Journal of Assisted Reproduction and Genetics (2019) 36:1127–1133
https://doi.org/10.1007/s10815-019-01459-0

ASSISTED REPRODUCTION TECHNOLOGIES

# Can follicular Emmprin and BMP 4 levels predict ICSI outcome?

**Ozguc Takmaz [1] · Dilara Yozgatli [2] · Selin Ozaltin [3] · Esra Ozbasli [1] · Yucel Kocyigit [4] · Sibel Bulgurcuoglu Kuran [5] · Huri Bulut [6] · Mete Gungor [1] · Faruk Buyru [4] · Ercan Bastu [1]**

Received: 28 January 2019 / Accepted: 23 April 2019 / Published online: 8 May 2019


## Abstract

**Purpose** To evaluate the relationship of clinical pregnancy rates with bone morphogenetic proteins 2-4-7 (BMP 2, 4, 7), growth differentiation factor 9 (GDF 9), and Emmprin levels in follicular fluid of intracytoplasmic sperm injection patients.

**Methods** Follicular fluid of 77 patients who underwent ICSI procedure was collected during the oocyte retrieval procedure. And follicular fluid levels of BMP 2, BMP 4, BMP 7, GDF 9, and Emmprin (Basigin) were measured and compared for clinical pregnancy rates.

**Results** Follicular levels of BMP 4 was significantly higher whereas Emmprin levels were lower in patients who had achieved clinically diagnosed pregnancy compared with those who did not achieve clinical pregnancy after ICSI procedure ($P = 0.007$ and $P = 0.035$, respectively). BMP 2, BMP 7, and GDF 9 levels were comparable for both groups.

**Conclusion** Clinical pregnancy rates after ICSI may be associated with follicular fluid levels of Emmprin and BMP 4. Follicular levels of Emmprin and BMP 4 can be used as a marker (as markers for predicting ICSI outcomes) for a better ICSI outcome.

**Keywords** Basigin · BMP 4 · Emmprin · Follicular fluid · Intracytoplasmic sperm injection · In vitro fertilization

## Introduction

Since the 1990s, the effect of follicular microenvironment on the development of the oocyte and the quality of embryo has been extensively investigated [1]. It has been shown that the quality of the oocyte and embryo strongly depends on hormonal and growth factors found in follicular fluid [2]. However, the mechanism of action of these molecules is not entirely understood.

Among the growth factors, bone morphogenetic proteins (BMPs) and growth differentiation factor 9 (GDF 9), members of the transforming growth factor beta (TGF-beta) superfamily, have critical roles in oocyte and follicular maturation [3–5]. Mutations affecting the function of these proteins lead to dysregulated signaling, thus interfering with the success of human conception [6].

BMP 4 along with BMP 7 is produced by ovarian stromal cells and theca cells and thought to enhance the transition from primordial to primary follicles [4]. BMP 4 correlates with the increasing size of primary and secondary follicles as well as with the increasing number of primary follicles [7]. BMP 2 acts as a heterodimer with BMP 7 in the process of folliculogenesis [8, 9]. GDF 9 in conjunction with BMPs functions in oocyte maturation too [10].

Emmprin (extracellular matrix metalloproteinase inducer), also called Basigin (CD147), is a transmembrane glycoprotein belonging to the immunoglobulin superfamily [11]. Lack of Emmprin has been shown to be related with infertility in both male and female mice [11, 12]. Emmprin plays roles in oocyte maturation, fertilization, implantation, and early embryonic development [11, 13].

