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Research Article

Apoptosis in Human Cumulus Cells and Intracytoplasmic Sperm Injection Outcomes

Eman Anwar Hassan[1], Mohamed SM Nasr[2] and Behery MA[3]*

[1]*Embryology unit, the International Islamic Institute for Population Studies and Research,Al-Azhar University, Egypt*

[2]*Department of Histology and Cell biology, Faculty of Medicine, Al-Azhar University, Egypt.*

[3]*ART unit, the International Islamic Institute for Population Studies and Research, Al-Azhar University, Egypt*

***Corresponding author:** Behery MA, ART unit, the International Islamic Institute for Population Studies and Research, Al-Azhar University, Heliopolis, Cairo, Egypt

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Abstract

Apoptosis as a genetically controlled form of cell death is closely associated with most of the reproductive processes. It has been reported that the incidence of apoptosis in granulosa or Cumulus cells may be used as a predictive factor in the outcome of assisted reproduction cycles. In this study it was revealed that the incidence of apoptosis in cumulus increased with increased female age and that influences fertilization rate, number of MII oocytes in addition to the number of Grade A embryos.

Keywords: Oocyte-Cumulus, Age; Fertilization; ICSI; Outcome

Abbreviations: CC: Cumulus cells; ICSI: intracytoplasmic sperm injection; cAMP: cyclic adenosine monophosphate; OCC: Oocyte-cumulus complexes; SOD: superoxide dismutase

Introduction

Apoptosis is defined as cell death form that involves variety of signaling pathways and has a major role in the normal function of all tissues. It is associated with reproductive process in view of high incidence of apoptosis in the human cumulus cells (CC) is associated with their alteration and in turn poor development of oocytes and embryos [1,2]. Many studies reported that increased apoptosis in CC is associated with poor reproductive outcome [3]. As the morphological feature of oocytes is correlated with fertilization rate and implantation rate during ICSI cycles. So the apoptosis in CC an affect oocyte maturation, fertilization and embryo development [4]. In the ovary, apoptosis can be initiated in four different cells, i.e., the theca cells, the granulosa cells, the cumulus cells, and the oocyte itself [5]. We can describe the process of apoptosis in four stages, stage one include DNA fragmentation, stage two, decrease in cell volume, stage three, loss of mitochondrial function, stage four, cell membrane alteration and blebbing and the last stage include cell breakage due to increased apoptotic bodies that is phagocytosed by phagocytes [6]. The rational of this study is to evaluate the effects of apoptosis on the oocyte quality and its relation to age and in turn, their effects on fertilization rate and ICSI outcomes (Table 1& Figure 1).

Table 1: Incidence of Cumulus Cells Apoptosis (mean± SD %) in relation to age.

Age	No of cases	Incidence of apoptosis	Significance		
			A &B	A&C	B&C
Group A (≤ 27)	30	40.7±4.28	P <0.001*	P <0.001*	P <0.01*
Group B (28-34)	30	45.7±5.00			
Group C (≥ 35)	30	48.7±4.28			
(*) =Indicate significant changes (P value<0.05)					

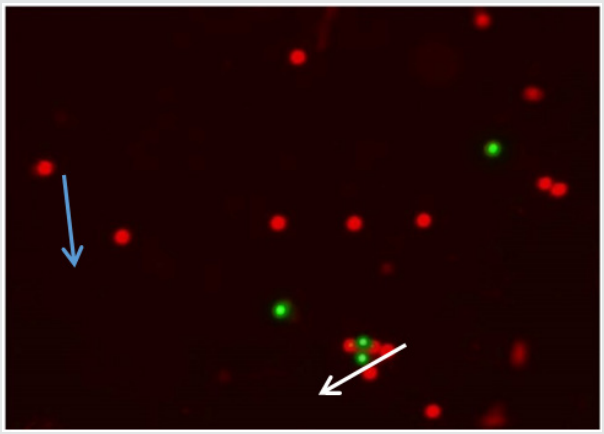

Figure 1: Immuno-fluorescent micrograph shows cumulus cells of group A. Apoptotic cumulus cells show green fluorescence (white arrow); whereas normal cells appear reddish color (blue arrow). (Magnification x 10) stained with Mitochondrial Apoptosis Detection Fluor metric Kit (Bio Vision).

Materials and Methods

This is a prospective clinical trial included 90 couples who were referred for assisted reproduction at the fertility clinic in International Islamic Center for Population Studies and Research, Al-azhar University, between May and December 2016 with the following criteria:

Inclusion Criteria

1-any subjects indicated for ICSI including couples with male factor infertility.

