Relationship of reactive oxygen species levels in day 3 culture media to the outcome of in vitro fertilization/intracytoplasmic sperm injection cycles

Mohamed A. Bedaiwy, M.D., Ph.D., a Reda Z. Mahfouz, M.D., b Jeffrey M. Goldberg, M.D., b Rakesh Sharma, Ph.D., b Tommaso Falcone, M.D., b Mohamed F. Abdel Hafez, M.S., b and Ashok Agarwal, Ph.D. b

a Department of Obstetrics and Gynecology, University Hospitals Case Medical Center, Case Western Reserve University; and b Center for Reproductive Medicine, Glickman Urological and Kidney Institute, & Ob-Gyn and Women’s Health Institute, Cleveland Clinic, Cleveland, Ohio

Objective: To examine the relationship of early human embryonic development parameters to day 3 reactive oxygen species (D-3 ROS) levels in culture media.

Design: Prospective study.

Setting: Tertiary care hospital.

Patient(s): Patients were undergoing IVF (n = 92; 36 with intracytoplasmic sperm injection [ICSI]).

Intervention(s): The D-3 ROS levels in sample and control of each embryo culture dish were measured by the chemiluminescence method using a luminol probe.

Main Outcome Measure(s): Embryo quality (days 3 and 5) and pregnancy rates (PR).

Result(s): The D-3 ROS level was significantly lower in pregnant cycles 26.8 ± 13.9 × 10^6 cpm (counted photon per minute) versus nonpregnant cycles 66.4 ± 39.4 × 10^6 cpm. This relationship was maintained when the cycles were stratified to conventional IVF (27.1 ± 14.95 vs. 67.0 ± 39.9 × 10^6 cpm) or ICSI (25.6 ± 12.75 vs. 65.5 ± 39.7 × 10^6 cpm). After controlling for all variables, D-3 ROS levels were negatively correlated with blastocyst development rate as well as PR. Odds ratio (OR) (95% confidence interval [CI]) of clinical pregnancy corresponding to a 10 × 10^6 cpm increase in D-3 ROS was 0.47 (0.30–0.74) for ICSI and 0.56 (0.37–0.85) for IVF.

Conclusion(s): During extended in vitro culture, ROS generated in culture media by day 3 may be an important biochemical marker for blastulation. An increase of 10 units in D-3 ROS may decrease the clinical pregnancy by 41%. (Fertil Steril 2010;94:2037–42. ©2010 by American Society for Reproductive Medicine.)

Key Words: Reactive oxygen species, fertilization, blastulation, ICSI, IVF, extended embryo culture

The evaluation and selection of embryos for fresh uterine transfer or cryopreservation is largely based on their morphology. Ideally, the selection of normal viable embryos with high implantation potential would be based on chromosomal integrity with expression of the appropriate developmental genes and normal metabolic function. The metabolic alterations of developing preimplantation embryos remains poorly understood (1–4). At present, specific early embryonic molecular markers indicative of optimal development are yet to be identified (5, 6). We identified reactive oxygen species (ROS) and total antioxidant capacity in day 1 culture media as possible metabolic markers of in vitro embryonic development (4, 7). Both markers, at certain levels, were significantly correlated with in vitro embryonic development parameters, as well as clinical pregnancy.

Oxygen species has also been shown to be involved in the etiology of defective embryo development and embryonic fragmentation. The ROS generation may be from embryo metabolism or embryo surroundings (8, 9). Although the embryo has several mechanisms of defense against ROS (10, 11), rapid embryonic development continues to generate more ROS (4). The exact role of oxygen species in early embryonic development has yet to be fully identified.

Several media that improve embryo culture to the blastocyst stage are available. Day 5 embryo transfer at the blastocyst stage permits full expression of the embryonic genome, which occurs after the eight-cell stage. Earlier transfer at the cleavage stage may cause unexpressed genetic defects to go unnoticed (12, 13). High implantation rates have been reported for blastocyst transfer by several investigators (14–18).

