Oocyte lysis following intracytoplasmic sperm injection: Association with measures of oocyte quality and technician performance

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Abstract
The purpose of this investigation was to assess the incidence of oocyte damage (oocyte lysis) following intracytoplasmic sperm injection (ICSI) and its association with oocyte quality and with the personnel performing the procedure. Data concerning damage were accumulated from a laboratory database that tabulated the results of each patient’s ICSI results. The incidence of lysis following ICSI was 4.8% of all injected oocytes. The number of oocytes injected and the incidence of intact (not lysed) oocytes that were fertilized by ICSI both were inversely correlated with the incidence of lysis. Individual technicians had distinctively different profiles of lysis. 1PN and 3PN embryos. Technicians in training had slightly elevated incidence of lysis. Whereas evidence is presented that both patient-specific oocyte quality and technician-specific performance are significantly related to the incidence of lysis, the small incremental incidence of lysis associated with each source leads us to believe that the incidence of lysis is largely unpredictable and unsystematic in nature.

Introduction
The development of intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992) revolutionized in vitro fertilization. It has become a universal tool to achieve fertilization of oocytes in assisted reproductive techniques. Since the inception of ICSI, there has been extensive investigation of the safety of the technique, with examination of the children born following fertilization using the technique (Palermo et al., 2000; Ponjaert-Kristofferson et al., 2004). Currently, it is generally accepted that some oocytes subjected to ICSI undergo damage. It is not clear what the typical incidence of damage is following use of ICSI or whether increased incidence of damage is attributable to unique, patient-specific properties of the gametes or to specific personnel performing the technique. In this paper, we attempted to assess the extent of oocyte damage (oocyte lysis), its variability and its association with measures of oocyte quality and with the personnel performing the procedure.

Materials and Methods
Patients
Patients between the ages of 20 and 46 years, presenting at our facility were treated with in vitro fertilization and embryo transfer. Following semen analysis, patients whose male partner had semen parameters with sperm count < 20 M/ml, motility < 50%, and/or normal morphology (strict criteria of Kruger) <12% were assigned treatment with ICSI. In addition, any patients with prior standard insemination resulting in poor fertilization were assigned treatment with ICSI. Patients diagnosed with unexplained infertility were assigned to treatment with ICSI for half of their oocytes (if 10 or more oocytes were retrieved) or to ICSI of all of
their oocytes (if fewer than 10 oocytes were retrieved).

Patient preparation, oocyte retrieval, embryo transfer and luteal support have been described previously by our group (Seungdamrong et al., 2008; Maseelall et al., 2009). Roughly 82.4% of the patient cycles were long cycles, downregulated with leuprolide acetate followed by administration of gonadotropins (either FSH alone or FSH plus hMG). Roughly 14% of the cycles were short cycles, utilizing the flare-up from administration of leuprolide acetate with administration of gonadotropins (either FSH alone or FSH plus hMG) beginning 1 or 2 days later. Roughly 2.4% of the cases were GnRH antagonist cycles with administration of gonadotropins (either FSH alone or FSH plus hMG). Gonadotropin administration was increased when patients were judged not to be responding adequately; and, gonadotropin administration was decreased when patients were judged to be responding too robustly. Roughly 1.0% of the cycles were natural (gentle stimulation with no gonadotropin administration) cycles.

**Preparation of Oocytes for ICSI.**

Oocytes were stripped of their cumulus oophorus and corona radiata cells by a combined treatment of exposure to hyaluronidase (30 – 80 IU/ml in HEPES-buffered medium) and subsequent mechanical removal of corona radiata cells. Oocytes were exposed to hyaluronidase while drawing the cumulus oocyte complexes into and out of a Pasteur pipette. When the majority of the cumulus oophorus was removed by this gentle trituration, individual oocytes were moved to HEPES buffered medium lacking hyaluronidase. Adherent corona radiata cells were removed by repeatedly drawing the oocyte into and expelling the oocyte out of a small diameter plastic pipette (Stripper tips, MidAtlantic Diagnostics). When the oocytes were cleaned they were returned to bicarbonate buffered culture medium inside a CO₂ incubator.

