Clinical and Experimental Obstetrics & Gynecology

IMR Press

Original Research

Defective expression of endometrial BMP-2 contributes to subfertility in women with unexplained infertility

Arzu Yurci[1,*], Nur Dokuzeylul Gungor[2], Tugba Gurbuz[3]

[1] Gynecology Obstetrics & Reproductive Medicine, Memorial Kayseri Hospital IVF Center, 38110 Kayseri, Turkey

[2] Bahcesehir University Goztepe Medical Park Hospital, Gynecology Obstetrics & Reproductive Medicine, 34363 Istanbul, Turkey

[3] Medistate Hospital Gynecology and Obstetric Clinic, 34363 Istanbul, Turkey

*Correspondence: arzuyurci2@gmail.com (Arzu Yurci)

DOI:10.31083/j.ceog4805176

Submitted: 9 January 2021 Revised: 13 February 2021 Accepted: 5 March 2021 Published: 15 October 2021

Background: This study was planned to measure bone morphogenetic protein 2 (BMP-2) levels in the endometrial samples of unexplained infertility (UEI) cases who had a history of unsuccessful *in vitro* fertilization - embryo transfer (IVF-ET) and therefore decided to have frozen-thawed embryo transfer (FET). *Methods*: Thirty patients diagnosed with UEI and decided to transfer frozen-thawed embryo transfer were included in the study. The UEI patients were selected among the patients whose previous IVF-ET trials were unsuccessful and were scheduled for FET this time. Thirty participants in the control group were selected among patients diagnosed with polycystic ovary syndrome (PCOS) and scheduled for FET. Serum total testosterone, fasting insulin, follicle-stimulating hormone and luteinizing hormone levels were measured. Homeostatic model assessment - insulin resistance (HOMA-IR) Formula was used for calculating insulin resistance. Endometrial samples were collected by pipelle during oocyte pick-up, washed three times with a sterile saline solution to remove blood and transferred into RNA stabilization buffer until analysis. Endometrial BMP-2 concentrations were measured by enzyme-linked immunosorbent assay. *Results*: Serum total testosterone, insulin levels and HOMA-IR of patients in the control group were significantly higher than the UEI group. BMP-2 levels in the endometrial supernatants of UEI patients were found to be about 2 times lower than the patients in the control group (984.5 (350) pg/mg wet tissue vs. 1720 (318) pg/mg wet tissue, $p < 0.000$, $Z = -6.6$). A strong and significant positive correlation was found between endometrial thickness, estradiol levels measured on HCG day and BMP-2 levels. A positive and significant correlation was found between endometrial BMP-2 levels and serum luteinizing hormone (LH), fasting insulin, glucose, testosterone, HOMA-IR and mature oocyte counts. *Conclusion*: BMP-2 is associated with UEI and mechanistically it may be useful to study this further to determine is this is causal or merely a biomarker.

Keywords

Unexplained infertility; Endometrium; BMP-2; Subfertility

1. Introduction

Despite one year of unprotected intercourse of a woman with normal ovulation and tubal functions and a man with normal semen analysis the absence of conception is called unexplained infertility [1]. In the light of this definition, we should consider that mechanisms other than tubal, ovulatory and semen parameters are responsible for the etiology of unexplained infertility (UEI) [2]. However, the range of data that we can ascribe to etiology is so wide that we have to include many disorders involving edocrinological, immunological, genetic and reproductive physiology [3]. This is one of the main factors that make it difficult to find a solution to the problem. Interestingly, the endometrium, which has a critical importance in embryo implantation, has not been adequately studied in UEI cases [2, 4]. One of the main reasons for this is that we do not have a non-invasive method other than ultrasonography (USG) to evaluate the endometrium [5]. On the other hand, endometrium evaluation with USG is a method with low sensitivity is about 30% [6]. We have very little data about what kind of changes occur in histomorphological and molecular levels in the endometrium of UEI cases. The fact that endometrial sampling is an invasive method is the main reason for very few studies on this subject.