This study was designed to quantify ROS in the embryo culture media 3 days after oocyte retrieval (day 3 media) in conventional IVF and intracytoplasmic sperm injection (ICSI) cycles and to assess the relationship of day 3 ROS (D-3 ROS) levels to embryo quality on day 3 and 5, and pregnancy rates (PR) for patients undergoing day 5 embryo transfer.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board (IRB) of the Cleveland Clinic. A total of 92 cycles (56 IVF cycles and 36 ICSI cycles) in 91 patients between 2000 and 2001 were included.

Controlled Ovarian Stimulation

All patients underwent pituitary down-regulation with the GnRH agonist leuprolide acetate (LA; Lupron; Tap Pharmaceutical,
Deerfield, IL) at a daily SC dose of 10 U (0.5 mg) initiated on cycle day 21. The dose was subsequently reduced to 5 U (0.25 mg) once serum E2 was suppressed to 50 pg/mL and continued until the day hCG was administered. Controlled ovarian stimulation with SC recombinant FSH (Gonal F; Serono, Randolph, MA) or Follistim (Organon, West Orange, NJ) was begun after pituitary down-regulation. The standard initial dose was 300 IU. Monitoring of the ovarian response was performed by serial serum E2 levels and transvaginal ultrasonograms beginning on the fifth day of stimulation. The subsequent FSH doses and monitoring frequency were individualized based on patients’ results. Stimulation was continued until at least two follicles reached a mean diameter of 18–20 mm, at which time hCG 10,000 IU SC was administered 36 hours before oocyte recovery.

Oocyte Retrieval
Oocytes were collected by transvaginal ultrasound (TVUS)-guided needle aspiration of the follicles. This procedure was performed under deep conscious sedation. The retrieved oocytes were rinsed, graded, and placed in N-2-hydroxyethylpiperazine- N’-2-ethanesulfonic acid (HEPES)-buffered human tubal fluid (HTF) (Irvine Scientific, Santa Ana, CA) at 37°C under 5% CO2, 5% O2, and 90% N2.

Sperm Preparation
After 2 days of abstinence, semen samples were collected from the male partner by masturbation in a sterile container. The sperm were prepared by double density gradient centrifugation technique. A single 2-mL layer of 90% Isolate (Irvine Scientific) was used for most specimens. After preparation, sperm were maintained in HTF medium with 5% synthetic serum substitute (SSS; Irvine Scientific) at room temperature until the time of IVF or ICSI. In conventional IVF, 150–200 × 103 sperm were added to each culture dish containing 4–5 oocytes. For ICSI, the single best looking spermatozoon was selected for microinjection of the oocytes.

Embryo Culture
After cumulus dissection and washing, the oocytes were placed in 1 mL of HTF supplemented with 6% SSS. Fertilization was confirmed 14–16 hours after insemination or ICSI by the presence of two pronuclei and the extrusion of the second polar body. The fertilized oocytes were cultured in groups of 4–5 oocytes in 1 mL of HTF with SSS until the early afternoon of day 3. They were then placed in 1 mL of blastocyst media (Irvine Scientific) after a five-drop rinse in the same medium. A second change to fresh blastocyst medium was done on the morning of day 5 after embryo evaluation and before embryo transfers. Embryo transfers were scheduled between noon and 2:00 PM on day 5.

Embryo Evaluation
On days 3 and 5 of development, the embryos were evaluated with an Olympus X 70 inverted microscope (Olympus America, Melville, NY) equipped with Hoffman Modulation Optics (Narisihge, Tokyo) at ×600 magnification. Cell number and degree and pattern of fragmentation were recorded on day 3 of development. The degree of fragmentation was expressed as a percentage and defined as the embryonic volume occupied by denucleated cytoplasmic fragments. Development on day 5 was recorded as well. An organized and distinct inner cell mass (ICM) and a cohesive layer of numerous tightly packed cells in the trophectoderm cells were considered normal. Highly irregularly arranged ICM or trophectoderm cells were considered abnormal. All abnormal embryos on days 5 and 6 were considered arrested.