**Selection of oocytes for ICSI**

After removing cumulus oophorus and corona radiata cells, and prior to performing ICSI, oocytes were assessed. Oocytes that contained a germinal vesicle, empty zona pellucidae, and oocytes that were considered atretic (dark, shrunken) were not injected with sperm. All other oocytes were injected (all apparently “living” oocytes whether a polar body was present or not). We chose to inject oocytes without a polar body in the hopes that upon extrusion of the polar body, they would gain the ability to respond to the injected sperm. Our hope was to achieve zygotes, in close synchrony with the other more advanced oocytes that had already extruded their first polar body by the time of sperm injection. In our experience roughly one-quarter of the oocytes injected with a sperm prior to polar body extrusion have 2 pronuclei the following day.

**ICSI**

ICSI was performed using two micromanipulators mounted on an inverted compound microscope. Manipulators were mounted on the microscope frame so that the stage could be moved independent of the micromanipulators. The micromanipulators were mounted so that the joystick controlled movements in the horizontal plane, parallel with the microscope stage. Vertical movements, perpendicular to the microscope stage were controlled by hydraulics controlled by the rotation of the knob forming the end of the joystick.

Holding pipette and ICSI pipette (both fabricated straight, with no bend) were purchased commercially. They were mounted in stainless steel pipette holders that were controlled by syringes for which the plunger was advanced by a threaded, screw-driven mechanism. Both pipette holders were mounted (holding pipette on the left and ICSI pipette on the right) at an angle of roughly 25° above the horizontal stage of the microscope. The pressure within the holding pipette was controlled by a 3 ml syringe. The syringe, the pipette holder and the intervening plastic tubing were filled with air.

The pressure within the ICSI pipette was controlled by a 1 ml syringe. The syringe and the plastic tubing were filled with sterile water up to within 3 cm of the connection between the tubing and the pipette holder.
Preparation of pipettes prior to injection
Prior to performing injections, the pipettes were mounted in the holders and the tips of the pipettes were filled by dipping the tips in a drop of HEPEs buffered medium in which the oocytes are maintained for injection. The holding pipette was dipped in medium allowing the tip to fill by capillary action. The level of solution in the ICSI pipette was adjusted so that the meniscus was positioned at a point inside the pipette where the inside diameter of the pipette was between 3 and 5 times the diameter at the tip of the pipette. This positioning of the meniscus along with the water filling the syringe and tubing allowed critical control of the sperm during injection.

Sperm pick-up
Sperm were pipetted into a drop of medium containing polyvinylpyrolidone under oil in the ICSI dish. During the performance of ICSI, sperm were immobilized by dragging the tip of the ICSI pipette across the sperm’s tail near the midpiece. A single sperm was drawn into the ICSI pipette, tail-first and was positioned several sperm lengths from the tip of the pipette.

Placement of the holding pipette
The microscope stage was then positioned so that the oocyte to be injected was in the center of the visual field. The holding pipette was lowered near the oocyte and suction was applied until the oocyte was held by the suction at the tip of the holding pipette. If a polar body was present, it was positioned near 12:00 or 6:00. If no polar body was present, the orientation was unknown. More suction was applied to assure that the oocyte was securely held in place. The holding pipette was raised, lifting the oocyte off of the bottom of the dish. Then the pipette was lowered until the oocyte first touched the bottom of the dish and then subsequently was seen rotating as the pipette forced the left side of the oocyte down as the right side rotated up. This rolling of the oocyte effectively positioned the oocyte so that both the pipette and the bottom of the dish provided stabilization of the oocyte for insertion of the ICSI pipette.