Failure of implantation despite healthy embryo transfer in UEI cases undergoing fertilization - embryo transfer (IVF-ET) generated the hypothesis that there may be a defect in the genes or growth factors that regulate endometrial receptivity. In addition to the presence of a decidua resulting from differentiation of endometrial stromal cells, adequate expression of steroid hormones and their receptors, cytokine and receptivity genes is required for successful implantation [7, 8]. The bone morphogenetic protein 2 (BMP-2) is one of the critical growth factors involved in embryo implantation. It belongs to the transforming growth factor-beta (TGF-β) superfamily and plays a role in endometrial proliferation and differentiation as well as embryo navigation [9, 10]. BMP-2 expression in endometrial cell is regulated by progesterone and critical for implantation and decidualization. Gradual expression of BMP-2 and its receptor in the endometrial stromal cells during early pregnancy indicates a possible link between BMP-2 expression and stromal cells differentiation [9, 10]. In exper-

imental models with BMP-2 ablation (BMP-$2^{d/d}$), both the implantation zone and decidua formation are almost completely blocked, and the animal becomes absolutely infertile [9]. In literature review, we could not find a study on endometrial BMP-2 levels of UEI patients who underwent IVF-ET. The aim of this study is to measure BMP-2 levels in endometrial samples of patients scheduled for IVF-ET with a diagnosis of UEI.

2. Materials and methods

2.1 Patient and control selection

This study was planned to measure BMP-2 levels in the endometrial samples of UEI cases who had a history of unsuccessful IVF-ET and therefore decided to have frozen-thawed embryo transfer (FET). When the sample size was calculated with the GPower 3.1 (http://www.gpower.hhu.de/) program, the total mean of two groups compared based on the Mann-Whitney test with the effect size of 6%, power of 80% and 0.1 type 1 error, was found to be at least 54 patients. Hence, 30 patients diagnosed with UEI and 30 control participants decided to frozen-thawed embryo transfer were included in the study. Unexplained infertility iwas defined as the absence of conception despite a year of unprotected intercourse, not explained by anovulation, tubal factor or male factor infertility. Hysterosalpingogram, mid-luteal progesterone and semen analysis, which are standard examinations, were performed in all couples. Endometrial thickness and antral follicle count were evaluated by ultrasonographic examination. Semen samples analysis of each group of participants was made according to the definition in the "WHO laboratory manual for the examination and processing of human semen" (fifth edition, 2010). The diagnosis of UEI is made when tubal patency, normal ovulatory function and normal semen analysis were established. The UEI patients participating in the study were selected among the patients whose previous IVF-ET trials were unsuccessful and were scheduled for FET this time. Thus, it was possible to take the endometrium samples of the patients under anesthesia by pipelle during oocyte pick-up. By performing endometrial sampling and OPU (oocyte pick-up) in the same session, no extra burden was placed on the patient in terms of both financial and anesthesia application. All of the participants in UEI group were required to meet the inclusion criteria: (1) clinical and laboratory diagnosis of UEI; (2) no hormonal medication or intrauterine device use within the past 3 months; and (3) absence of any gyencological or systemic disorders that may affect endometrial receptivity. Excluded cases were the ones with: (1) previous medication use; (2) endometrial pathology such as Asherman syndrome, endometrial polyp, submucous and/or intramural fibroids; (3) presence of pelvic inflammatory disease, endometriosis/endometrioma, or hydrosalpinx; (4) history of habitual abortion; (5) infertility etiology other than UEI.

We selected the control group patients among 30 patients diagnosed with polycystic ovary syndrome (PCOS) and scheduled for FET. The fact that the control group had anovulatory PCOS gave us two different advantages. (i) Frequent application of FET to prevent ovarian hyperstimulation syndrome (OHSS) in these cases facilitated us in terms of endometrial sampling, (ii) the control group was prevented from overlapping with the UEI group because the cases were anovulatory. All PCOS participants in the control group were subjected to progesterone induced withdrawal bleeding to determine their secretory phases. Preopertaive blood samples were taken from both groups of subjects for complete hormonal assays and insulin analysis. In addition to demographics characteristics of women in PCOS and control group age, body mass index (BMI) (kg/m^2), total testosterone, fasting glucose, insulin, serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were also measured. Serum glucose levels were measured in autoanalyzer by using photometric method (Roche Diagnostic c501, Tokyo, Japon). The fasting serum insulin, LH, FSH, testosterone, estrogen and progesterone levels were measured in autoanalyzer by using electrochemiluminescence immunoassay (Roche Diagnostic c601, Tokyo, Japon). Homeostatic model assessment [HOMA-IR] Formula was used for calculating insulin resistance [11]. This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local Ethics Committee of the Beykoz University (2020/4). Written informed consent was obtained from all participants at the time of enrollment.

