

Investigation of ADAMTS-1, BAX and IL-10 Expressions in Granulosa Cells Determinanting Fertilization Success in IVF

Investigación de las Expresiones ADAMTS-1, BAX e IL-10 en Célula de la Granulosa Determinando el Éxito de Fertilización en FIV

Yunus Çavus & Ugur Deger

ÇAVUS, Y. & DEGER, U. Investigation of ADAMTS-1, BAX and IL-10 expressions in granulosa cell determinanting fertilization success in IVF. *Int. J. Morphol.*, 38(2):427-434, 2020.

SUMMARY: Granulosa cells (GCs) are essential components of follicles and play a role in regulating follicle development. The aim of this study was to investigate certain cellular components involved in the proliferation, differentiation and functional characteristics of granulosa cells in the success of fertilization of human oocytes during invitro fertilization (IVF) via immunohistochemical techniques. In this study, 30 patients who were diagnosed as primary or secondary infertility, polycystic ovary syndrome in the IVF center of Memorial Hospital, Department of Obstetrics and Gynecology were included. The amount of Anti Müllerian Hormone (AMH) in blood and granulosa cell diameter and cell core diameter were measured in 20 cells collected from each patient. In addition, degeneration scoring and BAX, ADAMTS-1, IL-10 expressions in granulosa cells were evaluated ($p < 0.01$). It was thought that apoptosis induced by human GCs might be an indicator of egg quality. Moderate expression of ADAMTS-1 was thought to be related to failure of ovulation, deterioration of oocyte quality and decreased fertilization rate. This decrease in AMH levels may be associated with defects in granulosa cells. Therefore, significantly lower AMH secretion and increase in IL10 expression levels in healthy people can be explained by the increase of granulocyte cells.

KEY WORDS: Granulosa Cells; Fertility; ADAMTS-1; IL-10; BAX; immunohistochemistry.

INTRODUCTION

During in vitro fertilization, fertilization occurs when sperm is injected to micro-droplets containing secondary oocytes. Success rate depends on sperm-oocyte interaction, penetration through external cumulus layers and zona pellucida, oocyte binding and fertilization ability with plasma membrane (Varras, 2013). A proper successful ovulation required cumulus oocyte complex (COC) formation and rupture of epithelium lined the ovarian surface. During this step, extracellular matrix (ECM) components including versican and hyaluronic acid are elevated (Richards *et al.*, 2005).

Granulosa cells are somatic cells surrounding each developing oocyte necessary for follicle and oocyte development. The relation of GCs with the oocyte maintains essential nutrients for oocyte development and accumulation of oocyte secreted metabolites (Su *et al.*, 2009). Moreover, GCs are involved in hormonal activity with the production of estradiol during follicular growth and secretion of progesterone after ovulation (Dzafic *et al.*, 2013). GCs shape

changes into four different layers; the outermost layer is the membrana granulosa, the inner most layer is the periantral, the intermediate layer is the cumulus oophorus, and the layer aligned next to the oocyte corona radiata in a pre-ovulatory follicle. These layers have different function i.e. they secrete different molecules (Nguyen *et al.*, 2012).

ADAMTS-1 is a metalloproteinase that contain disintegrin domain and thrombospondin type I motifs (Kuno *et al.*, 1997). Disintegrin domain prevents integrins bind their ligands leading to blockage of intracellular signaling events. This suggests they may deteriorate interactions between cells and collagenous matrix or interactions of granulosa cells with each other (Fujiwara *et al.*, 1996). ADAMTS-1 is a critical molecule involved in degradation of versican, ovulation, vasculogenesis and expansion of COC formation. In an experiment on ADAMTS1-null mice, oocyte and ovarian vasculature was degenerated, ovarian network was less organized (Richards, 2005).

Cytokine function in the ovary has been described as promoting processes of follicular growth, steroidogenesis, recruitment and activation of leukocytes necessary for ovulation and tissue remodelling during ovulation, luteinization, and luteolysis (Büscher *et al.*, 1999). Ovarian interleukins (ILs) mediate follicle development, fertilization, embryo development and implantation. IL-10 and vascular endothelial growth factor levels promote angiogenesis and corpus luteum formation (Gómez *et al.*, 2010) IL-10 is a general anti-inflammatory molecule that is present at low levels in circulating blood. It exerts key functions for the maintenance of the pro-inflammatory and anti-inflammatory balance in the body to prevent inflammatory and autoimmune pathologies (Pestka *et al.*, 2004; Saraiva & O'Garra, 2010; Ouyang *et al.*, 2011).

The aim of this study was to investigate certain cellular components involved in the proliferation, differentiation and functional characteristics of granulosa cells in the success of fertilization of human oocytes during in vitro fertilization via immunohistochemical techniques.

MATERIAL AND METHOD

Experimental procedure. This study was performed between September 2018 and April 2019 in the IVF center of Memorial Hospital Obstetrics and Gynecology Clinic including 30 patients in the assisted reproduction program with the complaint of not having children, in addition to diagnosis of primary or secondary infertility, polycystic ovary syndrome (PCOS), male factor and unexplained infertility diagnosis when starting the Ovum-Pick-Up (OPU) procedure, the patient was first sedated. Before the procedure, the bladder was emptied in order to reach the ovaries more easily during transvaginal oocyte collection and to avoid possible bladder injury. After sedation, the patient was placed in the lithotomy position and covered sterile. Patients with a body mass index of between 18 and 35 kg/m² were selected for IVF or intracytoplasmic sperm injection for various indications between 18 and 45 years of age. When the mean diameter of the prominent follicle reached 18 mm, the final matured follicle was induced with 1000 IU of urinary Hcg or 250 mcg of recombinant hcg, and oocyte collection was planned after 34-48 hours. A gel was placed on the ultrasound probe and a sterile plastic sheath is passed before starting the OPU. A biopsy clip was inserted over the sheath, where the needle was passed and guided during aspiration. 16 or 17 gauge needles designed for OPU operation were used. The ideal aspiration pressure for the procedure was 100-150 mmHg.

