocyte as a small oval blue structure, while in the remaining 99 oocytes, sperm chromatin was seen inside the ooplasm. Sperm chromatin in our study exhibited 3 levels of condensation. In 48% of the oocytes studied, highly condensed sperm chromatin was seen as small oval structures (resembling the shape of the sperm head) with a high-intensity blue signal due to high DAPI concentration. In 36% of the studied oocytes, partial sperm chromatin decondensation was seen as larger rounded or oval structures with a moderate intensity blue signal, representing "swollen" sperm heads. In the remaining 16% of the oocytes, the male chromatin was highly decondensed, forming rounded, usually homogenous "pronuclei" of moderate to low intensity blue signal. The presence of a single polar body (PBI) was significantly associated with the developmental stages of anaphase-I, telophase-I, prometaphase-II, metaphase-II, and anaphase-II. The presence of 2 polar bodies (PBI and PBII) was significantly associated with the developmental stages of one pronucleus (1PN), two pronuclei (2PN), and syngamy. The presence of a typical meiotic spindle was confined to the metaphase-II developmental stage. Meiotic spindles were absent in all MII oocytes that showed condensed sperm chromatin and in 56% of MII oocytes with partially decondensed sperm chromatin.

DISCUSSION

In the context of IVF, the timing of trigger injection, OPU, and ICSI can affect the features and maturity of the oocyte and hence, the IVF outcome. In our study, the trigger-OPU time interval was within the recommended time interval mentioned in the literature. Generally, authors recommend that ova pickup (OPU) should be performed 34-36 hours after trigger administration [9]. A large recent study has concluded that a 37-hour trigger-OPU time interval was associated with the highest clinical pregnancy rate among other time intervals [11]. In our study, the time interval between OPU and ICSI was also within the range described in the literature, so this factor is not expected to affect the oocyte parameters in this study. Some authors believe that the hCG trigger-OPU time interval rather than the OPU-ICSI time interval correlates with ICSI outcomes, including fertilization and pregnancy rates [12]. Our results demonstrated that 27% of the injected oocytes have not actually reached the MII stage, despite the apparent extrusion of the first polar body. Here, some oocytes were found to be in anaphase-I, telophase-I, and prometaphase-II. For all these stages, especially the last two, the polar body had started to form (and hence, the oocyte was considered as MII) but was still not totally separated from the oocyte cytoplasm. On the nuclear level, the 2 sets of separating chromosomes were still connected to each other with the spindle microtubules.