ROS Measurement
After evaluating the embryos the morning of day 3, the culture media in the central well of the culture dish was collected as the test sample. The media in the outer well of the culture dish served as the control. Both were immediately centrifuged for 7 minutes at 600 × g, and the supernatant was removed. Aliquots of the supernatant were prepared for ROS measurement.

The ROS production was measured by the chemiluminescence assay using luminol (5-amino-2, 3-dihydro-1, 4-philazinedione; Sigma, St. Louis, MO) as the probe. Ten microliters of luminol (5 mM) prepared in dimethyl sulfoxide (Sigma) was added to 400 μL of the day 1 media. The ROS levels were determined by measuring chemiluminescence with a luminometer (LKB 953, Wallac, Gaithersburg, MD) in the integrated mode for 15 minutes, and results were expressed as 10⁶ cpm (counted photon per minute).

Implantation rate The implantation rate was calculated by dividing the number of gestational sacs on TVUS by the number of embryos transferred.

Clinical pregnancy rate The clinical pregnancy rate represents the number of cycles with fetal cardiac activity on ultrasound divided by the number of cycles initiated.

Statistical Methods
The D-3 ROS levels, PRs, and other studied parameters were analyzed separately and collectively for the IVF and ICSI groups. Descriptive statistics are presented as frequency (percentage) or mean ± SD. Associations with categorical variables were assessed using Fisher’s exact test or χ² test. Associations with quantitative and ordinal variables were assessed using logistic regression. Odds ratios (OR) of pregnancy were reported for quantitative variables. The association between D-3 ROS levels and PRs was assessed separately for the IVF and ICSI groups. Logistic regression was used to estimate an OR for clinical pregnancy with respect to a 10-fold increase in D-3 ROS. Multivariate modeling analysis was done to examine the effect of other predictive factors on D-3 ROS levels. Differences were considered significant if P ≤ 0.05. Analyses were performed using R software version 2.3.1 (19).

RESULTS
Ninety-two patients underwent 93 cycles of assisted reproduction technology (ART) during the study duration. Of those, 56 were conventional IVF cycles and 36 were ICSI cycles. Demographic features of the study population and the indications for ART are presented in Table 1. Cycle outcome parameters are presented in Table 2. Correlation between D-3 ROS levels and pregnancy are presented in Tables 3 and 4.

Comparison of Demographic Variables
Patients’ ages, height, weight, body mass index (BMI), parity, primary or secondary infertility, and infertility factors were not significantly different in pregnant versus nonpregnant overall (Table 1). There were no statistically significant differences in any of those parameters between the pregnant and nonpregnant patients within the IVF or ICSI groups (data not shown).
Comparison of Cycle Outcome
The number of days of FSH administration, total FSH units used, cycle day 3 E2 levels, peak E2 levels, number of mature follicles, number of retrieved oocytes, fertilization rate, blastocyst rate, and number of embryos transferred were similar between the pregnant and nonpregnant groups (Table 2). These parameters were also not significantly different within the IVF or ICSI groups (data not shown). The overall clinical PR was 51%, with a multiple PR of 45.1%.

Correlation Between D-3 ROS Levels and Cycle Outcome
The pregnant cycle group showed significantly lower D-3 ROS levels (log10 cpm) and log10 D-3 ROS overall as well as in the IVF and ICSI groups (Table 3). The D-3 ROS level was negatively correlated with high (>7) day 3 cell number in IVF and ICSI cycles. A significant negative correlation was also observed between D-3 ROS levels and worst fragmentation rate in both ICSI and IVF cycles. A significant correlation was seen between high D-3 ROS levels and low blastocyst development rates in conventional IVF and ICSI cycles (Table 4).