The follicle fluids and granulosa cells that came with these fluids were examined by histopathological and immunohistochemical methods

Histopathologic procedure. The granular cells obtained from the patients were preserved at + 4 °C. Materials were sent to the histology laboratory for routine histological procedures. For each sample, the fluid with granular cells was subjected to high-speed centrifugation, the supernatant was discarded then 10 % neutral formaldehyde was added (pellet:formaldehyde, 1:3). After a fixation of 2 h, the supernatant was discarded again and tube was reversed to filter paper. The pellet was placed on a filter paper using a spatula and Eosin staining solution was added using a Pasteur pipette. After the cell aggregate turned into red, it was wrapped in the filter paper and placed in a cassette. After routine histological follow-up, 4-5 µm sections were cut from the paraffin blocks with a microtome (Leica, Germany). Harris Hematoxylin and Eosin staining was applied to sections and examined under light microscope (Imager A2 Zeiss, Germany).

Immunohistochemical staining. Samples were fixed with 10 % formaldehyde solution, decalcified with 5 % ethylenediamine-tetraacetic acid (EDTA), dehydrated in a graded series of ethanol, and then embedded in paraffin wax. Then, 4-5 µm thick sections were cut with a microtome (Leica, Germany) and placed on coated slides. Sections were brought to distilled water and washed three times for 5 min in phosphate buffered saline (PBS, pH 7.4) (catalogue number # 10010023, Thermo Fisher Scientific, US). To unmask antigen sites, slides were incubated with EDTA solution in microwave for 110 minutes at 3x90 °C. The sections were washed in three times for 5 min in PBS and incubated with hydrogen peroxide (catalogue # TA-015-HP, Thermo Fisher Scientific, US) for 20 min. Ultra V block (TA-125-UB, Thermo Fisher Scientific, US) was applied to the sections for 8 min prior to the addition of the primary antibodies, which were left on overnight ADAMTS-1 monoclonal antibody 1:100, BAX monoclonal antibody 1:100, IL-10 monoclonal antibody 1:100. The sections were washed three times for 5 min in PBS and then were incubated with biotinylated secondary antibody (catalogue # TP-125-BN, Thermo Fisher Scientific, US) for 14 min. After washing with PBS, streptavidin peroxidase (catalogue # TS-125-HR, Thermo Fisher Scientific, US) was applied to the sections for 15 min. The sections were washed three times for 5 min in PBS. Diaminobenzidine (catalogue # TA-012-HDC, Thermo Fisher Scientific, US) was applied to sections for up to 20 min as a chromogen. Control slides were prepared using the same procedure, without primary antibodies. Counterstaining was done using Harris's haematoxylin for 45 s, dehydrated through

ascending alcohol and cleared in xylene (Product Number: HHS32 Sigma, hematoxylin solution, Harris Modified, Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO, 63103, USA). Slides were mounted with Entellan® (lot: 107961, Sigma-Aldrich, St. Louis, MO, USA) and examined under a light microscope (Olympus, Germany).

Statistical analysis. Age information was obtained from the patients in the study were evaluated statistically. AMH amount from blood samples taken from 30 patients (control 15 and experiment 15) and granulosa cell diameter and cell core diameter were measured in 20 cells from each collected cells. In addition, degeneration scoring and BAX, ADAMTS-1, IL-10 expressions were scored in granulosa cells. After the normal distribution test, independent t test was applied. P <0.05 was considered statistically significant.

RESULTS

Significant differences were observed in the morphometric parameters of granulosa cells in the experimental group compared to the control group (p <0.01). AMH in blood showed a significant decrease in the experimental group (Table I) (Figs. 1 and 2).

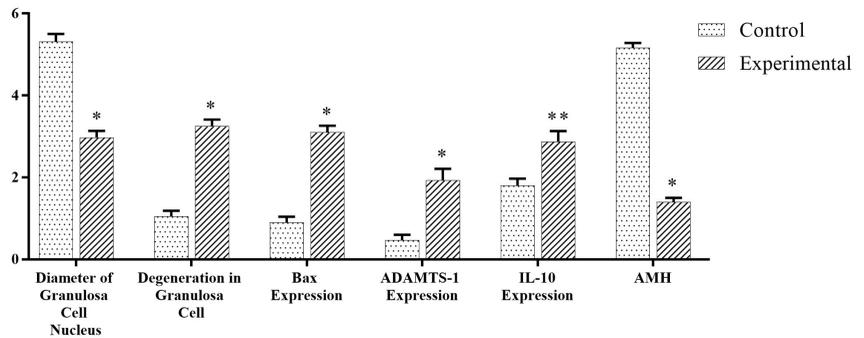


Fig. 1. Diagram of granulosa cell nucleus, degeneration in granulosa cell, Bax expression, ADAMTS-1 expression and AMH data independent t test analysis results graph. Different superscripts indicate significant difference between the control and experimental groups (*; p <0.01, **; p <0.05). Error bars indicate the standard error of mean values.