2-female ages ranged between 23 and 42 years with regular menstrual cycles.

Exclusion Criteria

Cumulus cells of atretic or empty follicles were excluded from the study. The 90 female subjects were divided into 3 groups according to age:

i. Group A: female partner ≤ 27 y (30 cases)

ii. Group B: female partner 28-34 y (30 cases)

iii. Group C: female partner ≥ 35 y (30 cases). The main outcome measure was the high quality embryos. The secondary outcome measures were the relation between Incidence of cumulus cells apoptosis and age, number and quality of oocytes and fertilization rate.

Assessment of Apoptosis in Cumulus Cells

In the retrieved oocytes cumulus cells were stained to detect apoptosis, we used the Mito Capture Mitochondrial Apoptosis Detection Fluor metric Kit (Bio Vision Incorporated, 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA).Disruption of the mitochondrial transmembrane potential is one of the earliest intracellular events that occur following induction of apoptosis. The Mito Capture Apoptosis Detection Kit provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells by detecting the changes in the mitochondrial transmembrane potential. The kit utilizes Mito-Capture TM, a cationic dye that fluoresces differently in healthy vs apoptotic cells. In healthy cells, Mito Capture accumulates and aggregates in the mitochondria, giving off a bright red fluorescence. In apoptotic cells, Mito Capture cannot aggregate in the mitochondria due to the altered mitochondrial transmembrane potential, and thus it remains in the cytoplasm in its monomer form, fluorescing green. The fluorescent signals were easily detected by fluorescence microscopy using a band-pass filter (detects FITC and rhodamine) (Table 2 and Figure 2&3).

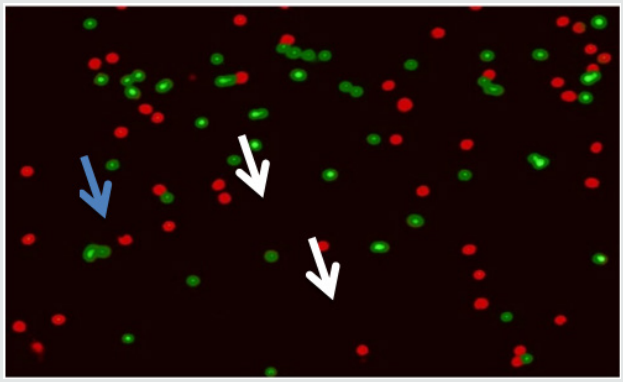

Figure 2: Immuno-fluorescent micrograph shows cumulus cells of group B. Apoptotic cumulus cells show green fluorescence (white arrows); whereas normal cells appear reddish color (blue arrow). (Magnification x 10) stained with Mitochondrial Apoptosis Detection Fluor metric Kit (Bio Vision).

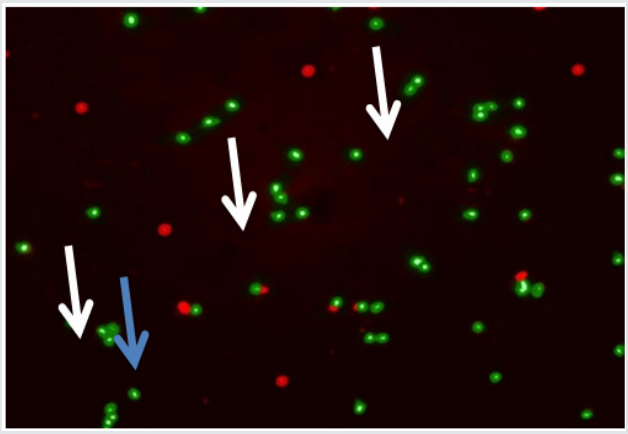

Figure 3: Immuno-fluorescent micrograph shows cumulus cells of group C. Apoptotic cumulus cells show green fluorescence (white arrows); whereas normal cells appear reddish color (blue arrow). (Magnification x 10) stained with Mitochondrial Apoptosis Detection Fluor metric Kit (Bio Vision).

Table 2: Relation between Incidences of Cumulus Cells Apoptosis & Fertilization Rate%.