Multivariate Modeling Analysis for Predictive Factors of Clinical Pregnancy
The covariate-adjusted odds ratio (95% confidence interval [CI]) for a 0.1-U change in Log10 (ROS) was 0.54 (0.39–0.74) (P<.001). Therefore, the univariable result (OR = 0.52; 95% CI 0.38–0.70; P<.001) was essentially unchanged, even after adjusting for age, BMI, diagnosis, total units, oocytes recovered, blastocysts at day 5, and worst observed fragmentation.

DISCUSSION
The ROS may block or retard early embryonic development by affecting key cellular organelles required for rapid cell division. The ROS may cause aggregation of cytoskeleton components, condensation of the endoplasmic reticulum, loss of membrane fluidity, and embryo fragmentation (20, 21). Free radicals have many pathological effects including DNA damage. The balance between ROS in the culture media and the ability of embryo to neutralize them may affect in vitro embryo development.

This study showed that slow development (<7 cells embryo on day 3), worst fragmentation, and reduced formation of morphologically normal blastocysts are associated with increased levels of D-3 ROS. Lower blastocyst development rates have been observed in ICSI cycles (22). The concern about sperm DNA damage by ROS is especially relevant during the ICSI procedure (23, 24). Lower clinical PRs were also associated with higher D-3 ROS levels in both conventional IVF and ICSI cycles. Day 3 embryos with reduced blastomere numbers and higher fragmentation due to the negative association with D-3 ROS would be predicted to develop into blastocysts at a lower rate. Such embryos have been shown to yield higher PRs after assisted hatching, fragment removal, and embryo transfer on day 3 rather than by extending culture to day 5 (25, 26).

### TABLE 1
Demographic features and indications for ART in both IVF/ICSI groups.

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant cycles</th>
<th>Pregnant cycles</th>
<th>P value</th>
<th>OR (95% CI) (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>42</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cycles</td>
<td>42</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>31.7 ± 3.6</td>
<td>32.5 ± 3.5</td>
<td>.2c</td>
<td>1.07 (0.95–1.21) (1)</td>
</tr>
<tr>
<td>Weight (kg), mean ± SD</td>
<td>70.0 ± 18.5</td>
<td>69.8 ± 13.3</td>
<td>.9c</td>
<td>0.99 (0.87–1.13) (5)</td>
</tr>
<tr>
<td>Height (m), mean ± SD</td>
<td>1.64 ± 0.07</td>
<td>1.7 ± 0.07</td>
<td>.1c</td>
<td>1.04 (0.98–1.10) (0.01)</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>25.85 ± 5.8</td>
<td>25.3 ± 4.9</td>
<td>.6c</td>
<td>0.91 (0.62–1.34) (5)</td>
</tr>
</tbody>
</table>

**Note:** Associations with categorical variables assessed using: OR = odds ratios of pregnancy reported for quantitative variables and pertains to a change of x units, where x is reported in parentheses; 95% CI = 95% confidence interval; No. of cycles = number of cycles for infertility and infertility factors; ART = assisted reproductive technology; ICSI = intracytoplasmic sperm injection; BMI = body mass index.

* Fisher’s exact test.
* χ² test.
* Associations with quantitative and ordinal variables assessed using logistic regression.

Actually we have done the multivariate modeling for other predictive factors including the embryo morphology/fragmentation and we got confirmation of our findings. The univariable result was essentially unchanged, even after adjusting for age, BMI, diagnosis, total units, oocytes recovered, blastocysts at day 5, and worst observed fragmentation. Worst fragmentation is defined as the greatest degree of fragmentation observed among the total embryos.

Reduction of the gametes exposure is the most proper way to reduce their oxygen species-induced damage. The IVF clinics are trying to minimize the gametes and embryos exposure time to conditions that allow high free radical generation. Eliminating exposure of gametes and embryos to air during handling can partially help. Exogenous ROS is mostly produced by spermatozoa that are used to inseminate oocytes in IVF.