Injection of the sperm
The microscope focus was adjusted so that the widest portion of the oocyte (the “equator”) was in clear focus. The ICSI pipette was then positioned near the right side of the oocyte and was raised or lowered so that the tip was in the same focal plane as the oocyte’s equator. The sperm was advanced nearer to the tip of the ICSI pipette (the head placed within 1 sperm length of the orifice). The ICSI pipette was advanced horizontally (using the joystick of the manipulator) pushing the pipette into the oocyte. The advancement was performed slowly, observing as the pipette pierced the oocyte’s zona pellucida. As soon as the zona was pierced (noted as a sudden pop-back of the zona pellucida) advancement of the pipette was halted. (The tip of the ICSI pipette was “optimally” near the center of the oocyte at this point.) With little delay, suction was applied to the ICSI pipette, aspirating the oolemma and some cytoplasm into the pipette. Aspiration was continued until a sudden rush of cytoplasm into the pipette signaled the breakage of the oolemma. Rapidly, the suction was released and, with control, the aspirated cytoplasm and the sperm were advanced out of the pipette and into the oocyte.

Confirmation of sperm injection
The entry of the sperm into the ooplasm was carefully examined. If the sperm was observed advancing well beyond the tip of the ICSI pipette into the ooplasm, then we concluded that the sperm was in the ooplasm.

If the sperm head abruptly halted as it exited the pipette (suggesting that it was obstructed at the end of the pipette), then more fluid from the pipette was injected.

If the fluid formed a delimited vacuole, then the sperm was confirmed not to be in the ooplasm. The sperm was reaspirated and the injection procedure was repeated. If the fluid formed a small bubble that rapidly dissipated (radially – not seeping up along the ICSI pipette) then the sperm was confirmed to be in the cytoplasm.

Once the sperm was confirmed to be in the ooplasm, the ICSI pipette was slowly removed from the oocyte, taking care to assure that the sperm was not pulled out during pipette withdrawal.

Oocyte lysis post-ICSI
Scoring the categories of oocytes following ICSI
The morning following ICSI (roughly 16 – 18 hours following ICSI), oocytes were examined and categorized as:

Oocytes with no polar body and no pronuclei (these were not analyzed in this study since they were extremely unusual and represent failure to mature),

1PB - Oocytes with one (or more) polar body and no pronuclei (considered undamaged but unfertilized),

Lysed - Oocytes that were lysed regardless of the number of polar bodies (considered damaged),

1PN - Oocytes with 1 pronucleus, regardless of number of polar bodies (considered activated but unfertilized, some may consider these to be damaged following ICSI),

2PN - Oocytes with 2 pronuclei, regardless of the number of polar bodies, (considered normally fertilized), and

3PN - Oocytes with 3 or more pronuclei regardless of the number of polar bodies (considered to be polyspermic or digynic, some may consider these to be damaged following ICSI).

Data Collection
Results of ICSI were recorded in a laboratory database (created using Microsoft Access) used to create laboratory reports. Data were extracted from the database to accumulate the numbers of oocytes for each patient in each category. Data were directly extracted into a spreadsheet (Microsoft Excel) for data ‘massage.’

Additional information compiled for patients in the database were: patient age at the time of oocyte retrieval, the numbers of follicles in three categories (<12mm, between 12 and 16 mm, and >16 mm) assessed in the morning prior to the evening injection of hCG, the total number of vials of gonadotropin (or vial equivalents if pens were used for injection), the identity of the technician(s) performing the sperm injection, and the live-birth outcome.

Statistics
Means, standard deviations, and standard errors were calculated in Excel. Comparisons of means were executed using Student’s t test within Excel. Linear Regressions and Correlations were performed using the Chart facility within Excel. Chi squared analyses were used as described in Tables 1, 2, and 3. Analysis of Variance (ANOVA) was performed for comparisons of the number of lysed oocytes per cycle and the percentage of all oocytes that were lysed per cycle when comparing more than 2 groups.

