Cumulus cell apoptosis changes with exposure to spermatozoa and pathologies involved in infertility

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Objective: To determine whether the incidence of apoptosis in mature oocyte cumulus cells changes after insemination related to infertility. 

Design: Prospective study. 

Setting: Public hospital and university. 

Patient(s): One hundred women undergoing in vitro fertilization and embryo transfer (IVF-ET). 

Intervention(s): Collection of cumulus cells from IVF-ET cycles with different infertility etiologies. 

Main Outcome Measure(s): Detection of apoptosis in cumulus cells; fertilization, embryo quality, and pregnancy rate. 

Result(s): The incubation of cumulus–oocyte-complexes with spermatozoa led to an increase in cumulus cell apoptosis from 34.2 ± 3.7 to 44.5 ± 6.3%. After insemination, cumulus cells of poor quality embryos showed a statistically higher apoptotic rate versus cumulus cells of good quality embryos (61.5 ± 6.4 vs. 40.6 ± 3.9%). Cumulus cells arising from oocytes with ≥50% fertilization rates after insemination showed higher apoptosis rates did cumulus cells from oocytes with <50% fertilization rates (46.0 ± 3.7 vs. 33.8 ± 4.0%). Patients with endometriosis presented higher apoptotic rates before insemination (77.6 ± 9.06%). Cumulus cells obtained after aspiration showed no differences in their apoptosis rates for the following factors: age of women, aspired oocytes, estradiol level, fertilization rate, and embryo quality or pregnancy. The apoptotic profile from pregnant women was less than (but not statistically significantly different from) profiles from nonpregnant women. 

Conclusion(s): These results suggest that the incidence of apoptosis in cumulus cells is associated with exposure to spermatozoa and the cause of infertility. (Fertil Steril® 2009;91:2061–8. ©2009 by American Society for Reproductive Medicine.) 

Key Words: Cumulus cells, apoptosis, spermatozoa, TUNEL assay

The mammalian oocyte and its surrounding cumulus cells are metabolically coupled through an extensive network of gap junctions, which provide a unique means of entry into the ooplasm for several metabolites (1–3). Cumulus cells provide essential support for in vivo maturation of oocytes during metaphase II and enhance cytoplasmic maturation, which allows for normal fertilization and subsequent development (4–7). Cumulus cells also facilitate sperm selection, capacitation, and acrosome reactions and promote male pronuclei formation and the acquisition of full embryonic developmental competence (8–12).

The apoptosis rate in mural cells has been proposed to be a predictor of ovarian reserves in women undergoing in vitro fertilization (during natural or gonadotropin-stimulated cycles), and high levels of apoptosis were evident in patients with low response, advanced age, and endometriosis (13–17).

The impact of granulosa cell apoptosis in in vitro fertilization and embryo transfer (IVF-ET) outcomes has already been studied extensively. Apoptosis in granulosa cells seems to have a negative effect on fertilization rate and embryo quality (18–20). Although Clavero et al. (21) did not find differences between apoptotic levels of granulosa cells/follicle and oocyte maturity and fertilization rates by intracytoplasmic sperm injection (ICSI), in a recent report Jancar et al. (22) showed that apoptosis and reactive oxygen species (ROS) production in granulosa mural cells had no significant impact on fertilization and did not correlate with the development of the blastocyst.

Because of the close association between the oocyte and the cumulus cells, it is likely that the DNA status of the cumulus cells can influence the development of the oocyte. Few studies have focused on the correlation between cumulus cells, apoptosis, and the outcome of IVF-ET. Because of the supportive role of these cells on the oocyte, it has been suggested that apoptosis in cumulus cells may be a better predictor of good oocyte quality, higher fertilization rate, and better embryo quality than the granulosa cells apoptotic rate.
Most reports support the idea that the degree of apoptosis in cumulus cells is negatively correlated with oocyte competence (23–28). Other results were controversial and showed a positive relationship between cumulus cell DNA fragmentation and fertilization by ICSI (29), whereas a lack of correlation was found between the incidence of apoptotic cumulus cells and the outcome of ICSI when analyzing only fertilization and embryo quality (30). A negative correlation was reported by Saito et al. (24) and Corn et al. (31) between embryo quality and apoptotic rates of cumulus cells, with no influence on pregnancy and implantation.

