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ASSISTED REPRODUCTION TECHNOLOGIES

Notch signaling pathway in cumulus cells can be a novel marker to identify poor and normal responder IVF patients

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Abstract
Purpose To identify expression of Notch signaling proteins and its ligands in human cumulus cells which were obtained by follicle aspiration and to compare the differences of this protein expression between the normal and poor responder patients.
Methods 47 patients who applied to the assisted reproductive treatments with various infertility problems were included to the study. Controlled ovarian hyperstimulation was performed by using GnRH agonist and gonadotropins. Serum hormon levels were measured by using Chemilluminescent Microparticle Immunoassay method for each patient. After ultrasonographic ovarian follicle screening, oocytes were retrievaled. Cumulus cells obtained from the follicles were cultured for 72 h and immunuhistochemistry were performed for Notch1, Notch2, Notch3, Notch4, Jagged1 and Jagged2 proteins. Histological score (HSCORE) were applied to all of the samples. The association between Notch and its ligands protein expressions and the oocyte-embryo quality and fertilization rates were investigated.
Results Significant differences were observed between the mean values of age, AMH and FSH in the 2 groups, respectively ($p<0.05$). However, the mean female infertility duration and total gonadotropin dose did not differ significantly between normal and poor responder groups. All the patients cumulus cells expressed Notch1, Notch2, Notch3, Notch4, Jagged1 and Jagged2. There was a significant difference ($p<0.05$) only for Notch2 between the 2 groups and a positive correlation between Notch2 and Notch3 ($r=547$, $p=0.00$) expressions were noted. Furthermore, no correlations were observed between the following: Notch1, Notch2, Notch3, Notch4, Jagged1, and Jagged2 expression; mature oocyte number; fertilization rates, and embryo quality percentage in both of the groups.
Conclusion Notch signalling proteins can be an indicator for understanding the ovarian response in ovulation induction.

Keywords Notch signalling · Cumulus cells · Poor responder · Ovary follicles · In vitro fertilization

This study was approved by the Etics Committe and Institutional Board of the Cerrahpasa School of Medicine (institutional review board number: 27568).

Capsule Our data indicates Notch and its ligands are expressed in cumulus cells of follicle in human ovary and Notch signalling proteins can be an indicator for understanding the ovary response.

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Introduction

During the reproductive lifespan of female mammals, continuous follicle development occurs within the ovary [1]. Follicles are the functional units of the ovaries. Each follicle consists of an oocyte surrounded by granulosa cell layers. Granulosa cells proliferate into the multilaminar epithelium and segregate upon formation of the fluid-filled follicular antrum; the subsequent differentiation occurs in granulosa cells [2]. Cells that line the follicle wall and reside very close to the basement membrane are called mural granulosa cells; cumulus cells form another group

of cells that are located directly adjacent to the oocytes [3]. These 2 cell populations are responsible for growth and maturation of the oocyte and for successful ovulation.

Cumulus cells communicate with each other and with the oocyte through specialized gap junctions (GJs) which provide a direct pathway for transporting signaling molecules. The growing oocyte supplies most of its substrates for energy metabolism and biosynthesis from the surrounding cumulus cells. Another critical aspect in the final stages of follicular development is cumulus expansion and it is an indicator for underastanding the oocyte maturity. Cumulus expansion is regulated by growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15) which belong to transforming growth factor beta (TGF-β) superfamily. GDF-9 and BMP-15 act as oocyte-secreted paracrine factors that regulate several genes expressions like cyclooxygenase-2 (COX-2), gremlin-1 (GREM-1), hyaluronic acid synthase-2 (HAS-2), and pentraxin-3 (PTX-3) that are expressed from cumulus cells and they are on the critical point on the folliculer developing and cumulus expansion. Oocytes of follicles with impaired cumulus expansion has a limited potential for implantation. So, it's clear that mammalian female fertility is critically dependent on timely coordinated intercellular communication between cumulus cells and oocyte [4, 5].

The Notch genes encode transmembrane receptors that are highly conserved evolutionarily and which control cell-fate decisions between tightly adjacent cells by modulating cell proliferation, differentiation, and survival [6]. Mammals have 4 types of Notch receptors (Notch1–4) and 2 ligand families (Jagged1; Jagged2; and Delta-like [DII] 1, DII 3, DII 4). The Notch signaling pathway is initiated by a receptor-ligand interaction between 2 neighboring cells. Cleavage of the receptors occurs after Notch receptors bind to their ligands. The intracellular domain of the receptor releases and translocates to the nucleus. In the nucleus, Notch forms transcriptional complexes with transcription factors of the CSL family (C-promoter binding factor 1/suppressor of hairless/Lag-1) [7].

Notch genes are actively expressed by cumulus cells during folliculogenesis [1]. However, it is not released by oocyte and atretic follicles. Several experimental studies involving animals have reported that Notch2, Notch3, and Jagged2 are released from granulosa cells and that Jagged1 is released more often from oocytes than from granulosa cells. The importance of Notch signalling in developing tissues has also been indicated in several studies. However, to our knowledge, no study has described Notch signaling in human granulosa cells, cumulus cells, and oocytes.