Results
Overall incidence of oocyte lysis
The incidence of lysis was 799/17475 (4.57% per oocyte). The mean incidence of lysis following ICSI for 1857 cases was 4.79% ± 0.22% per oocyte (mean ± SEM). Two patients had 100% of their oocytes undergo lysis following ICSI. One patient had 2 oocytes and the other had only 1 oocyte. Three additional patients had incidence of lysis greater than 50% (5/7 oocytes, 2/3 oocytes and 7/13 oocytes). These 5 patients amount to 5/1857 cases (0.27% of the cases). One thousand three hundred and three cases had no oocytes lysed following ICSI (1303/1857 = 70.17% of the cases).

The number of oocytes lysed per case is shown in Table 1 for a total of 1857 cases. This includes 810 cases for patients who had only one IVF case, 610 cases for 305 patients who had 2 cases, 276 cases for 92 patients who had 3 cases, 120 cases for 30 patients who had 4 cases, 35 cases for 7 patients who had 5 cases and 6 cases for 1 patient who had 6 IVF cases. The incidence of lysis was examined for 5 groups of patients (those with 1, 2, 3, 4, or >4 cases per patient) and the numbers of lysed oocytes per case were not significantly different between the 5 groups (Chi squared = 12.3 for 5 groups with 10 degrees of freedom, 0.1 < p < 0.5). In addition, the mean number of oocytes lysed per case (ANOVA, F = 1.39 for 4 / 1852 degrees of freedom, p > 0.05) and the mean percentage of oocytes lysed per case (ANOVA, F = 1.89 for 4 / 1849 degrees of freedom, p > 0.05) were not significantly different when comparing the five groups. Since there was no significant association between the number of IVF cycles per patient and the incidence of lysis, all of the
cycles were included in the remaining analysis.

The incidence of lysis was not distributed independently among all patients (Table 1, p << 0.005). Note that the number of patients with no lysis and the number of patients with \( \geq 4 \) oocytes lysed exceeded the numbers expected. The numbers of patients with 1, 2, and 3 oocytes lysed were less than the numbers expected. This occurs when a small number of patients have more oocytes lysed than would be anticipated by chance. Since the incidence of lysis per patient was not distributed independently, we surmise that some patients were more susceptible to lysis than others. The remainder of the commentary attempts to focus on what leads to a higher susceptibility to lysis.

Table 1: Incidence of oocyte lysis per patient following ICSI compared with binomial expectations

<table>
<thead>
<tr>
<th>No. oocytes lysed</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>( \geq 4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>1303</td>
<td>391</td>
<td>114</td>
<td>34</td>
<td>14</td>
</tr>
<tr>
<td>Expected</td>
<td>1079.3</td>
<td>562.8</td>
<td>167.4</td>
<td>37.8</td>
<td>8.7</td>
</tr>
</tbody>
</table>

1Numbers of oocytes lysed did not occur independently among all patients (test of independence: Chi-squared = 119.4 with 4 degrees of freedom, p << 0.005)
2Number of patients observed with the specified No. oocytes lysed.
3Number of patients expected to have the specified no. of oocytes lysed. For each patient, the expected number of oocytes lysed was determined using the mean incidence of lysis per oocyte (4.57%) and the number of oocytes injected. Expectations for each no. of oocytes lysed were summed across all patients and then divided by the total number of oocytes (17475) and multiplied by the total number of patients (1857).

We investigated the incidence of lysis among the several different stimulation protocols used. Stimulation protocol data were available for 1712 cycles: 1411 gonadotropin long cycles, 239 gonadotropin short cycles, 45 gonadotropin antagonist cycles and 18 gentle stimulation cycles. The distributions for numbers of lysed oocytes per cycle were not significantly different (Chi squared = 13.22 with 8 degrees of freedom, 0.1 < p < 0.5). In addition, neither the mean number of oocytes lysed per cycle (ANOVA, F = 2.86 with 3 / 1710 degrees of freedom, p > 0.05) nor the mean percentage of oocytes lysed per cycle (ANOVA, F = 2.58 with 3 / 1709 degrees of freedom, p > 0.05) was significantly different, comparing across the 4 groups. Therefore, we conclude that specific stimulation protocols were not related to the incidence of oocyte lysis.