Only a few investigators have studied the incidence of apoptosis in cumulus cells after co-incubation with spermatozoa in patients undergoing ICSI or IVF and correlated these results with fertilization rates and embryo quality. The rate of apoptosis in cumulus cells is influenced by the length of time of in vitro culture; apoptosis increases from 7% in freshly isolated cumulus cells to 32% at 24 hours after incubation, without the presence of spermatozoa (32). Moffatt et al. (33) found a significantly higher apoptosis rate in human cumulus cells co-incubated overnight with spermatozoa than cumulus cells incubated in culture medium without spermatozoa (59 ± 8% vs. 27 ± 6%); this was consistent with the results reported by Yuan et al. (27) for bovine cumulus cells.

To clarify whether the incidence of apoptosis in human cumulus cells could be used as a prognostic parameter of IVF-ET outcome in our patients, we assessed the apoptotic level of cumulus cells obtained from IVF-ET patients before and after being exposed to spermatozoa, related to causes of infertility. In addition we analyzed the relationship between cumulus cell apoptosis, the ages of the women, fertilization outcome, embryo quality, and pregnancy rate.

**MATERIALS AND METHODS**

**Patients and Follicle-Stimulation Protocol**

The study population included 100 patients undergoing IVF-ET and ICSI at the Maternal and Child Research Institute (IDIMI), at the San Borja-Arriarán Clinical Hospital. The average patient age was 32.87 ± 0.36 years (range: 23 to 42 years), and diagnoses included male factor infertility (MF) (n = 30), tubal factor infertility (TF) (n = 44), endometriosis (ENDO) (n = 9), and unexplained infertility (UI) (n = 17). Sixty-six patients were treated with IVF and 34 with ICSI. The study was approved by the institutional review board of the Faculty of Medicine at the University of Chile and the San Borja Arriarán Clinical Hospital. All patients gave informed consent.

Synchronization of ovarian cycles was achieved by administering a gonadotropin-releasing hormone agonist (Lupron; Abbott Laboratories, Chicago, IL): 14 IU subcutaneously for 7 days before menstruation, followed by an additional 7 IU subcutaneously between day 1 and the administration of human chorionic gonadotropin (hCG). Thereafter, ovulation was induced by sequential use of recombinant follicle-stimulating hormone (FSH, Puregon Pen; Organon Pharmaceuticals, West Orange, NJ; 200 IU, SC) and human menopausal gonadotropin (hMG; Instituto Massone S.A., Buenos Aires, Argentina; 150 IU, IM) daily for 3 days. After that, we used only 150 IU hMG daily until it was time to administer hCG.

The progression of follicular development was followed by daily ultrasound evaluation, and the expected changes in plasma estradiol (E2) levels were monitored in daily blood samples. When the development of at least three follicles (18 mm in diameter) was achieved, a single injection of hCG (Pregnyl, Organon Pharmaceuticals; 10,000 IU, IM) was administered. After 36 hours, oocyte retrieval was carried out using transvaginal ultrasound guidance (34).

**Sperm Preparation, Insemination, Culture, and Embryo Transfer**

Highly motile spermatozoa were collected using the isolate gradient (90%) method (Irvine Scientific, Santa Ana, CA), and these were used for insemination (100,000 motile sperm/mL) 4 to 6 hours after oocyte recovery. The ICSI was carried out on the stage of an inverted microscope (400× magnification; Nikon Diaphot-300, Tokyo, Japan) using the Hoffman modulation contrast system. This procedure was performed on all morphologically intact second metaphase (MII) oocytes. Immobilized motile spermatozoa were aspirated into the injection needle and injected into the oocyte (35).

Fertilization was confirmed 18 hours after insemination by visualization of two pronuclei. Fertilized oocytes were transferred to fresh human tubal fluid (HTF) medium (10% SSS, synthetic serum substitute) and cultured for an additional 48 or 72 hours. At the end of the culture period, the embryos were assessed morphologically, as described previously elsewhere (36–37). Embryo quality was graded mainly considering the number of blastomeres and the degree of fragmentation, defined as follows: good-quality embryos showed equal blastomeres and no fragmentation; poor-quality embryos showed >30% fragmentation and no equal blastomeres on the day of transfer.

