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Research Article

Comparıson of cumulus cells and follıcular fluıd obtaıned from ınfertile ındıvıduals dıagnosed wıth polycystıc ovary syndrome (PCOS) and endomethrıosıs wıth samples obtaıned from healthy ındıvıduals

Polikistik over sendromu (PCOS) ve endometriozis tanili infertil bireylerden elde edilen kumulus hücreleri ve foliküler sivinin sağlikli bireylerden elde edilen örneklerle karşilaştirilmasi

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Abstract

Aim: Investigating the relationship between Growth differentiation factor-9 (GDF-9), Bone morphogenetic protein-15 (BMP-15) markers, apoptosis levels in cumulus cells and total oxidant (TOS)/ anti-oxidant (TAS) stress levels, inflammation parameters (interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha)) in follicular fluid belonging to patients with polycystic ovary syndrome (PCOS), endometriosis (END) and male factor (MF) (control) groups.

Material and Methods: GDF-9 and BMP-15 markers are determined by immunohistochemical methods, apoptosis levels are studied with TUNEL. TOS and TAS statuses are investigated with spectrophotometry, IL-6 and TNF – alpha levels are examined by Enzyme-Linked Immuno Sorbent Assay (ELISA).

Results: According to the data obtained in the study; GDF-9 and BMP-15 levels are found to be lower in PCOS and END groups and apoptosis levels of cumulus cells were significantly higher at these groups . TOS levels were significantly higher in PCOS and END groups whereas follicular fluid TAS levels were not statistically significant for these groups. IL-6 and TNF – alpha levels of follicular fluid was significantly higher in PCOS. These parameters were also higher for END group, however the difference was not found to be significant.

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Conclusion: Our results imply that correlation between GDF-9, BMP-15 markers, apotosis levels, oxidative status, inflammation levels may be interpreted with improper environment for oocyte maturation for patients diagnosed with PCOS or END.

The need for further studies on subject proceeds. However, if similar datas are obtained in further studies, it is thought that evaluation of cumulus cell properties together with especially follicular fluid oxidative stress levels will contribute to the selection of the best oocyte.

Keywords: cumulus cell, follicular fluid, polycystic ovary syndrome, endometriosis, oxidative stress

Öz

Amaç: Polikistik over sendromu (PKOS), endometriozis (END) ve erkek faktör (MF) (kontrol) gruplarında bulunan hastalara ait kumulus hücrelerinde Büyüme farklılaşma faktörü-9 (GDF-9), Kemik morfogenetik protein-15 (BMP-15) belirteçleri, apoptoz seviyeleri ile foliküler sıvı inflamasyon parametreleri (interlökin-6 (IL-6), tümör nekroz faktör alfa (TNF-alfa), total oksidan (TOS)/anti-oksidan (TAS) stres seviyeleri arasındaki ilişkinin araştırılması.

Gereç ve Yöntemler: Kumulus hücrelerinde büyüme farklılaşma faktörü-9 (GDF-9) ve kemik morfogenetik protein-15 (BMP-15) belirteçleri immünohistokimyasal yolla değerlendirilmiş olup; hücre ölümü TUNEL yöntemi kullanılarak araştırılmıştır. Folikül sıvısı örneklerinde toplam oksidatif stres (TOS) ve toplam anti-oksidan düzey (TAS) spektrofotometrik olarak araştırılmış, interlökin-6 (IL-6) ve tümör nekrozis faktör alfa (TNF-alfa) düzeyleri ELISA (Enzyme-Linked ImmunoSorbent Assay) yöntemi ile incelenmiştir

Bulgular: GDF-9 ve BMP-15 düzeyleri sağlıklı gruba kıyasla PCOS ve END gruplarında düşük seviyede saptanırken, hücre ölümüne ilişkin veriler bu gruplarda daha yüksek gözlenmiştir. Endometriozis grubunda GDF-9, BMP-15 değerleri en düşük, hücre ölümü düzeyleri ise en yüksek olarak bulunmuştur. Sağlıklı gruba kıyasla PCOS ve endometriozis gruplarında folikül sıvısı TOS düzeyleri istatistiksel olarak anlamlı yüksek bulunmuştur. Folikül sıvısı TAS düzeyleri ise sağlıklı gruba kıyasla PCOS ve endometriozis gruplarında daha yüksek bulunmuş ancak gruplar arasındaki fark istatistiksel olarak anlamlı bulunmamıştır.

Sonuç: Sonuçlarımız, GDF-9, BMP-15 belirteçleri, apotoz seviyeleri, oksidatif durum, inflamasyon seviyeleri arasındaki korelasyonun PCOS veya END tanılı hastalarda oosit olgunlaşması için uygun olmayan mikroçevre ile yorumlanabileceğini düşündürmektedir.