2.2 Endometrial tissue sampling

Endometrial samples were obtained from 30 subjects with UEI and 30 PCOS subjects undergoing OPU. Since all embryos would be frozen in patients in the UEI and control group, endometrial sampling was performed with a pipelle cannula following OPU. Each endometrial tissue was rinsed with 1x phosphate-buffered saline (PBS) to remove excess blood and subsequently weighted as wet weight. Then, the clean endometrial samples were homogenized in 1 mL of 1 $\times$ PBS and stored overnight at a temperature $\leq$–20 °C. After 2 freeze–thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 g to get supernatant.

2.3 BMP-2 determination in the supernatant of endometrial tissues by enzyme-linked immunosorbent assay

In women with UEI and control participants, endometrial BMP-2 concentrations were measured by enzyme-linked immunosorbent assay (ELISA). This immunoassay kit (Quantikine, BMP-2 Immunoassay, R&D Systems, Minneapolis, MN, USA) allows for *in vitro* quantitative determination of human BMP-2 concentrations in cell culture supernatant, bone extracts, serum, plasma, other biological fluids, and tissue extracts. All endometrial samples were assayed according to the manufacturer's instructions and were tested in duplicate by personnel blinded to each patient's group. The optical density of each well was determined using a microplate reader. No interference and no cross-reactivity were ex-

pected based on the manufacturer's instructions. The results of BMP-2 concentrations were divided by the weights of endometrial tissue.

2.4 Statistical analysis

The Statistical Package for Social Sciences, version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. BMP2 and other individual group parameters were assessed with one-sample Kolmogorov–Smirnov Z test and were found to be abnormally distributed. Hence, statistical comparisons between groups were performed by nonparametric Mann-Whitney U test. Spearman's correlation analysis was used for detecting correlation between BMP-2 and other parameters. Data are presented as median (range). For all comparisons, statistical significance was defined by $p < 0.05$.

3. Results

The demographic and laboratory findings of the patients are shown in Table 1. The patients in the UEI group and control group were found to be similar in terms of age and body mass index values. Serum total testosterone, LH (luteinizing hormone) and estradiol levels of patients in the control group were significantly higher than the UEI group. similarly, fasting insulin, glucose and HOMA-IR levels of the control group were found to be significantly higher than the UEI group. The number of total oocytes, MII oocytes and 2PN embryos in the control group were significantly higher than in the UEI group. The probable reason for the difference between the two groups in metabolic and hormonal markers may be that the patients in the control group were selected from PCOS patients.

The mean BMP-2 level was significantly lower in the endometrium of women with UEI compared to control cases. BMP-2 levels in the endometrial supernatants of UEI patients were found to be about 2 times lower than the patients in the control group (984.5 (350) pg/mg wet tissue vs. 1720 (318) pg/mg wet tissue, $p < 0.000$, Z = –6.6). A strong and significant positive correlation was found between endometrial thickness, estradiol levels measured on hCG day and BMP-2 levels. A positive and significant correlation was found between BMP-2 levels and serum LH levels in the UEI group. A positive and significant correlation was found between endometrial BMP2 levels and fasting insulin, glucose, testosterone, HOMA-IR and mature oocyte counts. There was no significant correlation between other hormonal and reproductive parameters and BMP-2 levels in the UEI and control groups (Table 2).