The embryo transfer was performed 2 or 3 days after oocyte recovery. Each patient was monitored for evidence of clinical pregnancy.

**Cumulus Cell Removal and Preparation**

Cumulus cells were removed from mature cumulus–oocyte complexes derived from IVF-ET or ICSI procedures. The cumulus cells were examined immediately after aspiration and after 18 hours of incubation with or without spermatozoa. For the IVF procedure, a small portion of cumulus cell masses was removed manually from three to five cumulus–oocyte complexes using a 26-gauge needle. A pool of cumulus cells was collected after incubation of cell masses in a solution of hyaluronidase (80 IU, Type VII; Sigma Chemical, St. Louis, MO) in HTF HEPES 3% SSS (Irvine Scientific) by pipetting...
repeatedly for several minutes. All cumulus–oocyte complexes were inseminated, and they were examined for fertilization 18 hours later. Fertilized oocytes were removed from the insemination medium and transferred to fresh culture medium for their subsequent development. The remaining cumulus cells and spermatozoa were pooled, centrifuged, and processed.

To assess the apoptotic rate of cumulus cells in the absence of spermatozoa, cumulus–oocyte complexes were incubated for 18 hours in the insemination medium without spermatozoa. The cumulus cells were removed from the pool of oocytes after dispersion with hyaluronidase.

For the ICSI procedure (34 cycles), the surrounding cumulus cells were collected after denuding (80 IU/mL hyaluronidase; Type VII; Sigma Chemical) the oocyte for spermatozoa injection. The collected cumulus cells were centrifuged twice for 5 minutes at 400 × g, in phosphate buffered saline (PBS; Irvine Scientific). The cell pellet was fixed with 3.5% paraformaldehyde in PBS for 1 hours at 4°C. After washing twice with PBS (400 g for 5 minutes), the pellet was carefully pipetted and air-dried on a silanized glass slide for the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.

DNA Fragmentation by TUNEL Assay

The DNA damage was detected using the TUNEL assay with an in situ Cell Death Detection Kit with fluorescein isothiocyanate-labeled dUTP (Roche Diagnostics GmbH, Mannheim, Germany). The CC were permeabilized with 0.2% Triton X-100 (Sigma Chemical) in PBS (5 minutes at room temperature), and washed twice in PBS. Samples were then incubated in the dark at 37°C for 1 hours in the TUNEL reaction mixture containing labeled nucleotides and enzyme terminal transferase. After stopping the enzyme reaction, the slides were washed in PBS and counterstained with propidium iodide (1 µg/mL). The slides were mounted with DABCO (Sigma Chemical) and observed in a fluorescence microscope (Optipot; Nikon), with a 100 × oil-immersion objective.

For each sample, a total of 200 cells were evaluated and each cell was assessed for fragmented DNA (intense green nuclear fluorescence) or normal DNA (red nuclear fluorescence due to propidium iodide). Negative and positive controls were performed, respectively, by omitting the enzyme terminal transferase or by preincubating fixed and permeabilized cells with DNase I (1 mg/mL) for 20 minutes at room temperature (38).

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Results are expressed as mean ± standard error of the mean (SEM). Statistical comparisons were performed by using nonparametric Mann-Whitney U test, unpaired Student’s t-test, analysis of variance (ANOVA), and Tukey-Kramer tests. P < .05 was considered statistically significant.

RESULTS

Group Characteristics

The characteristics of the group undergoing IVF-ET are shown in Table 1. We studied 100 cycles of IVF-ET with at least one embryo transferred/cycle. The mean age of the women was 32.9 ± 0.4 years, and we recovered 10.7 ± 0.6 oocytes/cycle (95% MII), with a fertilization rate of 76.1 ± 2.2% and transferred a mean of 3.0 ± 0.1 embryos, producing a pregnancy rate/cycle of 21.0 ± 4.1%. The patient’s age is a crucial factor that may influence oocyte quality and the outcome of IVF-ET. As we have shown in Table 2, for a mean age of 32.9 ± 0.4 years, the incidence of cumulus cell apoptosis was 34.3 ± 3.7%. No statistically significant difference was found between patients <35 or ≥35 years old: 31.0 ± 4.5 versus 39.0 ± 6.0% cumulus cell apoptosis, respectively.