Search for parameters related to high incidence of lysis.

In order to identify parameters that might be associated with lysis, the group of patients was operationally divided into 2 unequal groups: one in which the incidence of lysis was 30% or greater (n = 56 patients) and the other in which the incidence of lysis was less than 30% (n = 1801 patients). There was no significant difference in the average age of the patients (35.8 ± 3.18 years) (mean ± standard deviation), the number of vials of gonadotropin per day per patient (4.5 ± 3.19 vials/day), the number (3.7 ± 2.7 follicles) or fraction (0.29 ± 0.16) of follicles greater than 16 on the day of hCG, or the number (3.9 ± 3.8 follicles) or fraction (0.28 ± 0.21) of follicles less than 12 mm on the day of hCG. The average numbers of oocytes with 1PN (0.37 ± 0.68) or 3PN (0.45 ± 0.86) were not significantly different.

The following were significantly less for patients with 30% or more lysis. The number of follicles between 12 and 16 mm was significantly less in the group with 30% or more lysis (4.5 ± 4.2 versus 6.9 ± 5.1, p = 0.0011). The number of oocytes injected with sperm was significantly less in the group with 30% or more lysis (6.1 ± 5.2 oocytes versus 9.5 ± 5.2 oocytes, p = 2 X 10-6). The number fertilized with 2 PN (2.3 ± 2.6 oocytes versus 6.8 ± 4.3 oocytes, p = 2 X 10-14) and percent fertilized (35% ± 23% versus 70.6 ± 20.8%, p = 2 X 10-34) were smaller in the group with 30% or more lysis. However, the number of oocytes undergoing lysis may have influenced the fertilization outcome when the lysed oocytes were included in the denominator. Therefore, the incidence of fertilization was determined using only the oocytes that were intact (not lysed) within each group. The incidence of fertilization (with 2 PN) was...

Oocyte lysis post-ICSI
The incidence of lysis was inversely correlated with the incidence of livebirth (Livebirth = 0.3078 - 0.2951 X fraction of oocytes lysed; R = -0.064; R² = 0.0041; p < 0.05).

Consideration of individual technicians performing the sperm injection.

The incidence of damage to oocytes was compared for oocytes injected by different personnel (Table 2). The incidence of normal fertilization (with 2 PN) varied between personnel, ranging from 55.48% to 72.13%. The incidence of poor outcomes varied significantly between the different personnel performing the injections. Lysis varied from 3.01% to 6.68%. Other poor outcomes (occurrence of oocytes with only 1 pronucleus or with more than 2 pronuclei also varied between personnel. The incidence of 1 PN oocytes ranged from 3.58% to 4.52%. The incidence of 3 PN oocytes ranged from 4.19% to 6.70%. The distribution of outcomes varied significantly between personnel performing the ICSI (p << 0.001). However, despite the statistical significance, it remains unclear whether the variation of incidence of lysis between technicians (overt damage to the oocytes associated with ICSI) is sufficient to be of clinically significance.

Table 2: ICSI outcomes for four different technicians

<table>
<thead>
<tr>
<th>Tech ID</th>
<th>No. of oocytes</th>
<th>2 PN (%)</th>
<th>3 PN (%)</th>
<th>1 PN (%)</th>
<th>Lysed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8305</td>
<td>5971 (71.89%)</td>
<td>297 (3.58%)</td>
<td>348 (4.19%)</td>
<td>250 (3.01%)</td>
</tr>
<tr>
<td>B</td>
<td>5851</td>
<td>4143 (70.81%)</td>
<td>241 (4.12%)</td>
<td>282 (4.82%)</td>
<td>376 (6.43%)</td>
</tr>
<tr>
<td>C</td>
<td>1238</td>
<td>893 (72.13%)</td>
<td>56 (4.52%)</td>
<td>83 (6.70%)</td>
<td>54 (4.36%)</td>
</tr>
<tr>
<td>D</td>
<td>694</td>
<td>385 (55.48%)</td>
<td>30 (4.32%)</td>
<td>36 (5.19%)</td>
<td>43 (6.20%)</td>
</tr>
</tbody>
</table>