Konu ile ilgili ileri çalışmalara ihtiyaç devam etmektedir. İleri çalışmalarda benzer verilerin elde edilmesi halinde kumulus hücre özelliklerinin, özellikle foliküler sıvı oksidatif stres düzeyleri ile birlikte değerlendirilmesinin, oosit seçimine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: kumulus hücresi, folikül sıvısı, polikistik over sendromu, endometriozis, oksidatif stres

Introduction

It is declared that approximately %17 of couples encounter with a failure to achieve a clinical pregnancy, after 12 months or more of regular unprotected sexual intercourse. Many factors are blamed to cause infertility but almost %50 of these are based on factors concerning female reproductive tract (1). Besides being an economic burden infertility has a big role as psychological effect. It is declared that desire to have a child can convert a depression inducement after being diagnosed as infertile is common in woman than man (2).

Under favour of all developments in intracytoplasmic sperm injection (ICSI) technique, a significant success has been gained. This improvement is accelerated by follicular monitoring, identification of top-quality embryos, embryo transfer and controlled ovarian hyperstimulation (COHS), procedures. Despite of all these progressions, still inefficiency at successful pregnancy outcome is seen at some of patients which had consulted assisted reproductive techniques (ART) (3). Undoubtfully, picking up the best quality oocyte has a significant importance in this area (3, 4).

Polycystic ovary syndrome (PCOS) effects almost %5-10 of women at reproductive age. It can be said that it is a common and complex disorder which provides a poor oocyte quality (5). Many authors defines PCOS as the most common endocrinological disorder in women at reproductive ages (6). Infertility is also seen at %40 of patients who are diognosed with PCOS (5, 7). As it is declared in literature parameters like oxidative stress, chronic inflammation, oocyte quality takes a significant importance in the success of ART techniques for PCOS patients (8, 9)

Endometriosis can be defined as the existance of endometrial tissue outside the uterine cavity. It is known that this chronic inflammatory condition affects approximately 6-10% of women of reproductive age worldwide. Although there are many different opinions about the etiology of the disease, it has been shared in the literature that the increase in inflammation and oxidative stress levels can have a big role in the pathogenesis (10-13).

Oocytes which are released with ovulation are surrounded closely with cumulus cells. It is known that bi-directional relationship between cumulus cells and oocyte has a significant importance on oocyte maturation. These cells provide the network which supplies the proper microenvironment for oocyte. The connection between oocyte and cumulus cells is bidirectional and this connection ensures capable oocyte maturation (14). Oocyte development is known to be essential for fertilization and embryo development processes. This maturation duration includes important steps such as nucleer maturation or changes of ooplasm (15). It has been demonstrated that the network between cumulus cells and oocyte is essential for competant oocyte maturation. Many studies have shown that molecules secreted from oocyte can be effective in many processes from ovulation to embryo development as a result of paracrine and autocrine interactions (16-18). Among these factors, especially GDF-9 and BMP-15, members of the TGF-β family, are of great importance (19). TGF-β is the largest family of extracellular protein groups found in mammals (20). These signal molecules which are secreted by the oocyte, provides a bidirectional connection with somatic cells which also plays a role in many critical earlier stages of follicle development, such as migration of germ cells (21-23).

In many studies number of apopitotic cumulus cells were interpreted with poor oocyte maturation. Pocar et al showed that cumulus oocyte complex (COC) cultured with enviromental toxin (polychlorinated biphenyls) caused an increase at number of apopitotic cumulus cells in company with poor maturated oocytes (24). It was demonstrated that attendance of cumulus cells can even modulate transcription and genomic remodelling of oocytes in mouse (25). Traditionally, apopitotic biomarkers of cumulus cells effect oocyte quality and clinic outcomes. In conclusion, apopitotic status of cumulus cell level is commonly interpreted with oocyte maturation, fertilization, healthy pregnancy outcomes in previos studies.(15, 18, 26).

Follicular fluid which is secreted by granulosa cells supplies the appropriate metabolites which are neccesary for oocyte maturation process. Oocyte quality is known to be essential for ART and this environment which houses this maturation directs the fate of follicular development process. Because of that the properties of this fluid has a profound effect at reproductive functions. It is also declared that the data gained from follicular fluid is important to determine the staus of follicle (5, 27). Oocyte cumulus complex maturates in the environment provided by follicular fluid, and oxidative stres levels in this area has an impulse on oocyte quality and clinic values like pregnancy outcomes, healthy placentation, implantation, embryological development (27). Oxidative stress which reflects an overbalance of reactive oxygen species (ROS) in contrast with antioxidant defense systems is a key factor that effects reproductive system. In many articles it is declared that this imbalance between oxidant and ant-oxidant parameters has a non-negligible effect on fertility (28). Compatibly anti –oxidant nutrient supplementation caused a decrease at oxidative status of follicular fluid besides an increase of number of good quality oocytes (29). These data is competibe with previous studies which have blamed oxidative stress as a significant agent for infertility (1, 27, 30).