For cumulus cells obtained 18 hours after insemination, the mean apoptotic level was 44.5 ± 3.5, and no differences were seen in these parameters between age groups (45.0 ± 5.0 vs. 39.6 ± 6.0%). As was reported previously, the number of oocytes retrieved/cycle for patients younger than 35 years old was statistically significantly higher than for women older than 35 years (12.0 ± 0.8 vs. 8.4 ± 0.9; P = .004). The incidence of apoptosis was the same for patients with <5 or ≥5 oocytes retrieved (34.0 ± 8.8 vs. 34.4 ± 4.1%; P > .05). Other parameters associated with IVF-ET results, such as fertilization rate, embryo transfer, and pregnancy rate, showed no statistically significant differences among the entire group or for different women ranked by age.

Fertilization and Embryo Quality

For all patients, the incidence of apoptosis in cumulus cells increased from 34.3 ± 3.7% to 44.5 ± 3.5% (P = .04) after

<table>
<thead>
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<th>TABLE 1</th>
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<tr>
<td><strong>Profile of infertile patients after IVF-ET.</strong></td>
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<tr>
<td><strong>Characteristic</strong></td>
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<tr>
<td>Patients (n)</td>
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<td>Age (years)</td>
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<td>Recovered oocytes/cycle (n)</td>
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<td>Estradiol on day of hCG (pg/mL)</td>
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<td>Fertilization rate (%)</td>
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<td>Total embryos transferred/cycle (n)</td>
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<td>Good-quality embryos (n)</td>
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<td>Pregnancy rate (%)</td>
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*Note: All values are expressed as mean ± standard error of the mean.*

insemination of cumulus–oocyte complexes with 100,000 spermatozoa/mL (see Table 2). Comparing patients with \( \leq 50\% \) and \( > 50\% \) oocyte fertilization rates, before or after insemination, no differences in their apoptotic rate were found: 37.0 ± 8.0 vs. 33.8 ± 4.0\% and 46.0 ± 3.7\% vs. 46.0 ± 3.7\%, respectively. Only the group with a fertilization rate higher than 50\% showed a statistically significant increase in cumulus cell apoptosis, from 33.8 ± 4.0 to 46 ± 3.7\% (\( P = .03 \)), after having been inseminated compared with the group with a fertilization rate of \( < 50\% \).

We also examined the percentage of spermatozoa that showed DNA fragmentation before and after being in contact for 18 hours with the cumulus–oocyte complexes. No differences were seen in the mean number of TUNEL-positive spermatozoa between both groups of cells: 17.5 ± 3.4 before versus 20.9 ± 1.9\% after being in contact with cumulus–oocyte complexes (\( P = .70 \)). Comparing patients with \( \leq 50\% \) or \( > 50\% \) fertilization, before and after being exposed to cumulus–oocyte complexes, no differences were found in spermatozoa DNA fragmentation (see Table 2).

The cumulus cells obtained from cumulus–oocyte complexes incubated for 18 hours at 37°C, without spermatozoa, showed apoptosis rates of 35.2 ± 8.3\%.

Eighty-one percent of the transferred IVF-ET cycles were performed with at least one good-quality embryo. The cumulus cells from cumulus–oocyte complexes that produced good-quality embryos showed an incidence of apoptosis of 33.0 ± 6.0 before and 41.0 ± 4.0\% after being in contact with spermatozoa (these differences were not statistically significant, \( P = .20 \)) (Fig. 1). The incidence of apoptosis in cumulus cells from cumulus–oocyte complexes that produced poor-quality embryos increased after 18 hours of exposure to spermatozoa (39.0 ± 9.0 vs. 61.0 ± 6.0\%), but the differences were not statistically significant (\( P = .054 \)). Comparing apoptosis rates of cumulus cells from poor-quality embryos with good-quality ones, after insemination a statistically significant increase in their apoptotic rates was seen (61.0 ± 6.0 vs. 41.0 ± 4.0, unpaired Student’s \( t \)-test and Mann-Whitney nonparametric test; \( P < .05 \)).