The purpose of the present study was to determine the expression of Notch and its ligands in cumulus cells obtained from aspiration of follicles and to compare differences between patients with poor responses and normal responses to assisted reproductive techniques. Another goal of the present study was to understand how Notch signaling regulates this granulosa-cumulus-oocyte cell microenvironment. Understanding this signaling mechanism would improve our understanding of good and poor responsiveness to assisted reproductive technique and help evaluate the different biological functions mediated by Notch genes in the ovarian follicles.

Materials and methods

Patients

In this prospective study, 47 infertile women referred to the In Vitro Fertilization Unit, Department of Obstetrics and Gynecology, Cerrahpasa School of Medicine, Istanbul University were included. After performing controlled ovarian hyperstimulation with GnRH analogues and gonadotropins, IVF-intracytoplasmic sperm injection procedures were applied to the patients, who were then divided into 2 groups. Eight patients were identified as poor responders and 39 were identified as normal responders. When the number of aspirated oocytes was 4 or less, the patient was considered to be a poor responder [8]. Follicular aspiration was performed after hCG injection. The study was approved by the Ethics Committee and Institutional Board of the Cerrahpasa School of Medicine (institutional review board number: 27568) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Obtaining cells

Following oocyte aspiration, aspirated follicular fluid was transferred into tissue culture tubes and the oocyte was separated from corona-cumulus cells by Hyase, which includes hyaluronidase enzyme. Cumulus cells obtained in this manner were centrifuged at 2,000 rpm for 10 min in order to remove the follicular fluid and other cells (erythrocytes, leukocytes, and epithelial cells). The supernatant of the centrifuged cumulus cells suspension was discarded. After centrifugation, the cell suspension was diluted with Dulbecco's Modified Eagle Medium (DMEM-F12; Sigma, Steinheim, Germany).

Cell culture

Cells were cultured on cover slips that were kept in 24-well plate culture dishes. After waiting for approximately 2 h for the cells to attach, they were cultured with 5 μL DMEM-F12 medium. Cultured cells proliferated in an incubator at 37 °C under 5 % CO_2 and 95 % humidity. After 72 h, the cells were observed under an inverted microscope (Olympus IX71; Olympus Corp., Tokyo, Japan). At the end of 72 h, the medium was removed from the well plates, and the cells were

fixed with 70 % alcohol. The cells were kept at 4 °C for immunohistochemical investigations.

Immunohistochemistry

The Histostain-plus-Broadspectrum-kit (85–9043; Invitrogen, San Francisco, CA, USA; used according to manufacturer's instructions) protocols were applied for immunohistochemical processing. Cells were treated inside the well plates. Before applying the protocols, alcohol was removed from the cell layer, and the cells were washed with phosphate-buffered saline (PBS) for 5 min and then treated with 3 % H_2O_2 for 5 min to inhibit endogenous peroxidase activity. The blocking solution was then applied for 5 min. Next, cells were incubated with primary antibodies directed against Notch1 (1:100 dilution; Santa Cruz sc-6010, CA, USA), Notch2 (1/500 dilution; Lifespan LS-B719, Seattle, WA, USA), Notch3 (1:100 dilution; Santa Cruz sc-5593, CA, USA), Notch4 (1:100 dilution; Santa Cruz sc-5594, CA, USA), Jagged1 (1:100 dilution; Santa Cruz sc-8303, CA, USA), or Jagged2 (1:50 dilution; Abcam ab60041-100, Cambridge, UK) for 1 h at 4 °C in a humidity chamber. The cells were then incubated with broad-spectrum secondary antibody solution and then with HRP-streptavidin conjugated solution (Invitrogen 85–9043, San Francisco, CA, USA; prepared according to manufacturer's instructions) for 30 min each. Finally, the cells were incubated with the AEC Substrate Kit (Life Science Division COI-12, WA, USA) for 5 min for immunolabeling analysis. All dilutions and thorough washes between stages were performed using PBS. Cells were counterstained with Mayer's hematoxylin (LabVision TA-125-MH; LabVision Corp., CA, USA). Then, the coverslips were mounted onto slides with the mounting medium (Zymed 00–8110, CA, USA). As for negative control, normal rabbit IgG (Santa Cruz Biotechnology, Santa Cruz CA, USA) was used. Positive controls were chosen according to the manufacturer's recommendation for each primary antibody.

Oocyte maturity analysis and embryo grading

Oocyte maturity classification and embryo grading were performed according to the procedures reported by Weeck et al. [9]. Oocyte-corona cumulus complexes were investigated under a stereomicroscope (Olympus BH-2; Olympus Corp., Tokyo, Japan), evaluated according to their maturation, and graded from 0 to 4. Embryos were graded from 1 to 5. Oocytes which have a large nucleus in their cytoplasm and a dense corona cumulus layer were regarded as immature "0"; oocytes which did not have a cytoplasmic germinal vesicle and a polar body, and had a corona layer that is relatively less dense and smaller than the total sizes of five oocytes were regarded as "1st degree"; oocytes which had radially placed crowded coronal cells in their vicinity, had cumulus cells spreading to a relatively wider area than the second group, contained a polar body and were easily removed from the cell group when manipulated were regarded as the "2nd degree"; oocytes which lost their tight adherence to the coronal cells and had cumulus cells which were quite scattered but still being cellular were regarded as "3rd degree"; oocytes which had pale cytoplasm and cumulus cells that lost their cellular image and became a gelatinous structure in its vicinity were regarded as "4th degree" mature in the classification [9]. Furthermore, oocyte which is regarded as "4th degree" calls M2 or late M2. M2 oocytes have clear cytoplasm, normal cell size, normal zona pellucida, and non-fragmented polar body. Late M2 oocytes are post-mature oocytes which have dark cytoplasm with cytoplasmic granulations, abnormalities in zona pellucida and fragmented polar bodies. M2 oocytes are considered to be better than late M2 oocytes [9].