In addition to oxidative stress levels of follicular fluid, inflammatory markers of this environment are also known to be effective in folliculogenesis and oocyte maturation. The impact of increased inflammation in many diseases of the female reproductive system has been shared. In accordance with this information, inflammatory marker levels with increased follicular fluid have been investigated in important diseases affecting female reproductive health such as PCOS(8) and END(10).

This research is a prospective study which aimed to investigate the relationship of GDF-9, BMP-15 markers and apopitotic status of cumulus cells with TAS/TOS levels and enflammatory status in folicular fluid in patients at PCOS, END or MF groups.

Material and Methods

Patient population

This prospective study was approved by the ethical committee of Gazi University Faculty of Medicine and included 30 patients (10 patients at each group) between March 2018 and December 2018. Two independent and blinded researchers had run experiments and analyzed the data. PCOS, END and MF patients with consent were recruited.

Immunohistochemical assay

Cumulus cells belonging to the groups taken by spreading method on slide were first kept in xylol and then rehydrated by passing through decreasing alcohol series. The cells were washed with PBS (Phosphate Buffer Saline, pH: 7.4). Samples applied for 10 minutes serum blocking solution (Cat: 54-003, Lot: 40522067, Acusine mause + rabbit HRP kit, Genemed Biotechnologies, USA) to prevent non-specific binding. Samples with primary antibodies GDF-9 (Cat: ab15640, Lot: 962605, Santa Cruz Biotechnology Inc., Europe) and anti-BMP-15 (Cat: ab198226, Lot: GR211311-2, Abcam) at 4 °C overnight incubated. Samples washed with PBS afterwards were applied with 3% hydrogen peroxide solution. After washing with PBS, biotin secondary antibody (Cat: 54-003, Lot: 40522067, Acusine mause + rabbit HRP kit, Genemed Biotechnologies, USA) were applied to the samples. Again, samples were washed with PBS, and chromogen (Cat: DABS-125, Lot: HD25395, Thermo Fisher Sci., CA) containing diaminobenzedin substrate was applied until a visible immune reaction occurred. Mayer's Hematoxylin was used as the Ground stain. Samples were dehydrated by passing through the alcohol series were kept in xylol and then covered with entellan. All samples were evaluated by taking pictures in Leica QVin3 program with the help of Leica DM4000 (Germany) computer aided imaging system. The presence of GDF-9 and BMP-15 were evaluated in the cell counts provided in 5 independent areas at 400X magnification selected for each slide.

TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method was used to assess DNA fragmentation in cumulus cells with ApopTag Aoptosis kit (Millipore). Cumulus cells belonging to the groups taken by spreading method on the slide were incubated with 20 µg/ml proteinase K (Roche Diagnostics, GmbH) for 15 minutes at room temperature. Then, cells were incubated with 3% hydrogen peroxide (LabVision, Fremont, USA) for the inhibition of endogenous peroxidase activity, in a humid environment for 5 min at room temperature. Equilibration buffer was applied for 5 min at room temperature. After the excess liquid was aspirated, the slides were incubated in TdT enzyme solution for 1 hour at 37 ° C in a humidity chamber. The slides were incubated at room temperature in the stop/wash buffer for 10 min, then slides were incubated in anti-digoxigenin peroxidase solution at room temperature for 30 min in a humidity chamber. Subsequent staining with diaminobenzidine (DAB) was used to determine TUNEL-positive cells. Methyl green was used for background. Slides were evaluated under a light microscope using a computer-supported imaging system, and the pictures were taken by using the Leica QVin3 programme.

Spechtrophotometry procedure

TAS (REL Assay Diagnostics, LOT: ST18083A) and TOS (REL Assay Diagnostics, LOT: AK17092O) levels were measured colorimetrically using a commercial kit. Throughout the experiment, the application was carried out as stated in the kit contents. Absorbance was determined using SHIMADZU UV-1601 spectrophotometer.

ELISA procedure

IL-6 (Cat. No. E0090Hu, Lot: E201904017) and TNF-alpha (Cat. No. E0082Hu, Lot: E201903008) levels were determined by ELISA method by following kit protocols.

Biotech ELISA reader was used for analysis of IL-6 (ng / L) and TNF-a (ng / L).