For the entire group, the pregnancy rate was higher but not statistically statistically different for patients with good-quality embryos versus poor-quality embryos: 23.0 ± 4.0 (\( n = 81 \)) versus 11.0 ± 7.0\% (\( n = 19 \)) (\( P = .3 \)).

### Infertility Factors and Pregnancy Rate

The incidence of apoptosis was analyzed by classifying patients into the following infertility factors: tubal factor, male factor, unexplained infertility, and endometriosis (Fig. 2). Patients with endometriosis showed a statistically

#### TABLE 2

<table>
<thead>
<tr>
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<th>Total (n = 100)</th>
<th>( \leq 50% ) (n = 12)</th>
<th>( &gt; 50% ) (n = 88)</th>
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<tr>
<td><strong>Cumulus cells</strong></td>
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<tr>
<td>Apoptosis (%)</td>
<td>34.3 ± 3.7\textsuperscript{a}</td>
<td>37.0 ± 8.0</td>
<td>33.8 ± 4.0\textsuperscript{b}</td>
</tr>
<tr>
<td>Apoptosis after insemination</td>
<td>44.5 ± 3.5\textsuperscript{a}</td>
<td>32.5 ± 10.0</td>
<td>46.0 ± 3.7\textsuperscript{b}</td>
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<tr>
<td><strong>Spermatozoa</strong></td>
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<tr>
<td>TUNEL (( \texttt{+} ))</td>
<td>17.5 ± 3.4</td>
<td>20.3 ± 7.9</td>
<td>16.8 ± 3.8</td>
</tr>
<tr>
<td>TUNEL (( \texttt{+} )) after insemination</td>
<td>20.9 ± 1.9</td>
<td>21.6 ± 5.8</td>
<td>20.8 ± 2.1</td>
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Note: All values are expressed as mean ± standard error of the mean. \( P \) value analyzed by Mann-Whitney.

\( \textsuperscript{a}P = .04 \), comparing cumulus cells apoptosis total before insemination versus cumulus cells apoptosis total after insemination.

\( \textsuperscript{b}P = .03 \), comparing cumulus cells apoptosis \( > 50\% \) before insemination versus cumulus cells apoptosis \( > 50\% \) after insemination.


#### FIGURE 1

The incidence of apoptosis (%) in cumulus cells immediately after aspiration (basal state) and 18 hours after insemination in patients with good-quality embryos or poor-quality embryos. Bars represent mean ± standard error of the mean. \( a, b: P < .05 \).

A significantly higher incidence of apoptosis in cumulus cells compared with the other groups before insemination \((P < .05)\) by multiple comparisons test (one-way ANOVA with Tukey’s test). We compared each group of pathologies before and after insemination; tubal factor and unexplained infertility patients showed a statistically significant increase in TUNEL-positive cells after insemination \((**P < .05)\). This increase was not seen in cumulus cells from patients in treatment for male factor infertility or endometriosis.

In those patients with cumulus cell samples in both conditions, before and after insemination (endometriosis, tubal factor, and unexplained infertility), we calculated the difference in the incidence of apoptosis before and after insemination \((D)\) per patient. Only the endometriosis group showed statistically significant differences versus tubal factor and unexplained infertility groups by multiple comparisons test \((P < .05)\).

Before the insemination of cumulus–oocyte complexes, the percentage of spermatozoa showing DNA fragmentation was statistically significantly higher in male factor infertility patients as compared with the mean value from the entire group: 36.0 ± 8.0% versus 17.5 ± 3.4% \((P < .05)\).

The incidence of apoptosis between pregnant and non-pregnant patients was analyzed in all patients and in the different infertility factor groups. The relationship between the incidence of apoptosis (%) in cumulus cells and pregnancy rate is shown in Figure 3. No statistically significant differences were seen in apoptosis levels between pregnant and nonpregnant patients for the entire group \((n = 100)\) or for the individual infertility factors. Pregnant patients have a tendency to present a lower apoptosis rate before \((24.9 \pm 7.8 \text{ vs. } 37.0 \pm 4.1)\) and after \((36.6 \pm 8.4 \text{ vs. } 46.9 \pm 3.8%)\) being inseminated than nonpregnant patients. In patients with endometriosis and with higher apoptosis levels before insemination \((63.13 \pm 7.3%)\), no pregnancy resulted.