4. Discussion

The main result we found in our study is a significantly lower BMP-2 levels in the endometrium of UEI patients. Decreased endometrial BMP-2 levels in UEI cases without tubal, ovulatory and male factor infertility may be one of the underlying causes of subfertility due to UEI. In order for this idea to be better understood, we need to explain the role of BMP-2 in implantation in more detail. BMP-2 is an essential growth factor that is released from endometrial stromal cells (ESC) and contributes to decidualization by acting paracrine on the same cell [9, 10, 12]. ESC cells contain BMP membrane receptors (BMPRs) type I and type II. BMP-2 phosphorylates smad-1 in the cytoplasm by attaching to both receptors in the cell membrane [8, 13]. Smad-1p then interacts with smad-4 to form smad-4p. Smad-4p enters the nucleus of the ESC and initiates implantation by increasing the expression of homeobox A10 (HOXA 10) and leukemia inhibitory factor (LIF), the main genes involved in decidualization [7, 13]. When compared with the patients in the control group, we found 2-fold decreased endometrial bmp-2 levels in patients in the UEI group. This significant decrease in BMP-2 levels may lead to insufficient decidualization and negatively affect the implantation of the transferred embryos. Sinclair *et al.* [13] reported that in the case of decreased BMP-2 levels or resistance to BMP-2, HOXA 10 and LIF gene expression decreased. Although receptivity genes were not evaluated in our study, we can argue that the significant reduction in BMP-2 may contribute to UEI-related subfertility by disrupting receptivity gene expression and subsequent decidualization [12].

Another critical finding we obtained in our study is the positive correlation between endometrial BMP-2 levels and LH, estradiol and endometrial thickness. This finding is important in that it supports the close relationship of BMP-2 synthesis and release with estrogen synthesis. Estrogen is critical for establishment of blastocyst attachment, implantation, and proliferation [14]. The lack of difference between the UEI and the control group in terms of endometrial thickness and estradiol levels on the day of hCG suggests that the decrease in BMP-2 levels in UEI cases occurs by a different mechanism. An example of this idea is that endometrial stromal cells of patients with myoma secrete less BMP-2 than those without fibroids. Similarly, myomas secrete TGF-β3 and block BMP-2 from acting through ESC [13]. When these findings and our findings are evaluated together, significantly lower endometrial BMP-2 levels in UEI cases may be due to the presence of a neglected small myoma, intrauterine adhesions, endometrial polyp or peritoneal or ovarian endometriosis/endometrioma rather than hormonal factors [15, 16]. For this reason, we recommend that ovaries, fallopian tubes, endometrium and myometrium should be evaluated in detail, as well as HSG, semen analysis and ovulatory evaluation in patients with UEI with recurrent implantation failure. Lower BMP-2 levels in UEI cases may indicate the presence of a non-clinical pathology located in the intrauterine cavity or cervical canal. Similar to the decrease in BMP-2 secretion in the presence of fibroids [13], lesions located in the cavity or in the cervical canal may impair BMP-2 secretion or the interaction between BMP-2 and endometrial stromal cells. Hysteroscopic evaluation of UEI cases may be important in this respect. Chiofaloa *et al.* [17] reported that office hysteroscopy is a highly effective di-

Table 1. Demographic and clinical characteristics of UEI and control groups.

	UEI (n = 30)	Control (n = 30)	*Z*	*p*
	Median (Range)	Median (Range)		
Age (year)	26 (11)	22.5 (14)	–0.05	0.5
BMI (kg/m^2)	26 (7)	25.5 (14)	–0.5	0.5
Infertility duration (month)	65 (74)	60 (60)	–0.3	0.7
IVF-ET attempt	2 (4)	1.5 (5)	–0.6	0.5
Total rFSH dose	2525 (2700)	2509.5 (3100)	–1	0.3
Endometrial thickness (mm)	10 (3)	10 (3)	–0.6	0.5
FSH (IU/L)	7 (2.1)	6.9 (4)	–0.4	0.6
LH (IU/L)	5 (3.2)	8.05 (2)	–6.6	0.000*
E2 (pg/mL)	49 (38)	49 (9)	–0.1	0.000*
P4	0.27 (1.05)	0.23 (1.05)	–0.1	0.8
E2 on hCG day	1914.5 (5845)	1635.5 (6164)	–1.5	0.1
P4 on hCG day	0.8 (0.8)	0.87 (1.5)	–0.7	0.4
Total oocyte	9 (8)	12 (19)	–2.6	0.007*
MII oocyte	3 (19)	7.5 (15)	–2.6	0.007*
2 PN	3 (13)	7 (11)	–3.06	0.002*
BMP-2	984.5 (350)	1720 (318)	–6.6	0.000*
Insulin	6.98 (9.7)	12.3 (12.8)	–3.4	0.001*
HOMA-IR	1.12 (5.9)	3.44 (8.86)	–3.7	0.000*
Glucose	89 (8)	90 (11)	–1.4	0.1
Testosterone	0.4 (0.9)	0.85 (0.9)	–4.3	0.000*

*: depicts $p < 0.05$.