Statistical analysis

The consistency of continuous variables to normal distribution was examined graphically and by Shapiro-Wilk test. Mean ± standard deviation to define the number of viable cells that provide normal distribution condition, brightness and sample volume; For other variables that do not meet the normal distribution condition, the median (width between quarters) value was used.

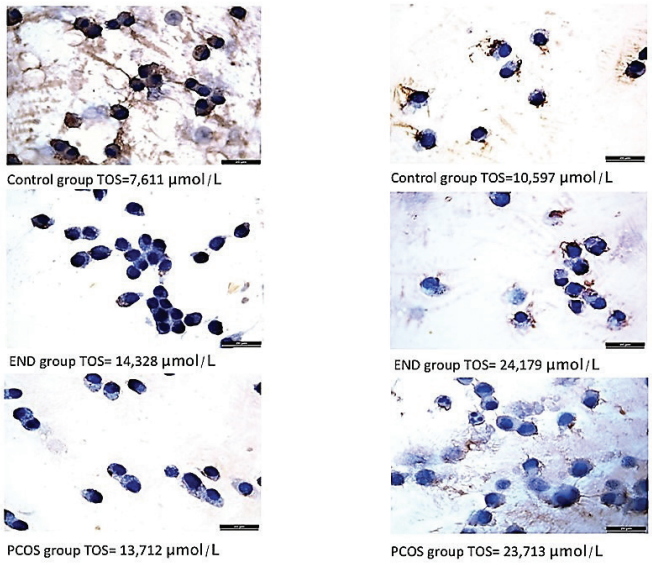
Student's t test was used to compare the variables that provide parametric test assumptions, and one-way analysis of variance (ANOVA) was used to compare sample volume by treatment groups. When a difference was found as a result of ANOVA, the source of the difference was investigated with Bonferroni post-hoc test.

Mann-Whitney test was used to compare distorted data (nonparametric) (variables). Kruskal-Wallis nonparametric for binary comparison of distorted data variance analysis was used. Post-hoc binary comparisons were made with the Bonferroni-corrected Mann-Whitney test to identify the different group when a difference was found.

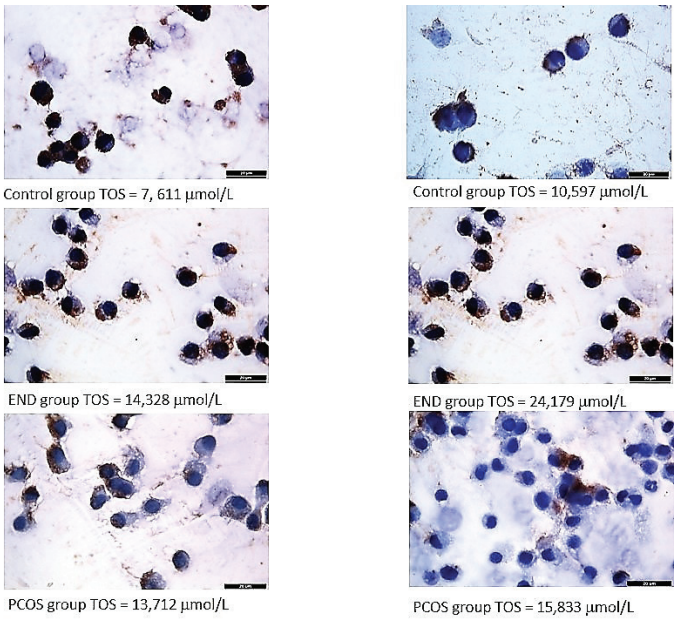
Results

GDF-9, BMP-15 markers

Both GDF-9 and BMP-15 markers were detected at lowest level for END group and at highest levels for MF group (Picture 1,2). The difference between PCOS-MF groups and END-MF groups was found to be statistically significant for both GDF-9 and BMP-15 ($p < 0,05$ (ANOVA)). Besides that difference of GDF-9 between the PCOS and END groups was also found to be significantly different ($p= 0,004$ (t test)). None the less BMP-15 levels were not evaluated with a significant difference between PCOS-END groups (Figure 1).