After the 40th hour of the intracytoplasmic sperm injection (ICSI) process, morphology of the dividing embryo was observed and 'embryo grading' was done. Embryos with equally-sized blastomeres and without cytoplasmic fragmentation were regarded as "grade 1", embryos with equally-sized blastomeres and minor cyto- plasmic fragmentations were regarded as "grade 2", embryos without equally-sized blastomeres and without cytoplasmic fragmentations were regarded as "grade 3", embryos with or without equally-sized blastomeres and with major cytoplasmic fragmentations were regarded as "grade 4", embryos with blastomeres that cannot be distinguished and with major cytoplasmic fragmentations were regarded as "grade 5" [10].

Histological score analysis

An Olympus BX61 (Olympus Corp., Miami, FL, USA) research microscope was used for histological score (HSCORE) analysis, and the samples were photographed with an Olympus DP72 (Olympus Corp., Miami, FL, USA) digital microscope camera. Five different areas from each slide were investigated under the immersion objective (100× magnification) by 2 histologists in a blinded manner. The intensity of cumulus cell immunostaining was semiquantitatively evaluated using the following categories: 0 (no staining), 1+ (weak, but detectable staining), 2+ (moderate staining), 3+ (distinct staining), and 4+ (intense staining).

Statistical evaluation

The Statistical Package for Social Sciences (SPSS) 11.0 program for Windows was used, and statistical calculations were made after consulting a biostatistician at the Biostatistics Department, Cerrahpasa School of Medicine. The Student's t and Mann–Whitney *U* tests and Pearson and Spearman correlation analyses were used. All values have been expressed in

terms of mean ± standard deviation (SD), and $p<0.05$ was considered to be statistically significant.

Results

The causes of female infertility in our study were male-factor problems in 20 cases, unexplained infertility in 20 cases, ovarian failure in 3 cases, and polycystic ovarian syndrome together with the male-factor problems in 4 cases. Demographic, clinical, and laboratory features of normal responder and poor responder patients are presented in Table 1.

The mean female infertility duration and total gonadotropin dose did not differ significantly between the 2 groups. However, a significant difference was observed between the mean values for age ($p<0.05$) and AMH and FSH on the third cycle day and serum estradiol on the day of HCG injection in the 2 groups. Furthermore, the total oocyte number significantly differed between the 2 groups. However, the fertilization rate (fertilized oocytes/number of ICSI oocytes), percentage of good embryos (grade 1 embryo+grade 2 embryo/total embryo number), and oocyte maturation rate (M2 oocyte number/total oocyte number) were not significantly different between the groups (Table 1).

All the patients expressed Notch1, Notch2, Notch3, Notch4, Jagged1, and Jagged2 (Figs. 1 and 2). Weak or undetectable Notch1 staining was noted for both groups. Notch2 immunoreactivity was mostly nuclear; cytoplasmic immunoreactivity was also observed. Normal responder group cells showed remarkably strong Notch2 immunoreactivity (Fig. 1b), while Notch2 immunoreactivity was very weak and undetectable in poor responder cells (Fig. 2b). Cytoplasmic staining was noted for Notch3 (Figs. 1c and 2c), and nuclear and cytoplasmic staining was noted for Notch4, Jagged1, and Jagged2 (Figs. 1d–f and 2d–f); distinct and intense immunoreactivities were detected. The 2 groups showed a significant difference only for Notch2 (Table 2).

Table 1 Demographic data of normal and poor responder patients

	Normal responder (n=39)	Poor responder (n=8)	P
Age(years)	30.74±4. 53[a]	36.75±4.23	<.05
Infertility period (years)	5.79±3.64	8.75±5.80	NS[b]
Day 3 FSH (mIU/ml)	6.30±1.68	8.69±3.54	<.05
Day 3 AMH (ng/ml)	4.07±2.75	1.64±0.79	<.05
E2 on HCG day (pg/ml)	1692.74±895.07	640.87±244.69	<.05
Total gonadotropin dose (IU)	2492.92±1684.92	3909.37±2775.27	NS
Number of aspirated oocytes	10.28±4.10	3.00±1.19	<.05
Percentage of fertilization (%)	59.10±26.01	50.00±46.29	NS
Percentage of good embryo (%)	64.05±38.25	59.37±49.88	NS
Percenatage of oocyte maturation (%)	79.15±19.66	78.12±24.77	NS

AMH anti-Müllerian hormone; *FSH* follicle stimulating hormone; *E2* estradiol; *HCG* human chorionic gonadotropin

[a] The values are given as mean ± SD or percent (numbers)