✉ Ozguc Takmaz
ozguctakmaz@hotmail.com

1 Department of Obstetrics and Gynecology, Acibadem Mehmet Ali Aydinlar University, Buyukdere Cd. No:40, Maslak, 34457 Sariyer/İstanbul, Turkey

2 Cerrahpasa School of Medicine, Istanbul University, Istanbul, Turkey

3 Department of Obstetrics and Gynecology, Acibadem Maslak Hospital, Istanbul, Turkey

4 Department of Obstetrics and Gynecology, Acibadem Fulya Hospital, Istanbul, Turkey

5 Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Istanbul School of Medicine, Istanbul University, Istanbul, Turkey

6 Department of Biochemistry, Bezmi Alem University, Istanbul, Turkey

In the present study, we compared the levels of BMP 2, 4, and 7, GDF 9, and Emmprin proteins measured in the follicular fluid samples between those with and without clinical pregnancy after intracytoplasmic sperm injection-embryo transfer (ICSI-ET) procedure.

## Materials and methods

This study was the continuation to our previously published study with the title of "Follicular fluid levels of Cathepsin B, relaxin and AMH" [14]. The follicular fluid samples used in the previous study have been preserved for the purpose of conducting future research in our institution. Follicular fluid samples were collected in tubes containing heparin by cardiac puncture. Samples were removed by centrifugation for 10 at 2000 × rpm. The samples were maintained at − 80 °C before performing assays. These samples were thawed to measure the levels of BMP 2, 4, and 7, GDF 9, and Emmprin. Institutional review boards approved the study and informed consent forms had been obtained from all participants included in the study. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### Study population

The study analyzed the follicular fluid samples of 79 patients who underwent ICSI procedure in the Infertility Clinic of Istanbul University School of Medicine (Istanbul, Turkey) between March and December 2012. The follicular fluid samples had been obtained from women with a tubal factor or unexplained infertility. These women did not have a history of poor response to controlled ovarian hyperstimulation (COH) or ovarian surgery, endometriosis, hydrosalpinx, and ovulatory disorders including polycystic ovary syndrome. All women also had antral follicle count (AFC) $\geq 5$ and their ages were less than or equal to 39 years. The study population was divided into two groups based on the presence of clinical pregnancy after in vitro fertilization (IVF) procedure. Group 1 consisted of women who achieved clinical pregnancy and group 2 consisted of women who did not achieve clinical pregnancy.

### Controlled ovarian hyperstimulation

GnRH antagonist protocol with a daily 225–300 IU of recombinant follicle stimulating hormone (FSH) (Puregon (Schering-Plough, NJ, USA) or Gonal-f (EMD Serono, MA, USA)) was started on the third day of the cycle for COH. Oral contraceptive pills were not utilized before the COH cycle. Starting dose of the gonadotropins was determined for every patient individually on the basis of the patients' antral follicle count, body mass index (BMI), and response to prior stimulation regime. To prevent premature LH surge, when the leading follicle reached 14 mm in diameter and the serum estradiol levels reached > 600 pg/mL, daily cetrorelix (Cetrotide; Serono, Geneva, Switzerland; 0.25 mg, SC) injection had been administered until the human chorionic gonadotropin (hCG) injection day.

### Ovarian follicular development and oocyte retrieval

Follicular development was followed up at one-to-three-day interval vaginal ultrasonography. hCG was administered when three or more follicles reached 17 mm in diameter to achieve follicular maturation. Oocyte retrieval was performed under general anesthesia with a 17-guage needle 35 to 36 h after the hCG injection. Follicles, 14 mm and larger, were retrieved and the number of oocytes was recorded. ICSI was performed after denudation and a 2-h incubation of the oocyte–corona complexes.

### Embryo transfer and luteal phase support

In accordance with the Turkish legislation of elective single embryo transfer (SET); to the patients ≤ 35 years old and in their first two IVF attempts, one embryo was transferred. Two embryos were transferred to the patients who were older than 35 years old or had two or more failed IVF attempts. Grade-1/2 embryos were transferred. The transfer took place either on day 2 or day 3. The number of grade-3 embryos was recorded to be evaluated. Vaginal progesterone was administered for luteal phase support on the first morning after the oocyte retrieval and continued until the 12th week of pregnancy if pregnancy is achieved.