Picture 1. GDF-9 detection for Control, PCOS, END groups with TOS values



Picture 2. BMP-15 detection for Control, PCOS, END groups with TOS values

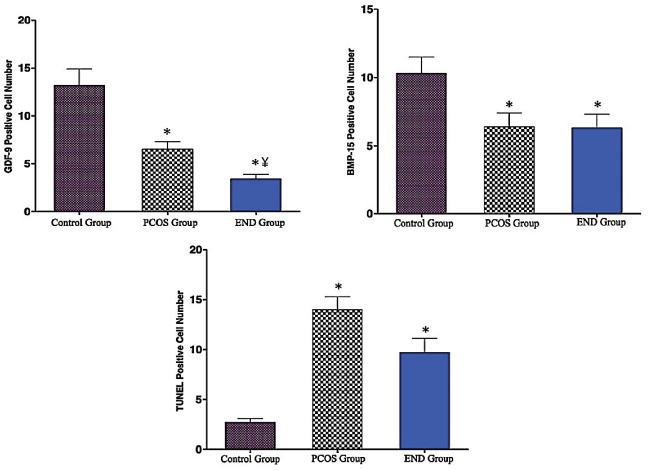


Figure 1. Comparing, PCOS and END groups in terms of GDF-9, BMP-15 markers and TUNEL positivity (* = different from Control group (Control - PCOS / Control - END: $p < 0.05$))

In all groups, both GDF-9 and BMP-15 markers levels were determined in relation to the TOS grades. The individuals at all gruops were sort ascended according to the TOS values in each group. Due to TOS values alignment each group had a subgroup with lower (5 patients) and higher TOS values (5 patients) in itself. Lower levels of GDF-9 and BMP-15 positivity were detected in cumulus cells from patients with higher TOS levels at follicle fluids. This relationship was found to be statisticallly significant (Figure 2).

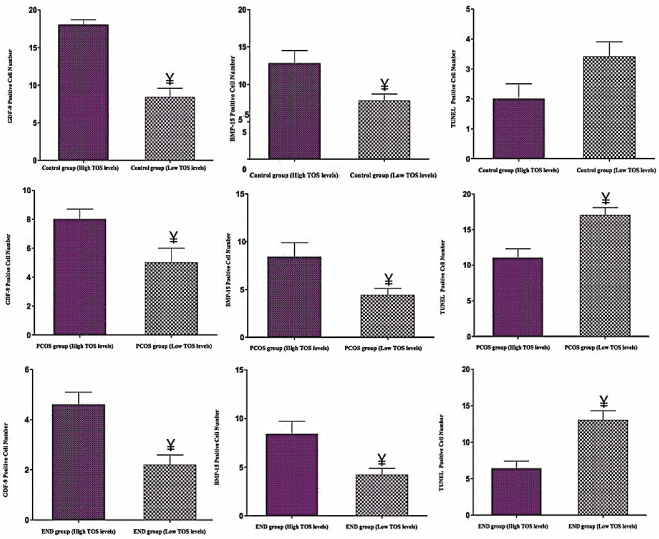
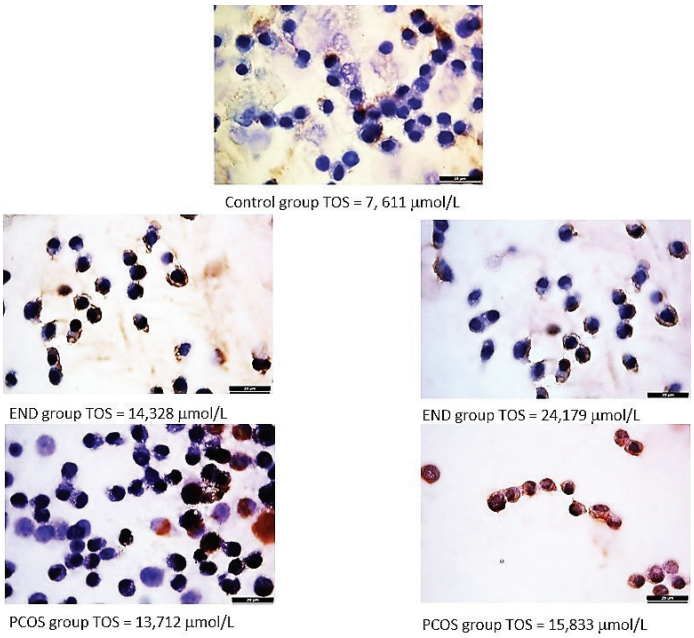


Figure 2. The relationship between GDF-9, BMP-15 markers and TUNEL positivity rates of cumulus cells and follicular fluid TOS values (¥: statistically different from control group, p<o.o5)

TUNEL results

TUNEL positive cumulus cells were found to be highest for

PCOS gruop and lowest for MF group (Picture 3). There was no statistically significant difference for TUNEL positivity between PCOS and END groups. However, a statistically significant difference was detected between both PCOS-MF and END-MF groups in terms of TUNEL positivity. (Figure 1) . Cumulus cells at PCOS and END groups showed, higher and statistically significant TUNEL positivity for patients with higher TOS levels (Figure 2).