[b] Not significant

The expression of Notch was compared with that of the ligands, and these results were also compared with hormone levels, fertilization rates, oocyte maturation, and embryo quality in each group. In the normal responder group, strong positive correlations were noted between Notch2 and Notch3 (r = 0.547, p = 0.00), Notch2 and Jagged2 (r = 0.857, p = 0.00), and Notch3 and Jagged2 (r = 0.538, p = 0.00) expression (Fig. 3) In this group, significant correlations were noted between AMH and Jagged1 expression (r = 0.332, p = 0.039) and also between AMH and Notch3 expression (r = 0.349, p = 0.039). However, the correlations between the following were not significant: Notch1, Notch2, Notch3, Notch4, Jagged1, and Jagged2 expression; mature oocyte number; fertilization rates; and embryo quality percentage. In the poor responder group, while there were positive correlations between Notch2 and Notch3 (r = 0.549, p = 0.159), Notch2 and Jagged2 (r = 0.807, p = 0.015), Notch3 and Jagged2 (r = 0.683, p = 0.062), and AMH and Jagged1 (r = 0.900, p = 0.002) were detected as in the normal responder group (Fig. 4). Furthermore, in this group, no correlations were observed between the following: Notch1, Notch2, Notch3, Notch4, Jagged1, and Jagged2 expression; mature oocyte number; fertilization rates, and embryo quality percentage.

Discussion

To our knowledge, this study is the first to demonstrate the expression of Notch and its ligands in cumulus cells of the human ovary in patients receiving intracytoplasmic sperm injections. Our results indicate that Notch and its ligands are expressed in the cumulus cells of human ovarian follicles. The normal and poor responder groups did not show significant differences in the expression of Notch1, Notch3, Nocth4, Jagged1, and Jagged2 in the cumulus cells. However, we found a significant difference in Notch2 expression between the 2 groups. Furthermore, there was a positive correlation between Notch2 and Notch3 expression in both groups.

Many studies have been performed on choosing good-quality oocytes and embryos for ICSI patients, and several articles have been published on growth factors, hormones, reactive oxygen species (ROS) production, apoptosis, and gap junctional complexes on the adjacent follicular cells in patients undergoing ICSI [8, 10–15]. Currently, there is increasing interest in signaling pathways (e.g., Wnt, Hedgehog, and Notch pathways) that are important for developing oocytes. However, these signaling pathways and associated molecules are not yet well understood in this context.

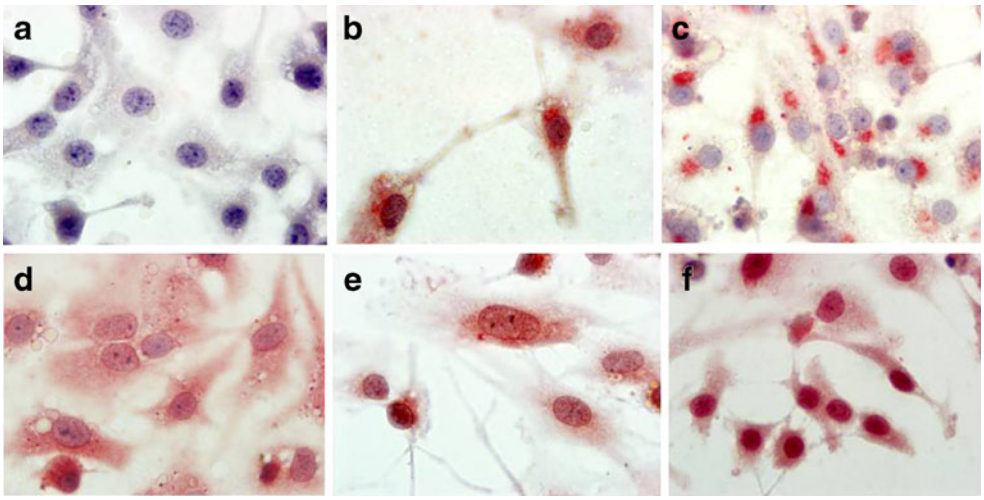

Fig. 1 Immunolocalization of Notch and ligand proteins in normal responder patients' cumulus cells. Weak or undetectable Notch1 staining (**a**) and strong Notch2 immunoreactivity (**b**) are detected. While cytoplasmic staining are noted for Notch3 (**c**), nuclear and cytoplasmic stainings are detected for Notch4 (**d**), Jagged1 (**e**) and Jagged2 (**f**). Original magnifcations of a, b, c, d, e & f: X100

The Notch genes encode transmembrane proteins involved in cell fate determination during embryonic development. These genes are evolutionarily conserved, and Notch homologs have been cloned from several vertebrate species. There are 4 Notch receptors (Notch1–4) and 2 families of ligands (Jagged1, Jagged2, Dll 1, Dll3, and Dll4); these proteins are also transmembrane proteins. The Notch signaling pathway is initiated by interaction between the extracellular domains of Notch receptors and their ligands on the surface of adjacent cells [16]. Although there are many publications on the importance of the Notch signaling for tissue development [1, 17–20], only a few studies have been performed on the effects of Notch signaling on oocyte growth. To date, no information is available on human cumulus cells and oocyte maturation. In the present study, we confirmed that Notch proteins are involved in human cumulus cells in the follicles of human ovaries.