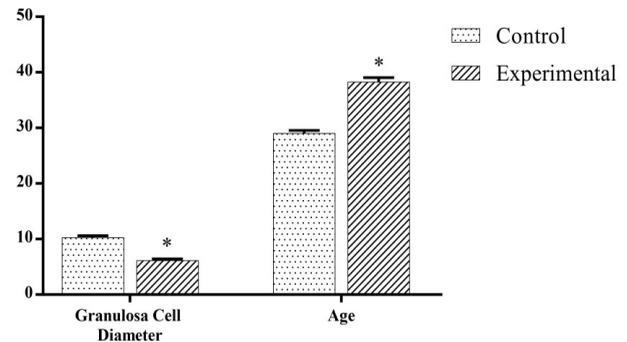


Fig. 2. Independent t test analysis result graph of granulosa cell diameter and age data. Different superscripts indicate significant difference between the control and experimental groups (*; p <0.01). Error bars indicate the standard error of mean values.

Table I. Statistical evaluation of parameters.

Parameter	Groups	N	Mean	Std. Error Mean	p value
Granulosa cell diameter	Control	20	10.29	0.30	<0.01
	Experimental	20	6.15	0.27	
Diameter of granulosa cell nucleus	Control	20	5.31	0.19	<0.01
	Experimental	20	2.98	0.16	
Degeneration in granulosa cell	Control	20	1.05	0.14	<0.01
	Experimental	20	3.25	0.16	
Bax expression	Control	20	0.90	0.14	<0.01
	Experimental	20	3.10	0.16	
AMH	Control	15	5.16	0.12	<0.01
	Experimental	15	1.40	0.10	
Age	Control	15	29.07	0.49	<0.01
	Experimental	15	38.33	0.73	
ADAMTS-1 expression	Control	15	0.47	0.13	<0.01
	Experimental	15	1.93	0.28	
IL-10 expression	Control	15	1.80	0.17	<0.05
	Experimental	15	2.87	0.26	

Histopathological observations. In the examination of histological sections taken from normal control group patients; the granulosa cells were found to be eosinophilic, with the chromosome-rich nucleus structures and the stoplasms of the polygonal cells where the nucleus settled out of the rounded cells. Cell size was found to be normal. In the samples taken from PCOS patients, there was a decrease in nucleus dimensions, changes in shape, small losses in stoplasmic areas and degeneration areas in granulosa cells. Deformations were observed with decrease in cell size (Figs. 3a and 3b).

Immunohistochemistry results. BAX expression was negative in stoplasma of granulosa cells and nuclei of the control group. Granulosa samples obtained from idiopathic patients showed a degerative changes in stoplasm of granulosa cells and chromatin particles in nucleus BAX expression (Figs. 4a and 4b). In the control group, ADAMTS-1 expression was observed in stoplasm of granulosa cells while ADAMTS-1 expression was negative in the nucleus. ADAMTS-1 expression levels were increased in the supranuclear region in idiopathic patients (Figs. 5a and 5b). IL-10 expression was moderate in granulosa cells in control group patients, whereas IL-10 expression was significantly increased in atypical flat granulosa cells in idiopathic patients (Figs. 6a and 6b).

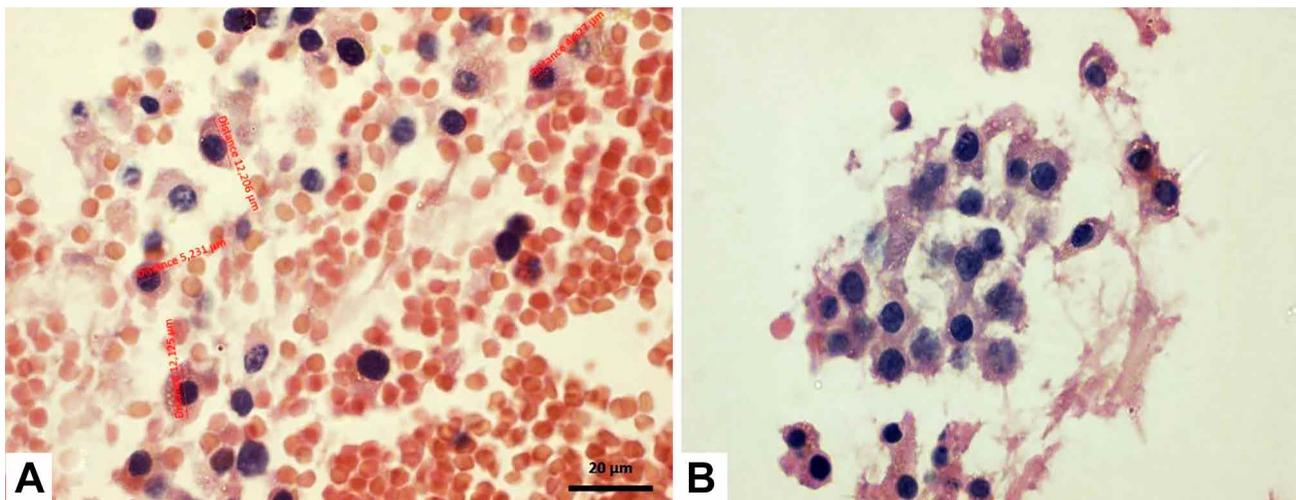


Fig. 3. A. In the examination of histological sections taken from normal control group patients; the granulosa cells were found to be eosinophilic, with the chromosome-rich nucleus structures and the stoplasms of the polygonal cells where the nucleus settled out of the rounded cells. Cell size was found to be normal. B. In the samples taken from PCOS patients, there was a decrease in nucleus dimensions, changes in shape, small losses in stoplasmic areas and degeneration areas in granulosa cells. Deformations were observed with decrease in cell size.