Picture 3. TUNEL positivity for Control, PCOS, END groups with TOS values

TAS/TOS values

TAS results were obtained as 1.221 ± 0.427 for MF group, 1.446 ± 0.408 for PCOS group, and 1.282 ± 0.230 for endometriosis group. When the obtained results were evaluated statistically, no significant difference was found between MF – PCOS groups (p = 0.2277), MF – END groups (p= 0.655), and PCOS - END groups (p = 0.225). TOS results were obtained as 10,632 ± 2,150 for MF group, 19,942 ± 3,060 for PCOS group, and 22,527 ± 3,410 for END group (Table 1). TOS results showed a significant differance between MF - PCOS (p = 0.002), and MF – END (p = 0.002). However, there was no statistically significant difference between PCOS – END groups (p = 0.225) (Table 1).

Table 1. TAS and TOS values for groups (* = different from the control group, p = 0.002)

Group name	TAS (mmol/L)	TOS (µmol/L)
MF	1.078±0.295	10.625±1.351
PCOS	1.686±0.322	11.111±1.547*
END	1.236±0.399	14.120±0.439*

IL-6/TNF-alpha values

The IL-6 findings were determined as 18.09 ± 7.19 for the MF group, 51.40 ± 43.32 for the PCOS group and 34.64 ± 38.11 for the END group. When the obtained results were evaluated statistically, a significant difference was found between the MF -PCOS groups (p = 0.012). In contrast with this finding, no significant difference was found between the MF – END (p = 0.074), as well as the PCOS - END (p = 0.246) groups for IL-6 results.

TNF-α results were obtained as 19.34 ± 23.65 for the MF group, 61.46 ± 52.13 for the PCOS group, and 28.34 ± 39.15 for the END group. When the TNF-α results of the PCOS group were compared with the MF group, a statistically significant difference was observed (p = 0.046). However, there was no significant difference between the MF – END (p = 0.753) and PCOS – END (p = 0.059) groups (Table 2).

Table 2. IL-6 and TNF-α values belonging for groups (* = different from control group p = 0.012, ¥ = different from control group p = 0.046)

Group name	IL-6 (ng/L)	TNF-α (ng/L)
MF	18,09 ± 7,19	19,34 ± 23,65
PCOS	51,40 ± 43,32*	61,46 ± 52,13 ¥
END	34,64± 38,11	28,34 ± 39,15

Discussion

PCOS Group

The amount of GDF-9 in cumulus cells obtained from individuals diagnosed with PCOS were decreased compared to the cumulus cells belonging to MF group. This result was consistent with the research data discussed when compared with similar studies in the current literature, which were tend to conclude that GDF-9 affects oocyte maturation processes (31-36).

A reduction was observed for the amount of BMP-15 in cumulus cells obtained from PCOS group compared to the cumulus cells belonging to MF group. This data on BMP-15 levels is consistent with the literatüre (31, 32, 35-37). The synergistic relationship between GDF-9 and BMP-15 has been presented in the literature. As a result of the decrease detected in both marker levels, it can be concluded these components may have an effect on oocyte quality. (16, 31, 38).

ZHAO et al shared that cumulus cells from individuals diagnosed with PCOS showed a decrease in the amount of GDF-9, while a significant difference was not observed in BMP-15 levels compared to the control group. This decrease in GDF-9 levels has been associated with premature lutinization and increased risk of luteal dysfunction, which can be seen in patients diagnosed

with PCOS. It was also emphasized in the study that GDF-9 and BMP-15 can be supplemented in order to support ovulation if the deficiency of paracrine factors is detected (33)

Karagül et al. found a decrease in GDF-9 and BMP-15 levels in patients diagnosed with PCOS and reported that this decline was associated with follicle development, zona pelusida maturation, as well as subfertility and infertility that can be observed in these individuals (32). Vireque et al. declared that the increase in GDF-9 and BMP-15 transcription levels in mature oocyte samples obtained from volunteers diagnosed with PCOS was associated with follicle fluid androgen levels. With same study it was shared that GDF-9 and BMP-15 levels affected progesterone release from follicular cells, preventing early luteinization in cumulus cells (39).

In our research results, increased apoptosis levels were obtained in cumulus cells belonging to PCOS patients are compatible with many data presented(40-42). Song et al declared that insulin levels in patients with PCOS were compatible with the apopitosis levels of the cumulus cells (41). Likewise, Ding et al detected high levels of apoptosis in patients belonging to the PCOS group compared to the control group. Obesity and insulin resistance parameters of these patients were also evaluated in relation to granulose cell death(42). Due to obtained resulst of our study it was hypothesied that increase in the insulin levels, obesity or insülin resistance of the patients diagnosed with PCOS may have an affect on the female fertility by showing a synergistic effect with the follicular fluid TOS level and the apoptosis levels of the cumulus cells.