Many etiological factors have been proposed to explain poor ovarian response. Decreasing ovarian reserves, breakdown of the hypothalamic-pituitary-adrenal/ovary axis, imbalance of ovarian-regulating factors, imbalance in gonadotropin receptor regulation, increasing granulosa cell apoptosis, and increasing auto-antibody levels against granulosa cells can be associated with poor ovarian response [21, 22]. The classification criteria for poor response patients reported in the literature are basal FSH levels [23], number of aspirated oocytes [24], the woman's age [24, 25], and the E_2 level measured on the day of HCG administration [24]. Most studies have emphasized that age and depleted ovarian reserves are the principle factors underlying poor ovarian response, and age-related decrease in ovarian function has been attributed to reduction in the number of follicles available for stimulation.

An increasing number of studies are being performed on granulosa and cumulus cell function in the ovarian response. Seifer et al. reported that increased apoptosis can be related to decreased ovarian reserves and poor ovarian response [26]. Cepni et al. emphasized that the junctional complex on the granulosa cells can be important for obtaining information about the ovarian response; they also labeled connexin 37

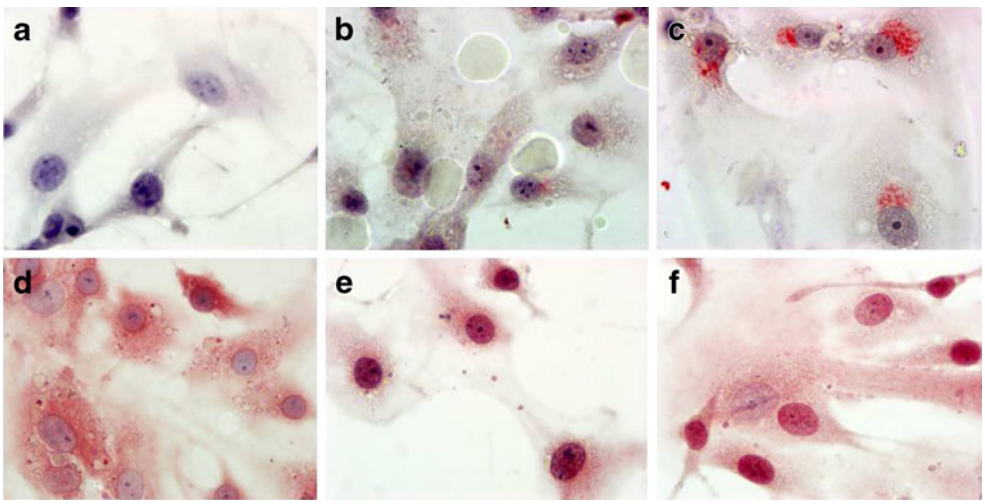

Fig. 2 Immunolocalization of Notch and ligand proteins in poor responder patients' cumulus cells. There is a weak or undetectable Notch1 (**a**) staining. Nuklear and cytoplasmic Notch2 immunoreactivities (**b**) are seen weakly in comparison to normal responder group. While cytoplasmic staining are noted for Notch3 (**c**), nuclear and cytoplasmic stainings are detected for Notch4 (**d**), Jagged1 (**e**) and Jagged2 (**f**). Original magnifcations of a, b, c, d, e & f: X100

Table 2 Comparison of HSCORE result

	Normal responder (n=8)	Poor responder (n=39)	P
Notch 1	0.21±0.37	0.12±0.35[a]	NS[b]
Notch 2	2.30±1.21	1.31±0.92	<.05
Notch 3	2.91±1.00	2.87±1.35	NS
Notch 4	3.32±1.16	3.06±1.01	NS
Jagged 1	2.33±1.43	1.18±1.22	NS
Jagged 2	2.53±1.36	1.75±0.88	NS

[a] The values are given as mean ± SD or percent (numbers)

[b] Not significant

(Cx 37) in the granulosa cells of poor and normal responder patients, but could not find any relation between Cx 37 and the ovarian response [8].

In 2 different studies in mice, it has been shown that Notch1, Notch2 and Jagged1 mutations cause embryonic lethality [27, 28]. In contrast, other studies have shown that mice survived in the absence of Notch3 and Notch4, but they were infertile [18, 19].

Johnson et al. showed differing expression levels of Notch and its ligands for different follicle sizes in an immunohistochemical study; they also emphasized that Notch2 immunopositivity was noted in all the stages of folliculogenesis and that Notch2 immunopositivity was strongly correlated with that of Notch3 [1]. They also reported that mature oocyte development can be disturbed unless Notch2 is absent; this disturbance can be a cause of infertility.

Recently, Javanovic et al. emphasized the importance of Notch expression in follicle development in an animal model also. Their results suggest that Notch is a novel intraovarian regulator that regulates folliculogenesis through vascular and nonvascular mechanisms [29].

Our results indicate that Notch signalling, especially Notch2 and Notch3 expression, can be a useful determinant for the ovarian response. We found a significant increase in Notch2 expression (p=0.034) in our normal responder group in comparison to that in the poor responder group. We could not find any significant differences between Notch3 expression for our 2 groups. However, we obtained a strong correlation between Notch2 and Notch3 expression (r=547, p=0.00). This result indicates that Notch2 and Notch3 are essential molecules for completion of folliculogenesis and a good ovarian response.