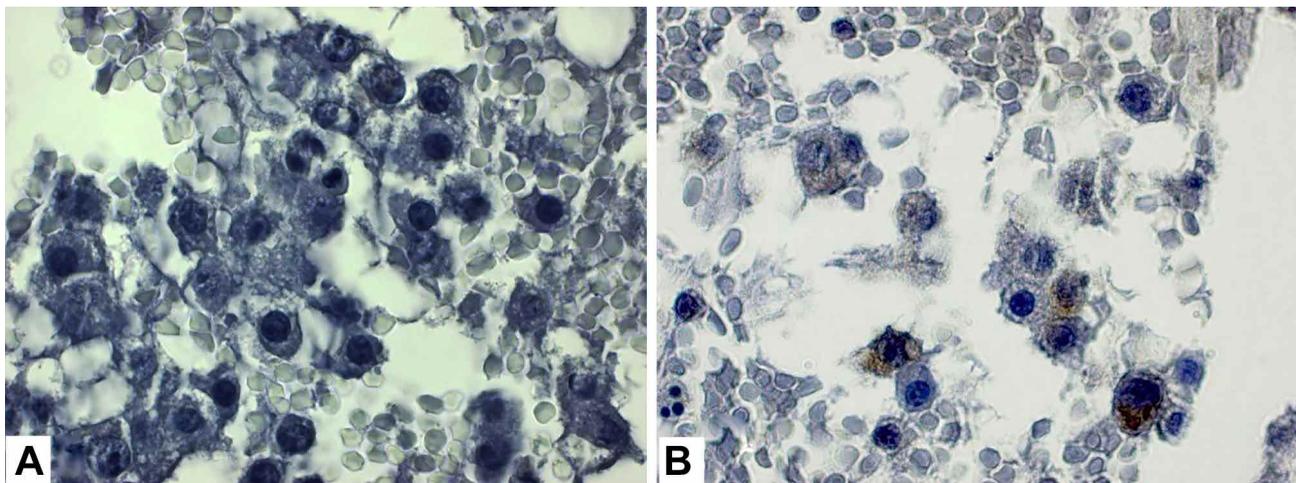


Fig. 4. A. BAX expression was negative in stoplasma of granulosa cells and nuclei of the control group. B. Granulosa samples obtained from idiopathic patients showed a degerative changes in stoplasm of granulosa cells and chromatin particles in nucleus BAX expression.

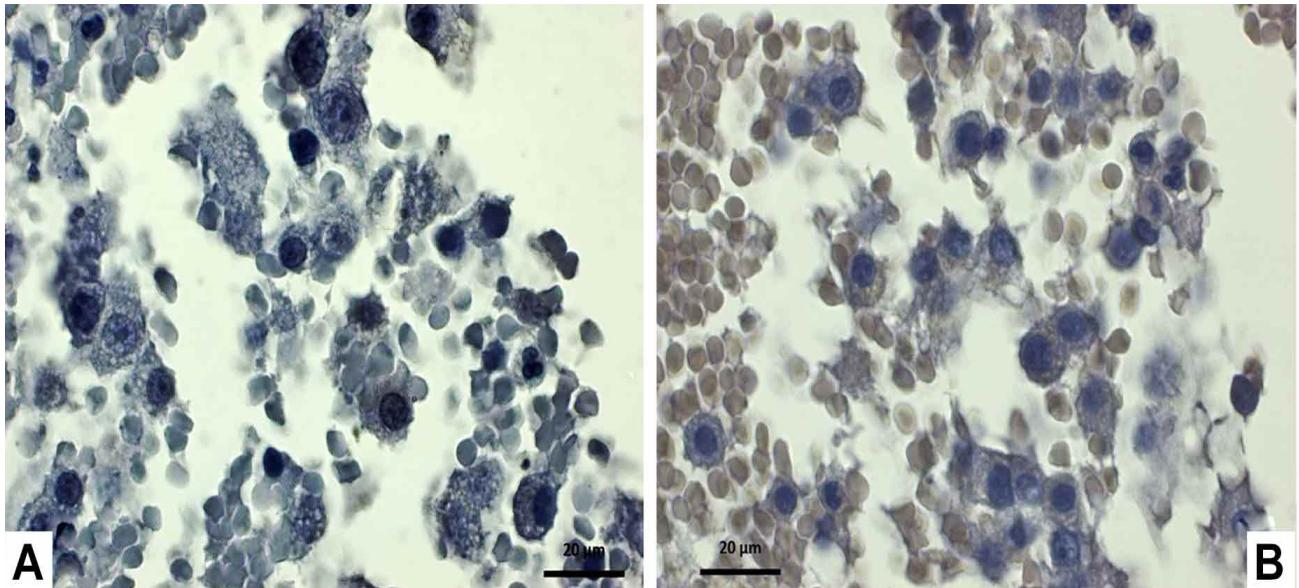


Fig. 5. A. In the control group, ADAMTS-1 expression was observed in stoplasm of granulosa cells while ADAMTS-1 expression was negative in the nucleus. B. ADAMTS-1 expression levels were increased in the supranuclear region in idiopathic patients.