With the results of the study, follicular fluid TOS values were significantly higher in the PCOS group compared to the control group. This result is congruous with previos datas in the literatüre (5, 8, 9, 27) The obtained result was interpreted to show the effect of total oxidative capacity on oocyte maturation.

It is known that the quality of the follicle fluid exerts an influence on even the relationship of oocyte with sperm and implantation. Therefore, follicle fluid is considered to affect even embryonic development levels closely (5, 27). Our results concluded that, lower levels of GDF-9 and BMP-15 and higher numbers of TUNEL (+) cumulus cells were observed belonging to patients with high TOS levels in follicular fluid for both PCOS and END groups. These data pointed out that it GDF-9 and BMP-15 molecules synthesized by the oocyte, and the TUNEL positivity of cumulus cells may have an impact in subfertility or infertility cases for patients with PCOS or END with the reciprocal cooperation of oxidative stress levels at folicular fluid. This impact may be efficient at a wide range from fertilization to many processes of embryonic development. These results brought to mind the need to elaborate the relationship between follicular fluid oxidative stress levels and oocyte markers. However, TAS values did not differ significantly in the PCOS group compared to the control group. This was associated with the need for further research with larger patients.

Another parameter associated with insulin resistance in individuals with PCOS is increased inflammation. We had significantly higher results of IL-6 and TNF-alpha at folicular fluid of the patients diagnosed with PCOS. It has been reported that certain levels of cytokines known to have an effect in the inflammatory process, such as IL-6, TNF-a, were found to be higher in follicular fluid samples of PCOS patients compared to healthy individuals (8). It was stated by Artimani et al's study that increased inflammation may have an effect on insulin resistance as well as impaired oxidative stress balance (8). In the light of these data, it could be suggested that increased inflammation should also be considered in the clinical approach if insulin resistance is detected unbalenced in patients diagnosed with PCOS. Besides this vision, the need for studies with larger number of patients, including different inflammatory markers continues.

End Group

With the study results, lower rates of GDF-9 and BMP-15 data were obtained in the cumulus cell samples belonging to individuals in the END group compared to the MF group and this result is compatible with several previous datas. The mentioned parameters have been associated with many steps that affect fertility, such as oocyte quality, implantation, pregnancy rates, or even embryo development (17, 18). Considering the synergistic relationship between both factors, these results seem to be consistent with the dynamics of the relationship between the cumulus cell and oocyte (16, 31, 38). With the study conducted by Kawabe et al., it has been reported that progesterone and estrogen levels, which have a large place in the endometriosis clinic, changed in accordance with the amount of GDF-9 (17). In the light of the cumulus cell data obtained from our research results, it can be thought that the decrease in the GDF-9 synthesis plays a more effective role in the pathogenesis of END compared to the pathogenesis of PCOS and ultimately leads to endocrinological disorders in the clinic of END.

High apoptosis levels observed in the cumulus cells belonging to individuals in the END group are compatible with the

literature (27, 43, 44, 45). With the results of the study, it was thought that cell death due to increased oxidative stress may be effective in endometriosis pathology by observing higher apoptosis in the cumulus cell samples of individuals with high TOS levels at folliculer fluid. With certain data presented to the literature, it supports the effect of increased oxidative stress on cumulus cell death and suggests the relationship of this effect with female infertility (11, 45,46, 47).

In our research results, both the individuals diagnosed with PCOS and END, GDF-9 and BMP-15 values were obtained lower comparing with MF group, while the number of apoptotic cells was higher. Another task of oocyte secreted molecules is to increase the synthesis of apoptosis-inhibiting B-cell lymphoma 2 (Bcl-2) proteins in somatic cells surrounding the oocyte and to create a protective effect by inhibiting the synthesis of Bcl-2-associated X (Bax) proteins known for their pro-apoptotic activity (16, 48). This result suggested that anti-apopitotic system formed by the synergistic effect of GDF-9 and BMP15 molecules could be insufficient for patients diagnosed with PCOS or END.

TOS values, which were significantly higher than the MF group in the END group, are also compatible with the literature. In contrast with this finding, TAS levels were significantly higher in the END group comparing with MF group but the difference was not significant. This result differs with some studies reporting decreased TAS values for patients diagnosed with END (27, 43, 44). The limited number of patient groups in our research data is thought to be a cause of this situation. In addition, in our research, total level of oxidant and antioxidant levels were obtained. Specific markers on these parameters could also evaluate oxidant and anti-oxidant differences at detailed ranges. Prieto et al. investigated specific parameters such as Vitamin C, Vitamin E, malonildialdehyde (MDA), superoxide dismutase (SOD) in patients diagnosed with END comparing with control group. They shared decreased antioxidant data alongside higher oxidative stress level at END group.