### Table 2

Comparison of the overall outcome of pregnant versus nonpregnant cycle parameters in the study population.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Nonpregnant cycles (n = 42)</th>
<th>Pregnant cycles (n = 51)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR (95% CI) (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of stimulation</td>
<td>9.3 ± 1.5</td>
<td>9.1 ± 1.5</td>
<td>.5</td>
<td>0.92 (0.69–1.22) (1)</td>
</tr>
<tr>
<td>FSH used (IU)</td>
<td>2,383.8 ± 922.2</td>
<td>2,311.1 ± 885.3</td>
<td>.7</td>
<td>0.99 (0.95–1.04) (100)</td>
</tr>
<tr>
<td>Day 3 E&lt;sub&gt;2&lt;/sub&gt; (pg/mL)</td>
<td>41.1 ± 18.2</td>
<td>36.4 ± 15.9</td>
<td>.2</td>
<td>0.85 (0.65–1.11) (10)</td>
</tr>
<tr>
<td>+ on day of hCG administration (pg/mL)</td>
<td>2,401.3 ± 932.2</td>
<td>2,350.15 ± 960.3</td>
<td>.8</td>
<td>0.99 (0.95–1.04) (100)</td>
</tr>
<tr>
<td>Number of mature follicles (&gt;15 mm) on day of hCG administration</td>
<td>15.2 ± 6.4</td>
<td>16.7 ± 6.1</td>
<td>.3</td>
<td>1.04 (0.97–1.12) (1)</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>17.1 ± 6.0</td>
<td>17.8 ± 6.1</td>
<td>.6</td>
<td>1.02 (0.95–1.09) (1)</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>67.3 ± 17.3</td>
<td>70.3 ± 15.4</td>
<td>.3</td>
<td></td>
</tr>
<tr>
<td>No. of blastocyst</td>
<td>4.5 ± 2.1</td>
<td>4.6 ± 1.6</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>No. of cryopreserved blastocyst</td>
<td>2.0 ± 2.2</td>
<td>2.3 ± 1.8</td>
<td>.28</td>
<td></td>
</tr>
<tr>
<td>No. cryopreserved embryos</td>
<td>1.7 ± 3.3</td>
<td>2.9 ± 3.6</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.47 ± 0.63</td>
<td>2.35 ± 0.59</td>
<td>.3</td>
<td>0.72 (0.36–1.41) (1)</td>
</tr>
<tr>
<td>Total embryos</td>
<td>10.9 ± 3.9</td>
<td>12.2 ± 4.4</td>
<td>.1</td>
<td>1.08 (0.98–1.20) (1)</td>
</tr>
<tr>
<td>Normal fertilization</td>
<td>11.1 ± 4.1</td>
<td>12.3 ± 4.4</td>
<td>.1</td>
<td>1.07 (0.97–1.18) (1)</td>
</tr>
<tr>
<td>High cell number</td>
<td>5.4 ± 2.1</td>
<td>5.3 ± 1.6</td>
<td>.6</td>
<td></td>
</tr>
<tr>
<td>Worst fragmentation</td>
<td>4.2 ± 2.7</td>
<td>4.1 ± 2.1</td>
<td>.7</td>
<td></td>
</tr>
<tr>
<td>No. of fetuses, events/trials (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28 (54.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21 (41.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 (3.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous gestations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term</td>
<td>0.28 ± 0.51</td>
<td>0.53 ± 0.77</td>
<td>.08</td>
<td>1.88 (0.93–3.81) (1)</td>
</tr>
<tr>
<td>Alive</td>
<td>0.25 ± 0.49</td>
<td>0.53 ± 0.77</td>
<td>.06</td>
<td>2.04 (0.98–4.22) (1)</td>
</tr>
<tr>
<td>Blastocysts at day 5</td>
<td>3.48 ± 2.57</td>
<td>4.14 ± 2.09</td>
<td>.17</td>
<td>1.13 (0.95–1.36) (1)</td>
</tr>
</tbody>
</table>

Note: All values mean ± SD unless otherwise indicated. OR = odds ratios of pregnancy reported for quantitative variables and pertains to a change of x units, where x is reported in parentheses; 95% CI = 95% confidence interval.

<sup>a</sup> Associations with quantitative and ordinal variables assessed using logistic regression.