Groups (Incidence of apoptosis)	Fertilization%	Significance		
		A&B	A&C	B&C
Group A (40.7±4.28)	74.3±29.4	P > 0.05	P < 0.001*	P < 0.05*
Group B (45.7±5.00)	65.7±30.01			
Group C (48.7±4.28)	49.9±29.03			
(*) =Indicate significant changes (P value<0.05)				

Discussion

The meiotic arrest of oocytes is regulated by cyclic adenosine monophosphate (cAMP), which is produced by cumulus cells. There is an intimate relation between a cumulus cell syncytium in conjunction with the oocyte involving large gap junctions. The gap junctions open and the large molecules move freely between the cumulus cells through the zona pellucida into the oocyte when stimulated with FSH, and before LH peak [7]. Because of the different physiologic effect of hormones on apoptosis, i.e. stimulatory (androgens, GnRH, or GnRH analogues) and inhibitory (E2, FSH, LH and hCG) effects, the results of many studies evaluating the correlation between the levels of apoptosis in granulosa and cumulus cells in relation to age and ICSI outcomes are contradictory [8-11]. Also this difference in study results may be attributed to the different methods of measuring apoptosis like Comet assay, flow cytometry, Hoestcht, Apodkit staining, or TUNEL assay that may have been used. The origin of cumulus cells either from the pool of collected follicles or from individual follicle may also affect results [12]. The incidence of apoptosis in CC is increasing with increased age and its relation to poor oocyte quality was reported in many studies [4,13]. On contrary to these results ,another studies reported no relation between the age of the subjects and apoptotic level in cumulus cells [14-16]. In this study, there was no concomitant relation observed between the number of oocytes retrieved and the incidence of cumulus cell apoptosis. If an oocyte falls to atresia, it might disappear during follicular development. As a result, cumulus cells are not collected from Oocyte-cumulus complexes (OCC) of atretic follicles at oocyte retrieval. Thus, the incidence of apoptosis of cumulus cells is not associated with the reduction of oocyte number resulting from follicular atresia. The same result was reported by many studies that revealed that no correlation observed between the number of oocytes retrieved (more or less than five) and level of cumulus cell apoptosis [14,15]. With different results, a study by Nakahara, et al. 1997 reported a relationship between the numbers of oocytes retrieved and the incidence of ovarian cells apoptosis [17]? They argued that the incidence of granulosa cell apoptosis was increased as the number of retrieved oocytes decreases. This decreased oocyte number was related to the increased number of empty follicles resulting from the apoptosis in granulosa cells during follicular development.

In our study, there was no concomitant relation observed between incidence of cumulus cells apoptosis and oocyte morphology. This finding may be due to the source of collected cumulus cells as they were derived from a pool of aspirated follicles. Our results differ from the data of a study by *Lee et al 2003* which suggest that the incidence of cumulus cells apoptosis could predict the age-related decline in fertility as well as oocyte quality [4] The greater incidence of cumulus cells apoptosis with reproductive aging may induce unfavorable environments for follicular oocyte development, which results in deterioration of oocyte quality, thus reducing fertilization rates and embryo development. As the fertilizing ability of oocytes is positively correlated with the cumulus cell's DNA status .And this status is affected by increased apoptosis .So we can explain the inverse correlation between the incidence of apoptosis and fertilization rate which was demonstrated by many studies and researches. Therefore the incidence of cumulus cells apoptosis is associated with decreased fertilization rate. Also our result confirm the data of related studies by Bosco, et al. 2005 [18]. which demonstrated that apoptosis in human oocytes determines fertilization failure after ICSI.

The incidence of cumulus cell apoptosis was significantly higher in unfertilized oocytes than in fertilized oocytes as evidenced by many studies. Also a lower incidence of apoptosis in cumulus cells from fertilized oocytes compared with non-fertilized oocytes had been reported by several investigators [4,14]. However, another study by Corn, et al. did not find any correlation between apoptosis in cumulus cells and fertilization rate [13].

In spite of inverse relationship between apoptotic rate in cumulus cells and embryo quality at day 2 of the cleavage stage that has been reported by Nakahara, et al, and Lee, et al. [4,14] our result did not support this finding. Similar results were reported by many studies [4-19] who reported no correlation between apoptosis in cumulus cells and embryo quality at day 3 of embryo cleavage. Factors other than apoptosis may contribute in ICSI failure such as: oxidative stress -induced digestion of DNA also increase with the increased female age and this is accompanied by decrease of CCs anti-oxidant level like superoxide dismutase (SOD) which protect the oocyte from damage caused by reactive oxygen species. Similar findings were reported by Matos, et al.. Also gap junction communication between the oocyte & CCs are required for normal oocyte and follicle

Conclusion

In this study it was revealed that the incidence of apoptosis in cumulus increased with increased female age and that influences fertilization rate, number of MII oocytes in addition to the number of Grade A embryos.

Conflict of interests

The authors reports no conflicts of interest.