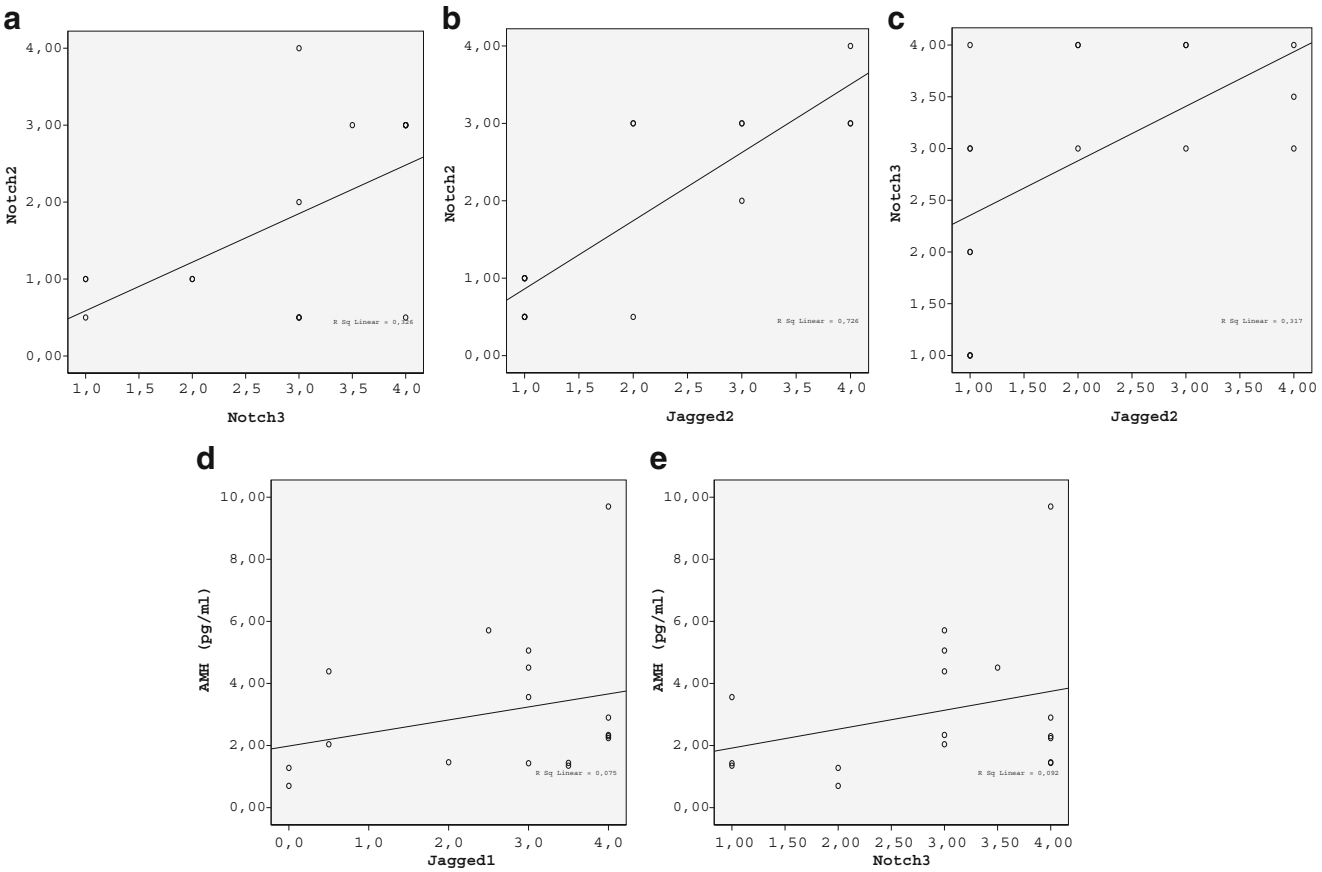

Fig. 3 Correlation graphics of normal responder group between Notch 2 and Notch 3 (**a**), Notch 2 and Jagged 2 (**b**), Notch 3 and Jagged 2 (**c**), AMH and Jagged 1 (**d**), AMH and Notch 3 (**e**)