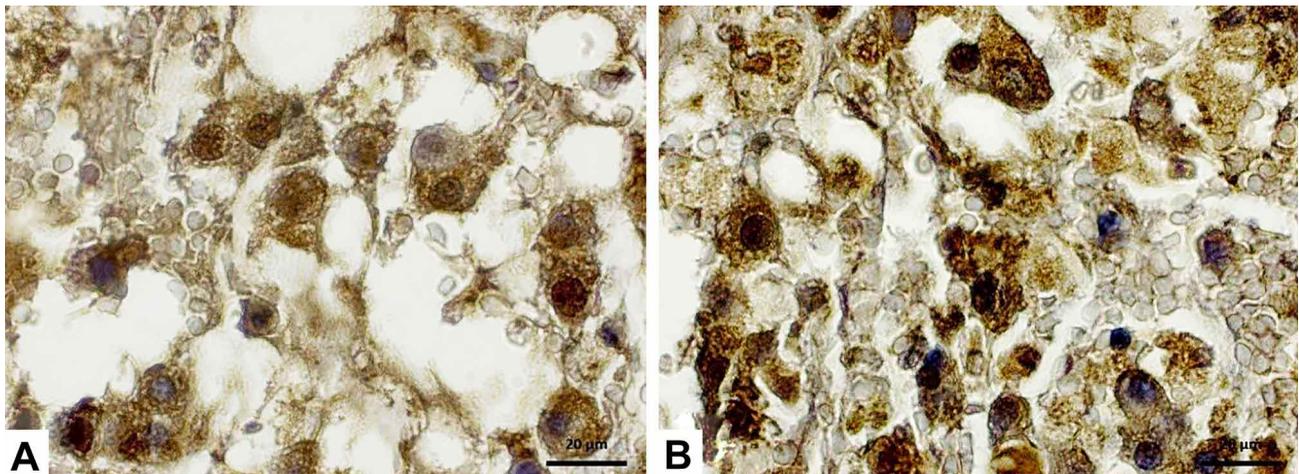


Fig. 6. A. IL-10 expression was moderate in granulosa cells in control group patients. B. IL-10 expression was significantly increased in atypical flat granulosa cells in idiopathic patients.

DISCUSSION

Ovulatory process is very similar to that of inflammation since inflammatory cytokines are produced, prostaglandins are induced, immune cells (leukocytes, neutrophils, and macrophages) are recruited (Care *et al.*, 2013). Oocyte develops in follicle fluid (FF) which may contain potential biomarkers for the development of follicle, fertilization of oocyte, development and quality of embryo, proper implantation and successful pregnancy (Revelli *et al.*, 2009). There is an important communication between granulosa cells and an oocyte in a developing follicle, and gene expression in maturing GCs is closely related to the production of

competent oocytes (Cillo *et al.*, 2007; Hamel *et al.*, 2008; Jiang *et al.*, 2010). GCs play a critical role in pathologic folliculogenesis not only in normal folliculogenesis but also in both benign disorders such as polycystic ovary syndrome (Yılmaz *et al.*, 2018), early ovarian failure (POF), also called early ovarian failure. Some authors have found that granulosa cell apoptosis is the primary factor leading to follicular atresia and affects oocyte structure and oocyte development potential (Han *et al.*, 2006; Elmore, 2007; Li *et al.*, 2009). The incidence of apoptotic bodies was considered as a predictive marker of assisted reproductive technology (ART) outcome.

It has been suggested that the emergence of apoptosis in the aspirated granulosa cells reflects the adequacy of the oocyte in pregnancy rates (Giampietro *et al.*, 2006). Oocytes with a low rate of apoptotic granulosa cells were observed to have normal morphology whereas high apoptotic cumulus granulosa cells were reported to have abnormal morphology (Assey *et al.*, 1994). Recent studies support the idea that an increase in apoptotic ratio of cumulus granulosa cells, a decrease in intracellular junction and loss of microvilli can cause low maturation and abnormal oocyte morphology and development so affect IVF or ICSI results. Moreover, studies on the issue also exhibited that egg maturation, fertilization, the quality of the corresponding embryos and also egg fertilization ability were closely related to the apoptosis of cumulus granulosa cells (Høst *et al.*, 2000; Raman *et al.*, 2001). Clavero *et al.* (2003) found that the rate of apoptosis in granulosa cells was not associated with the maturity of the oocyte and the ability for fertilization in ICSI or the quality of follicles during ovulation induction. In patients assisted with IVF, an increase in the number of apoptotic GCs isolated from follicular fluid pool was observed. Evidence from the previous studies suggested that ratio of apoptosis in GCs was increased in elderly IVF patients. This has been associated with the decreased oocyte quality, fertilization, pregnancy and live birth rates (Sifer *et al.*, 2002).