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References

1. Bo Zhaia, Huiyu Liua, Xu Lib, Lisheng Daia, Yan Gaoa, et al. (2013) Cell Physiol Biochem 32: 264-278.
2. Yeo CX, Gilchrist RB, Lane M (2009) Disruption of bidirectional oocyte–cumulus paracrine signaling during in vitro maturation reduces subsequent mouse oocyte developmental competence. Biol Reprod 80(5): 1072-1080.
3. Xu lei Sun, Hao Jiang, Dong xu Han, Yao Fu, Jian bo Liu, et al. (2018) The activated DNA double-strand break repair pathway in cumulus cells from aging patients may be used as a convincing predictor of poor outcomes after in vitro fertilization-embryo transfer treatment. PLoS One 13(9): e0204524.
4. Laura Rienzi, Gábor Vajta, Filippo Ubaldi (2011) Predictive value of oocyte morphology in human IVF: A systematic review of the literature. Hum Reprod Update 17(1): 34-45.
5. Yuting Fan, Yajie Chang, Lina Wei, Jianhui Chen, Jingjie Li, et al. (2019) Apoptosis of mural granulosa cells is increased in women with diminished ovarian reserve. Journal of Assisted Reproduction and Genetics 36(6): 1225-1235.
6. Yingying Zhan, Xin Chen, Cyril Gueydan, Jiahuai Han (2018) Plasma membrane changes during programmed cell deaths. Cell Research 28(1): 9-21.
7. Pilar FP, Xuan KN, Tomoki N, Thi TMB, Takuya W, et al. (2019) Removal of cumulus cells around 20 h after the start of in vitro maturation improves the meiotic competence of porcine oocytes via reduction in cAMP and cGMP levels. J Reprod Dev 65(2): 177-182.
8. Diego E, Peter H (2014) A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. Reprod Biol Endocrinol 12: 95.
9. Kaneko T, Saito H, Toya M, Saito T, Nakahara K, et al. (2000) Hyaluronic acid inhibits apoptosis in granulosa cells via CD44. J AsssistReprod Genet 17(3): 162-167.
10. Daniele Santi, Livio Casarini1, Carlo Alviggi, Manuela Simoni (2017) Efficacy of Follicle-Stimulating Hormone (FSH) Alone, FSH + Luteinizing Hormone, Human Menopausal Gonadotropin or FSH + Human Chorionic Gonadotropin on Assisted Reproductive Technology Outcomes in the "Personalized" Medicine Era: A Meta-analysis. Front. Endocrinol 8: 114.
11. Ruvolo G, Bosco L, Pane A, Morici G, Cittadini E, et al. (2007) Lower apoptosis rate in human cumulus cells after administration of recombinant lutelizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. FertilSteril 87(3): 542-546.
12. Marina Diaz Fontdevila, Ricardo Pommer, Rosita Smith (2009) Cumulus cell apoptosis changes with exposure to spermatozoa and pathologies involved in infertility. Fertility Sterility 91(5): 2061-2068.
13. MG Da Broi, VSI Giorgi, F Wang, DL Keefe, D Albertini, et al. (2018) Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. J Assist Reprod Genet. 35(5): 735-751.
14. Sheena LP, Regan, Phil G Knight, John LYovich, Yee Leung, (2018) Granulosa Cell Apoptosis in the Ovarian Follicle-A Changing View. Front Endocrinol (Lausanne) 9: 61.
15. Bárbara Lourenço, Ana Paula Sousa, Teresa Almeida Santos, João Ramalho Santos (2014) Relation of Cumulus Cell Status with Single Oocyte Maturity, Fertilization Capability and Patient Age. J Reprod Infertil. 15(1): 15-21.
16. Abu Hassan D, Koester F, Shoepper B, Schultze-Mosgau A, Asimakopoulos B, et al. (2006) comet assay of cumulus cells and spermatozoa DNA status, and the relationship to oocyte fertilization and embryo quality following ICSI. Repord Biomed online 12: 447-452.
17. Bosco L, Ruvolo G, Morici G, Manno M, Cittadini E, et al. (2005) Apoptosis in human unfertilized oocytes after intracytoplasmic sperm injection. Fertil Steril 84(5): 1417-1423.
18. Marina Diaz Fontdevila, Ricardo Pommer, Rosita Smith (2009) Cumulus cell apoptosis changes with exposure to spermatozoa and pathologies involved in infertility. Fertility Sterility 91(5): 2061-2068.
19. Matos L, Stevenson D, Gomes F, Silver-Carvalho JL, Almeida H (2009) Superoxide Dismutase expression in human cumulus oophorus cells. Mol Hum Reprod 15: 411-419.

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