Increased cytokine levels in follicular fluid samples have been shared in individuals diagnosed with END with many data presented to the literatüre (10, 11, 44). In contrast with this finding, within the results of the study no significant difference was obtained for IL-6, TNF-alpha levels in END group.

This the situation is related to the limited number of volunteers in the research group. It is believed further studies in larger groups will provide a wider perspective for the effect of inflammatory cytokines at follicular fluid.

Limitations

It was made out for all authors that, a further research with a larger number of patient groups and long-term patient follow-up would provide wiser results. Also the necessity of adding clinical data such as embryo development and neonatal findings was also absorved at the end of the study. This enterprise would supply a larger contribution to the affect of examined paramaters on clinical processes.

Conclusion

The relationship of cumulus cells with female infertility continues to be researched with increasing interest. Common sight on the relationship between oocyte and cumulus cells is that communication is effective on many parameters of female fertility from fertilization, implantation to to the newborn health. In this communication, which attracts a great deal of attention, which molecule / or which molecules has a wider effect through its own pathway is still not fully known. To find out more of which molecules are involved in this interaction and which pathophysiological mechanisms contribute will provide reflection of new parameters in the selection of healthy oocytes to the clinic.

It is known that oocyte quality is of great importance in obtaining pregnancy. The healthiest oocyte can be effective as one of the biggest goals for the success of obtaining pregnancy. Levels of GDF-9 and BMP-15 which are of great interest in molecules synthesized by oocyte, or the cell death rates of cumulus cells can cause a decrease in certain clinical pathologies and this situation is associated with fertility.

In our study, cumulus cells were examined at both PCOS, END and MF groups. Besides GDF-9 and BMP-15, apoptosis levels were examined. Compared to healthy individuals in MF group cumulus cells belonging to individuals diagnosed with PCOS and END, GDF-9 and BMP-15 levels were evaluated low and apoptosis levels were high.

Compared to each other, GDF-9 and BMP-15 decline was more prominent in the END group, whereas the increase in apoptosis level was obtained more evident for the PCOS group. Based on these results it can be thought that, GDF-9 and BMP-15 has a clear effect on infertility for patients with END. Similarly, the higher rate of apoptosis levels of cumulus cells at PCOS group were more significant and this result was associated with the relationship of cumulus cell death rate and infertility for patients diagnosed with END. In the selection of the best oocyte within the ART techniques, it can be thought

that a different perspective can be brought in by evaluating the properties of cumulus cells. In the light of our research data, it can be thought that GDF-9 and BMP-15 molecules will be of greater importance especially in individuals diagnosed with END, while TUNEL positivity rates of endometrial cells can be evaluated in individuals diagnosed with PCOS.

Although within the data obtained from the results of our study, it can be said that cumulus cell evaluation could be supportive, the need for molecular studies to be conducted with larger patient groups continues in order to consider cumulus cells as a criterion in the selection of oocytes belonging to individuals diagnosed with PCOS and / or END.

It can be thought that, the evaluation of follicular fluid oxidative stress level could promote the determination of GDF-9 and BMP-15 markers and/or TUNEL positivity rates. These criterias could provide a more comprehensive selection for best oocyte treatment with ART for patients diagnosed with PCOS or END.

The results of the study were determined in relation to increased TOS value, decreased GDF-9, BMP-15 levels as well as increased TUNNEL positivity rate. Data highlighting the relationship between increased oxidative stress levels, cumulus cell and embryo quality are available in literatüre (13). In the light of the data obtained, when the follicular fluid properties of the cumulus cell properties are evaluated together with the oxidative stress levels, it can be thought that it can also provide data on embryo quality in individuals with PCOS and END. TNF-alpha and IL-6 levels at follicular fluid did not exhibit significantly evaluated rates between groups. Therefore it cannat not be said that the levels of TNF-alpha and IL-6 levels of follicular fluid could be an evaluation criteria of selecting best oocyte. Further studies are still needed for this perspective.

Many studies in the literature investigate the effect of oxidative stress in PCOS pathology. However, as provided in our study, it is the first data which evaluates both mentioned criterias in cumulus cells while examining the oxidative stress and inflammation levels together at follicle fluid. Considering the results of our study, it can be thought that more satisfactory results can be expected in embryo development by providing the necessary supplements (GDF-9 and BMP-15 molecules) to the media of embryos for patients diagnosed with PCOS or END. Folicular fluid oxidative stress level can have a big effect fort his clinical judgement.

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