Lobach *et al.* (2019) reported that to show apoptosis in human preovulatory follicles, caspase-3 expression in aspirated GC cells can be used an indirect marker since it negatively affects follicular growth and oocyte yield. The same group also predicted for future research that to illustrate all mechanism of apoptosis in preovulatory follicles and explain the reason of high cell loss which leads to poor ovarian response. Different biomarkers should be examined and that was the starting point in our study to examine bax proapoptotic gene expression. A significant increase in BAX expression was observed in chromatin and degenerative cytoplasmic structures in granulosa cell nuclei obtained from idiopathic patients. Granulosa cells (GC) apoptosis may cause ovarian atresia and GC dysfunction may play a role in PCOS pathology. Increased increase in apoptotic granulosa cells will have a negative effect on embryo development with low oocyte quality and low oocyte quality.

Reported in studies, cytokines and inflammatory proteins regulate expansion of cumulus cell oocyte complex for successful ovulation. These events begin with changes in LH level and activation of downstream molecules (Richards *et al.*, 2015). Furthermore, infertility studies reveal that GCs increased expression of proinflammatory cytokines (Köks *et al.*, 2010). Adams *et al.* (2016) stated that there was a significant difference in the proinflammatory gene

expression between control and PCOS groups. Besides elevated GCs cytokine expression and increased number of immune cells, these results also showed that GCs interact with each other by cellular and paracrine ways in their in vivo periovulatory environment. Interleukins have been shown to play an important role in the evaluation of ovarian reserve function of intermittent hormone in immune diseases (Knauff *et al.*, 2009). Sun *et al.* (2018) reported that the higher the IL-6 and IL-21 levels, the lower the AMH level, the worse the ovarian reserve function. In our study, IL-10 expression was found to be very high in idiopathic patients and AMH value was found to be low compared to control (Table I).

An extracellular metalloprotease ADAMTS-1 we investigated here is induced by ovarian hormones in ovarian follicles and is required for fertility. The ADAMTS-1 protease domain mediates directions for cellular degradation and dissolution of the follicle wall, while the thrombospondin and disintegrin regions control their localization and the directions of inflammation, differentiation, and neovascularization, and thus have been reported to provide for the co-ordination of many follicle ruptures and the formation of the corpus luteum (Robker *et al.*, 2000). It has been shown that ADAMTS-1 is primarily secreted by wall granulosa cells, localized to the ECM of expanded cumulus oocyte complex, and induced significantly by LH in ovulating follicles (Russell *et al.*, 2003). It has a critical role in the expansion of ADAMTS-1 versican (a large extracellular matrix proteoglycan), expansion of COC formation, ovulation and angiogenesis. In ADAMTS-1 null mice, oocyte and ovarian vascular degeneration and a decrease in the organization of the ovarian network have been identified (Brown *et al.*, 2006). Sun *et al.* stated that ADAMTS-1 has reshaped the dynamic structure of versican catabolism required for subsequent degradation of the COC matrix in order to ensure successful fertilization of the ovulation-associated follicle wall and tissues. They also investigated ADAMTS-1 expression in granulosa cells of patients with polycystic ovary syndrome by immunocytochemistry and reverse transcription polymerase chain reaction. In this context, they showed a reduced expression of ADAMTS-1 in patients with PCOS compared to women laying eggs. In our study, ADAMTS-1 expression was moderate in the supranuclear layer of granulosa cells from idiopathic patient groups

Despite the results, there are a few limitations in clinical contribution. Apoptosis of granulosa cells help ovulation mechanisms by paracrine signaling and endocrine pathways, may disrupt the ovulation process in humans. It was thought that apoptosis induced by human GCs might be an indicator of egg quality. Moderate expression of

ADAMTS-1 was thought to be related to failure of ovulation, deterioration of oocyte quality and decreased fertilization rate. A reduction in AMH levels may be associated with defects in granulosa cells and correlated with increased granulocytes secreting significantly lower AMH than healthy people and increasing IL10 expression levels.

ÇAVUS, Y. & DEGER, U. Investigación de las expresiones ADAMTS-1, BAX e IL-10 en células de la granulosa determinando el éxito de fertilización en la FIV. *Int. J. Morphol.*, 38(2):427-434, 2020.

RESUMEN: Las células de la granulosa (GC) son componentes esenciales de los folículos y tienen un papel en la regulación del desarrollo de éste. El objetivo del estudio fue investigar ciertos componentes celulares involucrados en la proliferación, diferenciación y características funcionales de las células de la granulosa en el éxito de la fertilización de los ovocitos humanos durante la fertilización *in vitro* (FIV) a través de técnicas inmunohistoquímicas. En este estudio, se incluyeron 30 pacientes diagnosticados con infertilidad primaria o secundaria, síndrome de ovario poliquístico en el centro de FIV del Departamento de Obstetricia y Ginecología del Hospital Memorial. La cantidad de Hormona Anti Mülleriana (AMH) en la sangre, el diámetro de las células de la granulosa y el diámetro del núcleo celular se midieron en 20 células obtenidas de cada paciente. Además, se evaluó la puntuación de degeneración y las expresiones BAX, ADAMTS-1, IL-10 en células de granulosa ($p < 0,01$). Se estimó que la apoptosis inducida por los GC humanos podría ser un indicador de la calidad del huevo. Se estimó que la expresión moderada de ADAMTS-1 estaba relacionada con el fracaso de la ovulación, el deterioro de la calidad de los ovocitos y la disminución de la tasa de fertilización. La disminución en los niveles de AMH puede estar asociada con defectos en las células de la granulosa. Por lo tanto, el aumento de las células de granulocitos puede explicar la disminución significativa de la secreción de AMH y el aumento de los niveles de expresión de IL10 en personas sanas.