**DISCUSSION**

Previous studies have suggested that the incidence of apoptosis in cumulus cells may be a good indicator of oocyte developmental competence and a prognosis factor in an IVF-ET program. However, the detrimental threshold for the incidence of apoptosis has yet to be established, and therefore the consequences for embryo quality are difficult to assess.

In our study, the mean percentage of apoptotic cumulus cells in mature cumulus–oocyte complexes was detected by TUNEL assay. Before insemination, the percentage was 34.3 ± 3.7%. This result was higher than the 3.0% to 22.6% reported by other investigators \((20, 28, 30, 31, 33, 39)\).

The reports associating levels of apoptosis in granulosa cells and cumulus cells with respect to age, fertilization rate, embryo quality, and pregnancy rate are contradictory. The discrepancies in results among investigators may be due to three major factors:
1. The stimulation protocol. It is known that hormones such as E2, FSH, LH, and hCG produce an inhibitory effect on granulosa cell apoptosis, but other hormones like androgens, GnRH, or GnRH analogues increase the apoptotic rate compared with cycles without stimulation (40, 15, 28).

2. Different methods of measuring apoptosis. Comet assay, flow cytometry, Hoechst, Apodkit staining, or TUNEL assay may have been used.

3. Different origin of cumulus cells. The cells may have been collected from individual follicles or derived from a pool of aspirated follicles.

In spite of a relationship between patient age and apoptotic rate that has been reported (26, 31), our data do not support this finding because neither the age of the patients (older or younger than 35 years) nor the number of oocytes retrieved (more or less than five) were related to the apoptosis levels in cumulus cells. Similar results were reported by Nakahara et al. (18), Raman et al. (29), Høst et al. (39), and Abu-Hassan et al. (30).

A lower incidence of apoptosis in cumulus cells from fertilized oocytes compared with nonfertilized oocytes had been reported by several investigators (18, 26, 39). In contrast, Raman et al. (29) showed that the fertilizing ability of oocytes (after ICSI) was positively correlated with the cumulus cell DNA status (Comet assay). In a recent report, Corn et al. (31) did not find any correlation between apoptosis in cumulus cells and fertilization rate. This finding is consistent with our results, using the same method and cell types. In addition, our studies have confirmed earlier reports about cumulus cells of human oocytes being equipped with a mechanism to undergo apoptosis, and that exposure to spermatozoa may alter their profile (33).

The incubation of cumulus–oocyte complexes with spermatozoa led to an increase in cumulus cell apoptosis (34.3 ± 3.7% vs. 44.5 ± 3.5%), but no increase was seen in cumulus cells incubated for 18 hours in culture medium without spermatozoa. In contrast, José de los Santos et al. (32) reported that the rate of apoptosis was influenced by the length of time of in vitro culture, increasing from 7% in freshly isolated cumulus cell complexes to 32% after 24 hours of incubation in culture medium without spermatozoa. This increase was correlated with a decrease in the expression of Bcl-2 mRNA after 24 hours of incubation (32).

Our findings in 100 patients in our IVF-ET program and the results of Moffatt et al. (33), who used the TUNEL assay in over 23 IVF-ET patients, found an increase in cumulus cell apoptosis after insemination. These results may be related to a deleterious effect of spermatozoa on cumulus cells. However, no differences were found in spermatozoa DNA fragmentation before or after being exposed to cumulus–oocyte complexes after 18 hours, in spite of the higher DNA fragmentation in spermatozoa seen in patients in treatment for male infertility with respect to the entire group (36.0 ± 8.0 vs. 17.5 ± 3.4%; P=.04). Our study, similar to Moffatt et al. (33), showed that cumulus cells incubated overnight with spermatozoa had higher apoptosis rates than cumulus cells incubated overnight with no spermatozoa (59 ± 9 vs. 27 ± 6%). Moffatt et al. (33) also reported an increase in TIAR and Fas expression in cumulus cells after insemination. In another recently published study, the investigators found reduced s-Fas anti-apoptotic activity in cumulus–oocyte complexes in the culture medium where oocytes were fertilized (40).