BMI, body mass index; IVF-ET, *in vitro* fertilization - embryo transfer; FSH, follicle-stimulating hormone; LH, luteinizing hormone; P4, progesterone; E2, estradiol; hCG, human chorionic gonadotropin; MII, oocyte maturation; PN, pronuclear; BMP-2, bone morphogenetic protein-2; HOMA-IR, homeostatic model assessment - insulin resistance.

Table 2. Spearman's correlations between BMP-2 and other parameters.

	Correlation (r)	*p*-value
Age (year)	0.1	0.4
BMI (kg/m^2)	–0.07	0.5
Infertility duration (month)	0.03	0.8
IVF-ET attempt	–0.009	0.9
Total rFSH dose	–0.15	0.2
Endometrial thickness (mm)	0.88	0.01*
FSH (IU/L)	–0.05	0.6
LH (IU/L)	0.75	0.000*
E2 (pg/mL)	0.07	0.5
P4	0.04	0.7
E2 on hCG day	0.70	0.02*
P4 on hCG day	0.14	0.2
Total oocyte	0.23	0.06
MII oocyte	0.27	0.03*
2 PN	0.33	0.01*
Insulin	0.45	0.000*
HOMA-IR	0.41	0.001*
Glucose	0.26	0.04*
Testosterone	0.49	0.000*

*: depicts $p < 0.05$.

agnostic and therapeutic intervention for the management of cervico-isthmic and intrauterine adhesions, as well as for small polyps. Further, surgical removal of these lesions detected in hysteroscopy may contribute positively to receptivity [15, 16]. Unlu *et al.* [16] reported that myomectomy has a favorable effect on endometrial home box gene expression. Celik *et al.* [15] reported that surgical removal of endometrioma improves endometrial receptivity suggesting that there is a functional link between reptoductive tractus lesions and endometirum receptivity. When our findings and literature data are evaluated together, we can conclude that decreased BMP-2 levels in UEI cases may coexist with other pathologies [13, 15, 16]. Surgical treatment of these pathologies may lead to improvement in endometrial receptivity in UEI cases, but this idea needs to be confirmed by further studies.

We do not have sufficient data on how metabolic parameters affect receptivity in UEI cases. Since our control group patients were selected among those with PCOS, we evaluated serum insulin, testosterone and HOMA-IR in both groups. High testosterone, insulin and HOMA-IR values we found in control group may cause subfertility directly or indirectly [18]. The positive correlation between BMP-2 and HOMA-IR, testosterone and fasting insulin levels suggests that metabolic parameters may affect endometrial receptivity in UEI cases. For this reason, we can say that the synthesis and release of BMP-2 from endometrial

stromal cells can be affected by the metabolic parameters in women with UEI. This data is important in terms of providing clues that the subfertility-causing effects of metabolic diseases such as PCOS are through BMP-2 [19]. Similarly, disturbed metabolic parameters may have an effect on BMP-2 release and leading to subfertility in UEI patients. However, although there was no significant difference between estrogen and progesterone levels measured on hCG day between the two groups in our study, the lower BMP-2 levels in the UEI group suggests that BMP-2 was regulated differently in this patient group. This paradoxical situation needs to be clarified because it is a known fact that both estrogen and progesterone stimulate synthesis and release of receptivity molecules [18].

5. Conclusions

Expression levels of BMP-2, one of the most important regulators of the endometrium at the implantation stage, were significantly lower in UEI cases. Endometrial BMP-2 is associated with UEI and mechanistically it may be useful to study this further to determine is this is causal or merely a biomarker. Finding the underlying reason for the significanlty lower endometrial BMP-2 levels and arranging treatment accordingly may improve reproductive outcome in women with UEI.