PALABRAS CLAVE: Células de Granulosa; Fertilidad; ADAMTS-1; IL-10, BAX; Inmunohistoquímica.

REFERENCES

Adams, J.; Liu, Z.; Ren, Y. A.; Wun, W. S.; Zhou, W.; Kenigsberg, S.; Librach, C.; Valdes, C.; Gibbons, W. & Richards, J. Enhanced Inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome. *J. Clin. Endocrinol. Metab.*, 101(9):3459-68, 2016.

Assey, R. J.; Hyttel, P.; Greve, T. & Purwantara, B. Oocyte morphology in dominant and subordinate follicles. *Mol. Reprod. Dev.*, 37(3):335-44, 1994.

Brown, H. M.; Dunning, K. R.; Robker, R. L.; Pritchard, M. & Russell, D. L. Requirement for ADAMTS-1 in extracellular matrix remodeling during ovarian folliculogenesis and lymphangiogenesis. *Dev. Biol.*, 300(2):699-709, 2006.

Büscher, U.; Chen, F. C.; Kentenich, H. & Schmiady, H. Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? *Hum. Reprod.*, 4(1):162-6, 1999.

Care, A. S.; Diener, K. R.; Jasper, M. J.; Brown, H. M.; Ingman, W. V. & Robertson, S. A. Macrophages regulate corpus luteum development during embryo implantation in mice. *J. Clin. Invest.*, 123(8):3472-87, 2013.

Cillo, F.; Brevini, T. A. L.; Antonini, S.; Paffoni, A.; Ragni, G. & Gandolfi, F. Association between human oocyte developmental competence and expression levels of some cumulus genes. *Reproduction*, 134(5):645-50, 2007.

Clavero, A.; Castilla, J. A.; Núñez, A. I.; García-Peña, M. L.; Maldonado, V.; Fontes, J.; Mendoza, N. & Martínez, L. Apoptosis in human granulosa cells after induction of ovulation in women participating in an intracytoplasmic sperm injection program. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 110(2):181-5, 2003.

Dzafic, E.; Stimpfel, M. & Virant-Klun, I. Plasticity of granulosa cells: on the crossroad of stemness and transdifferentiation potential. *J. Assist. Reprod. Genet.*, 30(10):1255-61, 2013.

Elmore, S. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.*, 35(4):495-516, 2007.

Fujiwara, H.; Maeda, M.; Honda, T.; Yamada, S.; Ueda, M.; Kanzaki, H.; Suginami, H. & Mori, T. Granulosa cells express integrin alpha 6: possible involvement of integrin alpha 6 in folliculogenesis. *Horm. Res.*, 46 Suppl. 1:24-30, 1996.

Giampietro, F.; Sancilio, S.; Tiboni, G. M.; Rana, R. A. & Di Pietro, R. Levels of apoptosis in human granulosa cells seem to be comparable after therapy with a gonadotropin-releasing hormone agonist or antagonist. *Fertil. Steril.*, 85(2):412-9, 2006.

Gómez, R.; Soares, S. R.; Busso, C.; Garcia-Velasco, J. A.; Simón, C. & Pellicer, A. Physiology and pathology of ovarian hyperstimulation syndrome. *Semin. Reprod. Med.*, 28(6):448-57, 2010.

Hamel, M.; Dufort, I.; Robert, C.; Gravel, C.; Leveille, M. C.; Leader, A. & Sirard, M. A. identification of differentially expressed markers in human follicular cells associated with competent oocytes. *Hum. Reprod.*, 23(5):1118-27, 2008.

Han, Z. B.; Lan, G. C.; Wu, Y. G.; Han, D.; Feng, W. G.; Wang, J. Z. & Tan, J. H. Interactive effects of granulosa cell apoptosis, follicle size, cumulus-oocyte complex morphology, and cumulus expansion on the developmental competence of goat oocytes: a study using the well-in-drop culture system. *Reproduction*, 132(5):749-58, 2006.

Høst, E.; Mikkelsen, A. L.; Lindenberg, S. & Smidt-Jensen, S. Apoptosis in human cumulus cells in relation to maturation stage and cleavage of the corresponding oocyte. *Acta. Obstet. Gynecol. Scand.*, 79(11):936-40, 2000.

Jiang, J. Y.; Xiong, H.; Cao, M.; Xia, X.; Sirard, M. A. & Tsang, B. K. Mural granulosa cell gene expression associated with oocyte developmental competence. *J. Ovarian Res.*, 3:6, 2010.

Knauff, E. A. H.; Eijkemans, M. J. C.; Lambalk, C. B.; ten Kate-Booij, M. J.; Hoek, A.; Beerendonk, C. C. M.; Laven, J. S. E.; Goverde, A. J.; Broekmans, F. J. M.; Themmen, A. P. N.; *et al.* Anti-mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. *J. Clin. Endocrinol. Metab.*, 94(3):786-92, 2009.

Köks, S.; Velthuis, A.; Sarapik, A.; Altmäe, S.; Reinmaa, E.; Schalkwyk, L. C.; Fernandes, C.; Lad, H. V.; Soomets, U.; Jaakma, U.; *et al.* The differential transcriptome and ontology profiles of floating and cumulus granulosa cells in stimulated human antral follicles. *Mol. Hum. Reprod.*, 16(4):229-40, 2010.