All these details are not visible under the inverted light microscope, and this is the reason that these oocytes were considered as MII stage, & their relative immaturity may be the reason for fertilization/cleavage failure. These findings strongly agree with a number of previous studies [13-16] that showed similar results in having a visible PBI in apparently mature oocytes that are actually still in anaphase-I, telophase-I, interkinesis, or prometaphase-II stages. Ideally, the oocytes collected for ICSI should have reached the MII stage before being injected by the sperm. The current standard evaluation of oocyte maturity in IVF labs depends on 2 features to confirm MII oocytes: the absence of a germinal vesicle and the extrusion (appearance) of the first polar body. However, the second feature may not be enough to indicate the MII stage of the oocyte, as shown by our study and the studies mentioned above. The time interval between OPU and ICSI in our study was about two hours. It is generally accepted that retrieved oocytes should be incubated for some time before being denuded and injected, to allow immature and apparently mature oocytes to proceed to the MII stage before being injected. However, the exact duration of incubation is controversial, with authors recommending a minimum of 1 hour [17], 3 hours [18], or even 6 hours [19]. So, since a high percentage of oocytes in our study did not actually reach the MII stage within 2 hours of incubation, it is possible that the OPU-ICSI incubation period is not sufficient for oocyte maturation. In our study, the meiotic spindle was visible in 66% of oocytes that had actually reached the MII stage. The published literature reported the percentage of MII oocytes with a detectable MS to range from 60% to 90% in oocytes collected for ICSI [20-23], while 80% of MII oocytes that failed to fertilize after ICSI had detectable MS [24]. According to these studies, fertilization rates and good-quality embryo rates were significantly lower in oocytes without a detectable MS. In our study, we have excluded cases of MS absence due to oocyte immaturity, as oocytes that have not reached the MII stage were identified by the immunofluorescence study. So, MS absence in MII oocytes may be attributed to inappropriate laboratory conditions where the oocytes were handled. The absence of MS in these MII oocytes may itself be a possible reason for fertilization arrest in the MII stage. Moreover, this study showed that 6% of the oocytes had one pronucleus (1PN). This is comparable to the frequency reported in the literature, which ranges between 1.6% and 7.7% among all injected oocytes [25], indicating that our study included only oocytes that failed to cleave. The large, centrally located single pronuclei seen in some of the 1PN oocytes in our study may be explained by the fusion of male and female pronuclei. The remaining 1PN oocytes with small, peripherally located pronuclei may be explained by the female spindle aspiration hypothesis, where the oocyte spindle with its attached chromosomes may be incidentally aspirated into the injecting pipette, leading to its destruction & the failure of the female pronucleus to form. This hypothesis is supported by the absence of any other chromatin material in the cytoplasm other than the single pronucleus, in addition to its smaller size and peripheral location. Two pronuclei (2PN) were seen in 9% of oocytes in our study. This percentage is larger than what was reported in a recent similar study. 24 Previous studies reported the occurrence of zygote arrest at the 2PN stage, describing a number of maternal gene defects as a cause of the arrest [26-28]. However, the wide versatility in pronuclear size, position, and spatial relation of the two pronuclei seen in our study makes it unlikely to attribute fertilization arrest to a single specific cause. One zygote was found to be in the syngamy stage in our study. Zygote arrest may occur in this developmental stage by the same mechanisms described for pronuclear arrest [26-28]. Regarding the sperm chromatin, almost half (48%) of our studied oocytes had sperm chromatin decondensation failure. Previous similar studies reported the same finding at lower percentages [24,29]. As we excluded cycles with abnormal sperm morphology, it is obvious that injecting the oocyte with apparently normal sperm cannot eliminate the possibility of chromosomal defects in the sperm and, hence, its failure to decondense inside the oocyte. The ability of the sperm to undergo chromatin decondensation can be a measure for the sperm fertilization competence [30]. Partial sperm chromatin decondensation was seen in over one third (36%) of our studied oocytes, a percentage lower than what was reported by previous similar studies (58.2% and 61.2%) [24,29]. Partial sperm chromatin decondensation can occur due to pathogenetic oocyte activation 24, or may be caused by severe sperm abnormalities [31,32]. High sperm chromatin decondensation was seen in oocytes exhibiting pronuclei (one or two). In cases of 1PN oocytes, actually, we couldn't know whether the single pronucleus belonged to the sperm, the oocyte, or both (fused male and female pronuclei). High sperm chromatin decondensation characterizes the formation of pronuclei and is a sign of successful oocyte activation (defined at least by the formation of the female pronucleus) [29]. Interestingly, we have noticed that all MII oocytes with condensed sperm chromatin didn't show a meiotic spindle, and only 44% of MII oocytes with partially decondensed sperm chromatin had a detectable MS. As the fertilizing sperm has no role in the formation of the oocyte meiotic spindle, this finding supports the hypothesis that developmentally competent oocytes are crucial for initiating sperm chromatin decondensation, as the majority of oocytes in our study showed either no or only partial sperm chromatin decondensation. The important finding in our study is that the vast majority (84%) of oocytes that failed to fertilize had oocyte activation failure, evident by condensed or partially decondensed sperm chromatin and failure to form pronuclei.

Study limitations

The relatively small number of oocytes studied, plus the lack of knowledge of the oocytes' chromosome/spindle status prior to insemination by the sperm, are the main limitations of this study.

Conclusions

In oocytes retrieved for ICSI following controlled ovarian hyperstimulation, the mere appearance of the first polar body does not necessarily indicate the maturity (i.e., reaching MII stage) of the oocyte. A visible first polar body may still be connected to the ooplasm, and thus the oocyte is still in the first meiotic division and cannot be considered a metaphase-II oocyte. The meiotic spindle may not be detectable in all retrieved oocytes, either due to oocyte immaturity or the disintegration of the existing spindle. Human oocytes that fail to cleave following ICSI may be arrested in any developmental stage of fertilization, from sperm injection to syngamy. The majority of oocytes that fail to fertilize exhibit sperm chromatin decondensation defects, in which the sperm chromatin either fails to decondense or partially decondenses.

Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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