1 Contingency Chi Squared (comparison of all four technicians with the overall averages for 2PN, 1PN, 3PN, and Lysed) = 246.4. p << 0.001
2 Four different technicians identified as A, B, C, and D performed ICSI independently for cases. The values tabulated here are limited to those cases for which each technician completed the entire case.
Consideration of trainees performing the sperm injection
Comparison was made between experienced personnel and personnel who were in training. Personnel in training were permitted to inject no more than one-half of the oocytes for any particular patient. The remaining oocytes were injected by an experienced technician. Only following a minimum of 20 cases of shared injection with no significant difference in the incidence of normal fertilization, were personnel permitted to perform ICSI unassisted.

The incidence of damage was compared between personnel who were qualified to perform ICSI independently (single tech in Table 3) and cases that were performed shared by an experienced technician and a trainee (multiple techs in Table 3).

The incidences of outcomes were significantly different between Single and Multiple Techs (0.01 < p < 0.025). The higher incidence of 1 PN, 3 PN and Lysed and the lower incidence of normal fertilization (2 PN) for oocytes of patients injected by multiple technicians (one of whom was training) suggests that there is a component of experience associated with improved success and decreased damage (lysed, 1 PN and/or 3 PN) oocytes.

The row in Table 3 labeled Inexperienced Tech Deduced represents the contribution of the training technician to the incidence. Roughly one half of the oocytes were injected by the tech in training. The deduced incidences were deduced by assuming that half of the oocytes had the same incidence as the oocytes injected by experienced technicians. No statistical analysis was performed on the deduced values. Deduced values are provided to estimate the risk of damage for technicians in training.

Discussion
Lysis of oocytes following ICSI occurred in a manner that was not independent among all patients. This lack of independence suggests that some patients’ oocytes were more susceptible to lysis than other patients’ oocytes. Damage to oocytes can occur in association with the technician performing the sperm injection and/or in association with a specific cohort of oocytes that may be of better or poorer quality. Damage to oocytes was not related to the number of IVF cycles per patient or to the type of stimulation protocol used. Oocyte survival has been discussed in relation to oolemma characteristics and the difficulty of injection (Palermo et al., 1996).

The incidence of lysis considering all patients and oocytes was 4.79% per oocyte. Lysis of at least one oocyte occurred for fewer than 30% of the patients. Lysis of at least 30% of the oocytes occurred for only 3.02% of the patients. Therefore, lysis of less than 30% of the oocytes occurred for over 96.9% of the patients. While vanishingly few patients suffered from lysis of all the oocytes, this occurred in cases with very few oocytes, indeed. Unfortunately, for these few patients, the issue of lysis was a very major issue. This incidence of lysis reveals the incidence in our program, using

Table 3: ICSI outcomes compared for experienced (Single Tech) technicians and technicians in training (Multiple Technicians)

<table>
<thead>
<tr>
<th>ID 2</th>
<th>No. of oocytes</th>
<th>2 PN (%)</th>
<th>1 PN (%)</th>
<th>3 PN (%)</th>
<th>Lysed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Tech (Experienced)</td>
<td>16099</td>
<td>11398 (70.80%)</td>
<td>624 (3.87%)</td>
<td>750 (4.66%)</td>
<td>723 (4.49%)</td>
</tr>
<tr>
<td>Multiple Techs (Inexperienced plus Experienced)</td>
<td>1370</td>
<td>938 (68.46%)</td>
<td>67 (4.89%)</td>
<td>76 (5.55%)</td>
<td>77 (5.62%)</td>
</tr>
<tr>
<td>Inexperienced Tech Deduced</td>
<td>[685]</td>
<td>[66.22%]</td>
<td>[5.91%]</td>
<td>[6.44%]</td>
<td>[6.75%]</td>
</tr>
</tbody>
</table>