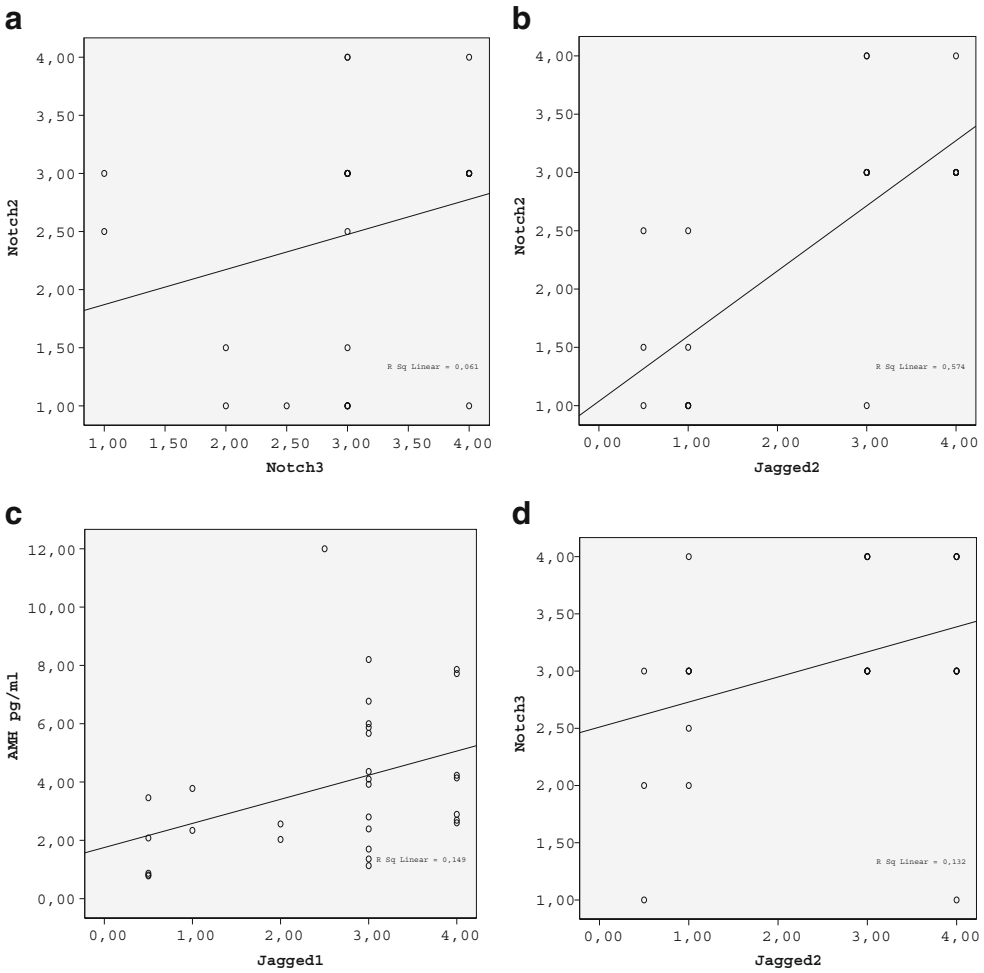

Fig. 4 Correlation graphics of poor responder group between Notch 2 and Notch 3 (**a**), Notch 2 and Jagged 2 (**b**), AMH and Jagged 1 (**c**), Notch 3 and Jagged 2 (**d**)

Another related hormone within the follicle pool is anti-Müllerian hormone (AMH). AMH expression occurs first in the follicle's granulosa cells in humans, and its expression level is highest for preantral and small antral follicles (diameter≤4-mm). When the size of the follicles increases, the AMH level decreases gradually and AMH disappears completely by the time the follicle size is nearly 8 mm. These results indicate that AMH plays a role in selection of the dominant follicle [30].

Several studies have emphasized that AMH could be a strong indicator for determining the ovarian response. Choi MH et al. found a close correlation between the retrieved oocyte number and AMH levels. They also reported a significantly decreased cutoff value for the serum AMH levels of poor responder patients [31].

In our study, we found a significant difference in the serum AMH levels (p=0.018) of the 2 groups. The mean serum AMH levels for the poor responder and normal responder groups were 1.64±0.79 and 4.07±2.75, respectively. Our results support the literature.

On analyzing the relationship between serum AMH levels and Notch receptors and ligands, we obtained some positive correlations. There was a very strong correlation between AMH and Jagged1 in the poor responder group (r=0.900, p=0.002). A similar result was found between AMH and Jagged1 for the normal responder group (r=0.332, p=0.039), although the correlation was not as high as in the poor responder group. Considering these results, we conclude that Notch and its ligands might be useful for understanding the follicle pool.

Conclusion

Our study shows that Notch and its ligands were expressed in cumulus cells of the follicle in human ovary and that there was a significant increase in the expression of Notch2 in the cumulus cells of normal responder patients. An extremely strong correlation was noted between Notch2 and Notch3 expression in both groups. Based on these results, we think that Notch and its ligands are important markers for explaining the ovarian response and that Notch 2 might be a marker for follicle quality. Further studies on the communication between oocytes and granulosa cells are required in order to better understand the interactional mechanisms between oocytes and follicular cells. Such information may be beneficial for improving in vitro fertilization outcomes.

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Conflict of interest The authors state that they do not have any potential conflict of interest that would compromise the validity of the research conducted.