Author contributions

AY designed the research study and wrote the manuscript. NDG and TG performed the research and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the local Ethics Committee of the Beykoz University (2020/4), informed consent was obtained from all participants at the time of enrollment.

Acknowledgment

We thank participants, anonymous reviewers for excellent criticism of the article.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Buckett W, Sierra S. The management of unexplained infertility: an evidence-based guideline from the Canadian Fertility and Andrology Society. Reproductive BioMedicine Online. 2019; 39: 633–640.

[2] Gunn DD, Bates GW. Evidence-based approach to unexplained infertility: a systematic review. Fertility and Sterility. 2016; 105: 1566–1574.e1.

[3] Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohi J, Simon C. The follicular and endocrine environment in women with endometriosis; local and systematic cytokine production. Fertility and Sterility. 1998; 70: 425–431.

[4] Mol BW, Tjon-Kon-Fat R, Kamphuis E, van Wely M. Unexplained infertility: is it over-diagnosed and over-treated? Best Practice & Research Clinical Obstetrics & Gynaecology. 2018; 53: 20–29.

[5] Ersahin A, Celik O, Acet M, Ersahin S, Acet T, Bozkurt DK, *et al.* Impact of Endometrioma Resection on Eutopic Endometrium Metabolite Contents: Noninvasive Evaluation of Endometrium Receptivity. Reproductive Sciences. 2017; 24: 790–795.

[6] Killick SR. Ultrasound and the receptivity of the endometrium. Reproductive BioMedicine Online. 2007; 15: 63–67.

[7] Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK, *et al.* Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. Proceedings of the National Academy of Sciences. 2001; 98: 1047–1052.

[8] Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnionchorion and cardiac development. Development. 1996; 122: 2977–2986.

[9] Lee KY, Jeong J, Wang J, Ma L, Martin JF, Tsai SY, *et al.* Bmp2 is Critical for the Murine Uterine Decidual Response. Molecular and Cellular Biology. 2007; 27: 5468–5478.

[10] Li Q, Kannan A, Wang W, Demayo FJ, Taylor RN, Bagchi MK, *et al.* Bone morphogenetic protein 2 functions via a conserved signaling pathway involving Wnt4 to regulate uterine decidualization in the mouse and the human. The Journal of Biological Chemistry. 2007; 282: 31725–31732.

[11] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004; 27: 1487–1495.

[12] Li Q, Kannan A, Das A, Demayo FJ, Hornsby PJ, Young SL, *et al.* WNT4 acts downstream of BMP2 and functions via beta-catenin signaling pathway to regulate human endometrial stromal cell differentiation. Endocrinology. 2013; 154: 446–457.

[13] Sinclair DC, Mastroyannis A, Taylor HS. Leiomyoma simultaneously impair endometrial BMP-2-mediated decidualization and anticoagulant expression through secretion of TGF-β3. The Journal of Clinical Endocrinology and Metabolism. 2011; 96: 412–421.

[14] Hong K, Choi Y. Role of estrogen and RAS signaling in repeated implantation failure. BMB Reports. 2018; 51: 225–229.

[15] Celik O, Unlu C, Otlu B, Celik N, Caliskan E. Laparoscopic endometrioma resection increases peri-implantation endometrial HOXA-10 and HOXA-11 mRNA expression. Fertility and Sterility. 2015; 104: 356–365.

[16] Unlu C, Celik O, Celik N, Otlu B. Expression of Endometrial Receptivity Genes Increase after Myomectomy of Intramural Leiomyomas not Distorting the Endometrial Cavity. Reproductive Sciences. 2016; 23: 31–41.

[17] Chiofalo B, Palmara V, Vilos GA, Pacheco LA, Lasmar RB, Shawki O, *et al.* Reproductive outcomes of infertile women undergoing "see and treat" office hysteroscopy: a retrospective observational study. Minimally Invasive Therapy & Allied Technologies. 2019; 30: 147–153.

[18] Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. The Journal of Clinical Endocrinology and Metabolism. 1999; 84: 1129–1135.

[19] Celik O, Aydin S, Celik N, Ugur K, Yavuzkir S, Hatirnaz S, *et al.* Molecular role of peptides/proteins in subfertility of polycystic ovarian syndrome. Cellular and Molecular Biology. 2019; 65: 32–40.