### Table 3

Correlation between D-3 ROS levels in pregnant and nonpregnant cycles.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Nonpregnant cycles, mean ± SD</th>
<th>Pregnant cycles, mean ± SD</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR (95% CI) (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, n</td>
<td>42</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 ROS level (× 10&lt;sup&gt;6&lt;/sup&gt; cpm)</td>
<td>66.4 ± 39.4</td>
<td>26.8 ± 13.9</td>
<td>&lt;.001</td>
<td>0.47 (0.30–0.74) (10)</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; (D-3 ROS) level</td>
<td>1.74 ± 0.29</td>
<td>1.36 ± 0.26</td>
<td>&lt;.001</td>
<td>0.59 (0.47–0.74) (1)</td>
</tr>
<tr>
<td>IVF, n</td>
<td>26</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 ROS level (× 10&lt;sup&gt;6&lt;/sup&gt; cpm)</td>
<td>67.00 ± 39.91</td>
<td>27.11 ± 14.95</td>
<td>&lt;.0001</td>
<td>0.47 (0.30–0.74) (10)</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; (D-3 ROS) level</td>
<td>1.75 ± 0.26</td>
<td>1.36 ± 0.27</td>
<td>&lt;.0001</td>
<td>0.52 (0.36–0.74) (1)</td>
</tr>
<tr>
<td>ICSI, n</td>
<td>16</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3 ROS level (× 10&lt;sup&gt;6&lt;/sup&gt; cpm)</td>
<td>65.53 ± 39.79</td>
<td>25.6 ± 12.75</td>
<td>.006</td>
<td>0.56 (0.37–0.85) (10)</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; (D-3 ROS) level</td>
<td>1.71 ± 0.34</td>
<td>1.34 ± 0.26</td>
<td>.005</td>
<td>0.66 (0.49–0.88) (1)</td>
</tr>
</tbody>
</table>

Note: OR = odds ratios of pregnancy reported for quantitative variables; the odds ratio pertains to a change of x units, where x is reported in parentheses; 95% CI = 95% confidence interval; D-3 ROS = day 3 reactive oxygen species.

<sup>a</sup> Associations with quantitative and ordinal variables assessed using logistic regression.

in conventional IVF cycles. Furthermore, other indicators of oxidative stress, such as lipid peroxides and total antioxidant capacity, could have provided a more comprehensive picture of oxidative stress in culture. It is unclear if the relationship between embryo quality and high day 3 ROS levels in culture media is one of cause or effect and is independent of whether single or multiple culture media is used. Actually we believe that it may be a cause-and-effect in the same time, as high culture media ROS may compromise the embryo quality through inducing uncorrectable oxidative stress damage. In addition, low embryo quality may produce a high ROS production and hence its ROS level may be used as a predictor for the embryo quality. In our study because we use the outer well as a negative control, our findings mainly indicate that the source of ROS is from the embryos themselves, whatever their quality. However, we did not isolate or remove low quality embryos to study the effect of such removal on the ROS levels. Our findings refer to the use of day 3 culture media ROS levels as predictor of embryo quality or clinical pregnancy outcome in both IVF and ICSI procedures. Another potential application of our findings is the use of day 3 culture media ROS as an indicator for culture media quality control and this may be used as an additional parameter for media quality testing and assurance.

In conclusion, increasing levels of ROS generation in day 3 in vitro embryo culture media may have a detrimental effect on the in vitro embryo growth parameters, as well as clinical PRs in both conventional IVF and ICSI cycles. Also, we can improve blastocyst development rates in the mouse embryo model by a short coincubation time of the gametes in conventional IVF cycles and supplementing the culture media with antioxidants to help scavenge excessive production of ROS in the culture media. These measures may be applied clinically in an attempt to improve ART outcomes.

Acknowledgments: The authors thank Kurt Miller, Ph.D., and Jeffery Hammel, M.S., for their valuable assistance during the preparation of the manuscript.

**REFERENCES**