References

1. Johnson J, Espinoza T, McGaughey RW, Rawls A, Wilson-Rawls J. Notch pathway genes are expressed in mammalian ovarian follicles. Mech Dev. 2001;109(2):355–61.
2. Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. Reproduction. 2006;132:191–206.
3. Russell DL, Robker RL. Molecular mechanisms of ovulation: co-ordination through the cumulus complex. Hum Reprod Updat. 2007;13:289–312.
4. Uyar A, Torrealday S, Seli E. Cumulus and granulosa cell markers of oocyte and embryo quality. Fertil Steril. 2013;99(4):979–97.
5. Teilmann SC. Differential expression and localisation of connexin-37 and connexin-43 in follicles of different stages in the 4-week-old mouse ovary. MolCell Endocrinol. 2005;234:27–35.
6. Trombly DJ, Woodruff TK, Mayo KE. Suppression of Notch signaling in the neonatal mouse ovary decreases primordial follicle formation. Endocrinology. 2009;150:1014–24.
7. Wang Z, Li Y, Banerjee S, Sarkar FH. Exploitation of the Notch signaling pathway as a novel target for cancer therapy. Anticancer Res. 2008;28:3621–30.
8. Cepni I, Kahraman N, Ocal P, Idil M, Uludag S. Expression and comparison of gap junction protein connexin 37 in granulosa cells aspirates from follicles of poor responder and nonpoor responder patients. Fertil Steril. 2008;89:417–20.
9. Veeck LL. Oocyte assesment and biological performance. Ann N Y Acad Sci. 1999;541:259–74.
10. Ocal P, Ersoylu B, Cepni I, Guralp O, Atakul N, Irez T, et al. The association between homocysteine in the follicular fluid with embryo quality and pregnancy rate in assisted reproductive techniques. J Assist Reprod Genet. 2012;29:299–304.
11. Idil M, Cepni I, Demirsoy G, Ocal P, Salihoğlu F, Senol H, et al. Does granulosa cell apoptosis have a role in the etiology of unexplained infertility? Eur J Obstet Gynecol Reprod Biol. 2004;112:182–4.
12. Jancar N, Kopitar AN, Ihan A, Virant Klun I, Bokal EV. Effect of apoptosis and reactive oxygen species production in human granulosa cells on oocyte fertilization and blastocyst development. J Assist Reprod Genet. 2007;24:91–7.
13. Tingen C, Kim A, Woodruff TK. The primordial pool of follicles and nest breakdown in mammalian ovaries. Mol Hum Reprod. 2009;15:795–803.
14. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF-9 and BMP-15 in ovarian function. Mol Reprod Dev. 2011;78(1):9–21. doi:10.1002/mrd.21265. Epub 2011 Jan 11. PubMed PMID: 21226076; PubMed Central PMCID: PMC3051839.
15. Edson MA, Nalam RL, Clementi C, Franco HL, Demayo FJ, Lyons KM, et al. Granulosa cell-expressed BMPR1A and BMPR1B have unique functions in regulating fertility but act redundantly to suppress ovarian tumor development. Mol Endocrinol. 2010;24:1251–66.
16. Vorontchikhina MA, Zimmermann RC, Shawber CJ, Tang H, Kitajewski J. Unique patterns of Notch1, Notch4 and Jagged1 expression in ovarian vessels during folliculogenesis and corpus luteum formation. Gene Expr Patterns. 2005;5:701–9.
17. Jiang R, Lan Y, Chapman HD, Shawber C, Norton CR, Serreze DV, et al. Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. Genes Dev. 1998;12:1046–57.
18. Krebs LT, Xue Y, Norton CR, Sundberg JP, Beatus P, Lendahl U, et al. Characterization of Notch3-deficient mice: normal embryonic development and absence of genetic interactions with a Notch1 mutation. Genesis. 2003;37(3):139–43. PubMed PMID: 14595837.
19. Krebs LT, Xue Y, Norton CR, Shutter JR, Maguire M, Sundberg JP, et al. Notch signaling is essential for vascular morphogenesis in mice. Genes Dev. 2000;14:1343–52.
20. Guo S, Liu M, Gonzalez-Perez RR. Role of Notch and its oncogenic signaling crosstalk in breast cancer. Biochim Biophys Acta. 1815;2011:197–213.
21. Feyles V, Gianetto-Berruti A. Poor responders in assisted reproduction cycles. Minerva Ginecol. 2005;57:1–14.
22. Pellicer A, Ardiles G, Neuspiller F, Remohi J, Simon C, Bonilla-Musoles F. Evaluation of the ovarian reserve in young low responders with normal basallevels of follicle-stimulating hormone using three-dimensional ultrasonography. Fertil Steril. 1998;70(4):671–5.
23. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, et al. Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. Fertil Steril. 1998;69(5):826–30.
24. Karande V, Gleicher N. A rational approach to the management of low responders in in-vitro fertilization. Hum Reprod. 1999;14:1744–8.
25. Vollenhoven B, Osianlis T, Catt J. Is there an ideal stimulation regimen for IVF for poor responders and does it change with age? J Assist Reprod Genet. 2008;25:523–9.
26. Seifer DB, Gardiner AC, Ferreira KA, Peluso JJ. Apoptosis as a function of ovarian reserve in women undergoing in vitro fertilization. Fertil Steril. 1996;66:593–8.
27. Xue Y, Gao X, Lindsell CE, Norton CR, Chang B, Hicks C, et al. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. Hum Mol Genet. 1999;8(5):723–30.
28. Hamada Y, Kadokawa Y, Okabe M, Ikawa M, Coleman JR, Tsujimoto Y. Mutation in ankyrin repeats of the mouse Notch2 gene induces early embryonic lethality. Development. 1999;126(15):3415–24.
29. Jovanovic VP, Sauer CM, Shawber CJ, Gomez R, Wang X, Sauer MV, et al. Intraovarian regulation of gonadotropin-dependent folliculogenesis depends on notch receptor signaling pathways not involving Delta-like ligand 4 (Dll4). Reprod Biol Endocrinol. 2013;11:43.
30. Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction. 2006;131(1):1–9.
31. Choi MH, Yoo JH, Kim HO, Cha SH, Park CW, Yang KM, et al. Serum anti-Müllerian hormone levels as a predictor of the ovarian response and IVF outcomes. Clin Exp Reprod Med. 2011;38:153–8.