Kuno, K.; Iizasa, H.; Ohno, S. & Matsushima, K. The exon/intron organization and chromosomal mapping of the mouse ADAMTS-1 gene encoding an ADAM family protein with TSP motifs. *Genomics*, 46(3):466-71, 1997.

Li, H. J.; Liu, D. J.; Cang, M.; Wang, L. M.; Jin, M. Z.; Ma, Y. Z. & Shorgan, B. Early apoptosis is associated with improved developmental potential in bovine oocytes. *Anim. Reprod. Sci.*, 114(1-3):89-98, 2009.

- Lobach, V. N.; Casalechi, M.; Dela Cruz, C.; Pereira, M. T.; Del Puerto, H. L. & Reis, F. M. Caspase-3 gene expression in human luteinized granulosa cells is inversely correlated with the number of oocytes retrieved after controlled ovarian stimulation. *Hum. Fertil. (Camb.)*, 22(1):33-8, 2019.
- Nguyen, T.; Lee, S.; Hatzirodos, N.; Hummitzsch, K.; Sullivan, T. R.; Rodgers, R. J. & Irving-Rodgers, H. F. Spatial differences within the membrana granulosa in the expression of focimatrix and steroidogenic capacity. *Mol. Cell. Endocrinol.*, 363(1-2):62-73, 2012.
- Ouyang, W.; Rutz, S.; Crellin, N. K.; Valdez, P. A. & Hymowitz, S. G. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu. Rev. Immunol.*, 29:71-109, 2011.
- Pestka, S.; Krause, C. D.; Sarkar, D.; Walter, M. R.; Shi, Y. & Fisher, P. B. Interleukin-10 and related cytokines and receptors. *Annu. Rev. Immunol.*, 22:929-79, 2004.
- Raman, R. S.; Chan, P. J.; Corselli, J. U.; Patton, W. C.; Jacobson, J. D.; Chan, S. R. & King, A. Comet assay of cumulus cell DNA status and the relationship to oocyte fertilization via intracytoplasmic sperm injection. *Hum. Reprod.*, 16(5):831-5, 2001.
- Revelli, A.; Delle Piane, L.; Casano, S.; Molinari, E.; Massobrio, M. & Rinaudo, P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod. Biol. Endocrinol.*, 7:40, 2009.
- Richards, J. S. Ovulation: new factors that prepare the oocyte for fertilization. *Mol. Cell. Endocrinol.*, 234(1-2):75-9, 2005.
- Richards, J. S.; Hernandez-Gonzalez, I.; Gonzalez-Robayna, I.; Teuling, E.; Lo, Y.; Boerboom, D.; Falender, A. E.; Doyle, K. H.; LeBaron, R. G.; Thompson, V.; *et al.* Regulated expression of ADAMTS family members in follicles and cumulus oocyte complexes: evidence for specific and redundant patterns during ovulation. *Biol. Reprod.*, 72(5):1241-55, 2005.
- Richards, J. S.; Liu, Z. & Shimada, M. Ovulation. In: Plant, T. & Zeleznik, A. J. (Eds.). *Knobil and Neill's Physiology of Reproduction*. 4th ed. Boston, Elsevier, 2015.
- Robker, R. L.; Russell, D. L.; Espey, L. L.; Lydon, J. P.; O'Malley, B. W. & Richards, J. S. Progesterone-regulated genes in the ovulation process: ADAMTS-1 and cathepsin L proteases. *Proc. Natl. Acad. Sci. U. S. A.*, 97(9):4689-94, 2000.
- Russell, D. L.; Doyle, K. M. H.; Ochsner, S. A.; Sandy, J. D. & Richards, J. S. Processing and localization of ADAMTS-1 and proteolytic cleavage of Versican during cumulus matrix expansion and ovulation. *J. Biol. Chem.*, 278(43):42330-9, 2003.
- Saraiva, M. & O'Garra, A. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.*, 10(3):170-81, 2010.
- Sifer, C.; Bénifla, J. L.; Bringuier, A. F.; Porcher, R.; Blanc-Layrac, G.; Madélenat, P. & Feldmann, G. Could induced apoptosis of human granulosa cells predict in vitro fertilization-embryo transfer outcome? A preliminary study of 25 women. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 103(2):150-3, 2002.
- Su, Y. Q.; Sugiura, K. & Eppig, J. J. Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Semin. Reprod. Med.*, 27(1):32-42, 2009.
- Sun, S.; Chen, H.; Zheng, X.; Ma, C. & Yue, R. Analysis on the level of IL-6, IL-21, AMH in patients with auto-immunity premature ovarian failure and study of correlation. *Exp. Ther. Med.*, 16(4):3395-8, 2018.
- Varras, M. The expression of genes in human ovarian granulosa cells during assisted reproduction technologies and their clinical significance. *Androl. Gynecol. Curr. Res.*, 1(1):1000e102, 2013.
- Yilmaz, B.; Vellanki, P.; Ata, B. & Yildiz, B. O. Metabolic syndrome, hypertension, and hyperlipidemia in mothers, fathers, sisters, and brothers of women with polycystic ovary syndrome: a systematic review and meta-analysis. *Fertil. Steril.*, 109(2):356-364.e32, 2018.

Corresponding author:

Yunus Çavus,
Department of Obstetrics and Gynecology at
Memorial Hospital
Diyarbakır
TURKEY

Email:ycavus1212@gmail.com

Received: 02-08-2019

Accepted: 17-09-2019