Moreover, our findings and previous work (29) suggest an indirect relationship between the fertilizing ability of the oocyte and the cumulus DNA status. Hakuno et al. (41) also agreed that “the increase in cumulus cell apoptosis may produce a fall away of cumulus cells from the oocyte, and would be the signal that the oocyte has attained competence for fertilization.” As Moffatt et al. (33) point out, “Probably this may be one of the mechanisms necessary to leave the surface of the developing embryo free of cumulus cells.” In addition, in porcine and bovine studies, some investigators (42–44) have suggested that “cumulus cells may be involved in alleviating stress, such as ROS on spermatozoa during fertilization, thereby increasing their apoptotic rate”.

An inverse relationship between apoptotic rate in cumulus cells and embryo quality at day 2 of the cleavage stage was suggested by Nakahara et al. (19) and Lee et al. (26). According to our results, at day 3 of embryo cleavage, no correlation between apoptosis in cumulus cells and embryo quality was found; this in agreement with Corn et al. (31) and Høst et al. (39). Nevertheless, Corn et al. (31) reported that, for cumulus cells from cumulus–oocyte complexes that produced normal blastocysts, a significantly lower apoptosis rate was found at day 5 of embryo cleavage.

Most of these previous reports show the relationship between cumulus cell apoptosis and embryo quality in embryos derived from the ICSI procedure and for cumulus cells obtained 2 to 4 hours after aspiration. In our study, the incidence of apoptosis in cumulus cells was also evaluated 18 hours after insemination and was correlated with embryo quality at day 3 of cleavage. After insemination, cumulus cells of poor-quality embryos showed a statistically significantly higher apoptotic rate versus cumulus cells of good-quality embryos. We studied the incidence of cumulus cell apoptosis before and after insemination for the most common pathologic conditions that require IVF-ET. For the majority of the infertilities (MF, TF, UI, and all patients), the basal level of apoptosis in cumulus cells was similar, but a higher apoptotic percentage was found in patients with endometriosis (P<.005) (14, 18, 45). Our results confirm the data of previous studies of Nakahara et al. (14, 18) that showed a higher level of apoptotic bodies in cumulus cells from patients with endometriosis than other infertility groups, especially those patients with MF. Seino et al. (46) found that patients with endometriosis showed a higher 8-OHdG index than did patients with other etiologies of infertility such as TF, MF, and UI. It was suggested that the high incidence of apoptosis in granulosa cells may be caused by a high level of...
oxidative stress produced by endometriosis lesions (47). Also, in patients with endometriosis a statistically signifi-
cantly lower CD44 expression was detected in cumulus cells compared with the UI group (48); Kaneko et al. (49) reported that hyaluronic acid, via CD44, reduced the incidence of apoptosis in granulosa cells. Several other studies have suggested that in patients with endometriosis the antiapop-
totic effect of hyaluronic acid may be reduced (50–52).

In our study, 9 of 100 patients presented with endometri-
osis; no pregnancies resulted for these patients. Confirming previous reports of Nakahara et al. (18), Lee et al. (26), and Raman et al. (29), no statistically significant differences were seen in apoptotic levels between pregnant and nonpreg-
nant patients.

We conclude that the incidence of apoptosis in cumulus

cells isolated from mature oocytes increases after the ex-
posure of the cumulus–oocyte complex to spermatozoa. This in-
crease was higher in patients with poor-quality embryos. We
confirm that in patients with endometriosis the cumulus cell
apoptosis after follicle aspiration was higher compared with
the rate for patients with infertilities of cause unknown or
from tubal or male factors. We also found that the patients
with lower apoptotic rates in cumulus cells have a tendency
to present higher pregnancy rates. The incidence of apoptosis
in cumulus cells isolated from mature oocytes is not related to
patient age, the number of oocytes recovered, or the fertiliza-
tion rate.

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cidence of apoptotic bodies in membrane granulosa can predict prognosis
of ova from patients participating in in vitro fertilization programs.
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