1 Contingency Chi Squared (Single Tech and Multiple Techs comparing numbers for 2PN, 1PN, 3PN and Lysed to the overall average) = 10.57 with 3 degrees of freedom, 0.01 < p < 0.025
2 ID indicates whether the case was performed exclusively by one single technician or was performed by multiple technicians sharing the case. Cases performed by multiple technicians were performed by an experienced technician and a technician in training. The total number of oocytes is smaller than 17475 because some cases lacked information on the technician(s).

Oocyte lysis post-ICSI
incidence of lysis is an appropriate benchmark for others remains to be seen.

Oocyte cohorts with smaller numbers of oocytes or with poorer incidence of fertilization are more prone to lysis. This suggests that oocyte cohorts from patients with poorer ovarian response (fewer oocytes retrieved or fewer oocytes fertilized are more likely to experience lysis, albeit at rather low incidence, generally less than 7% of the oocytes. While it is tempting to speculate that patients with fewer oocytes or fewer fertilized oocytes have poorer oocyte quality, we cannot directly test this notion. In addition, despite the significant association, the mean incidence of lysis at the maximum was less than 7.2% of the oocytes. In addition, the regression equation accounted for less than 1% of the variability in lysis (R² values for correlation were 0.0069 and 0.0065, respectively, for injected oocytes and fertilized oocytes). Whether controlled ovarian hyperstimulation parameters are associated with oocyte fragility or tendency to undergo lysis following ICSI is not clear. However, our observation that there was no significant association between follicle size distributions, the gonadotropin dose, the patient's age or the stimulation protocol utilized and incidence of lysis suggests that stimulation parameters are not systematically associated with damage. It is possible that our program’s strict adherence to empirically determined protocols and criteria for performing controlled ovarian hyperstimulation has led to oocytes of uniform resistance to lysis.

Different personnel performing ICSI have different characteristic patterns of damage, including lysis, 1 PN, 2PN and 3 PN embryos. Technicians in training have significantly higher incidence of oocyte lysis, 1 PN and 3 PN embryos. Whereas the incidence of these poor outcomes is significantly more prevalent with trainees, patients were protected from catastrophic loss by diversifying the trainees’ experience level with that of an experienced technician. Overall, the deduced risk of damage was roughly 1.5 times the risk of damage seen with experienced technicians. In no case was the risk of damage greater than 7% in any one category.

It is difficult to determine the root cause of lysis for an individual patient’s oocytes. The low incidence of lysis leads us to believe that the majority of oocyte lysis was inadvertent and that any systematic issue of patient or laboratory staff was not of major consequence. If oocyte quality affects the incidence of lysis, then oocyte quality within one patient’s cohort of oocytes must be quite diverse, such that one patient’s oocytes vary in quality and susceptibility to damage. Such diversity would also lead to differences in incidence of damage for different technicians.

Although the incidence of lysis was significantly inversely correlated with the incidence of livebirth (R = 0.064; p < 0.05), it is difficult to determine the root cause of this association between lysis and livebirth. Patients (1606) with < 30% lysis averaged 6.76 ± 4.3 fertilized oocytes available for embryo transfer. In contrast, patients (47) with > 30% lysis had 2.3 ± 2.7 fertilized oocytes available for embryo transfer. It is not clear whether oocyte cohort quality indicated by the incidence of lysis or the number of fertilized oocytes had a greater influence on the final live birth outcome. Livebirth is the ultimate demonstration of good oocyte quality. However, the contrary is not necessarily true. The failure to result in livebirth may be associated with multiple parameters unrelated to oocyte quality.

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