### Follicular fluid aspiration

Each follicle was recovered in a different tube during individual aspiration. To avoid contamination from blood, flush medium, or mixed follicular fluid, only the first retrieved leading follicle's fluid was collected. Thus, one follicular fluid sample per patient was used for analysis. Samples were centrifuged at 2000*g* for 10 min, and the supernatants were stored at − 80 C for further analysis.

### Assessment of fertilization, embryo quality, and pregnancy

Fertilization results were assessed 18 h after ICSI for the appearance of two distinct pronuclei and two polar bodies. Cleavage was evaluated 24 h after fertilization. Embryo quality was assessed on the second day of fertilization and graded as follows: grade I, symmetric blastomeres and no fragmentation; grade II, blastomeres different in size and shape with < 25% fragmentation; and grade III, blastomeres different in size and shape with > 25% fragmentation. Blood levels of b-hCG were measured on the 14th day after embryo transfer. Biochemical pregnancy was considered when the b-hCG level was > 5 mIU/mL. Clinical pregnancy was confirmed by the presence of a fetal heartbeat using vaginal ultrasound at 6 weeks of amenorrhea.

### Determination of BMP 2, BMP 4, BMP 7, GDF 9, and Emmprin levels in follicular fluid

Protein content of the follicular fluid was measured by the Bradford method to prevent potential bias owing to different volumes of the follicular fluid [15].

Human BMP 2, BMP 4, BMP 7, EMMPRIN/CD147, and GDF 9 enzyme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm Co. Ltd., China) was used for the quantitative measurement. Samples and standards were added to appropriate wells which are pre-coated with antihuman monoclonal antibody before incubation. Biotin was added to all wells and combined with streptavidin-HRP to form immune complex and then carry out incubation again and washing to remove the uncombined enzyme. Then chromogen solutions A and B were added when the color of the liquid changes into blue. At the effect of acid, the color finally becomes yellow. Optical density was read on a standard automated plate reader at 450 nm (Thermo Scientific Microplate Reader). BMP 2-4-7 levels were expressed as ng/mL and Emmprin and GDF 9 levels were expressed as ng/L. The detection range was 2–16 ng/mL for BMP 2, 7.5–480 ng/mL for BMP 4, 0.75–24 ng/mL for BMP 7, 100–3200 ng/L for EMMPRIN, and 75–4800 ng/L for GDF 9.

#### Statistical analyses

The assumption of normality was made using the Shapiro-Wilk test. The Mann-Whitney *U* test was used to evaluate non-normally distributed data, which is reported as median (minimum-maximum), and the Student *t* test was used to evaluate normally distributed dada which is reported as mean ± standard deviation. The Box-Cox transformation was applied to make the distributions more symmetric. The natural algorithm was used for Emmprin and GDF 9 and inverse transformation was used for BMP 2-4-7. The association between pregnancy results and the protein levels was modeled by logistic regression analysis. *P* value of < 0.05 was considered the threshold for statistical significance. All analyses were carried out in R Version 3.5.2 (R Core Development, 2018).

## Results

A total of 79 follicular fluid samples were examined. Of these, 23 (29.1%) were the ones obtained from women who achieved clinical pregnancy and the remaining 56 (79.9%) were the ones obtained from women who did not. Characteristics of these patients are presented in Table 1. The groups did not differ in terms of age, BMI, duration of infertility, day-3 FSH, and estradiol levels (Table 1).

Table 2 shows the comparisons of levels of BMP 2, 4, and 7, Emmprin, and GDF 9 between the groups. The median BMP 4 level was significantly higher in women who achieved clinical pregnancy than in those who did not ($P = 0.03$), whereas the median *Emmprin* level was significantly lower in women who achieved clinical pregnancy compared with those who did not ($P < 0.001$). The levels of other proteins studied were similar between the groups.

BMP 4 was found to be associated with the probability of pregnancy ($P = 0.03$). Area under the curve (AUC) of BMP 4 for prediction of pregnancy was found to be 0.61 (95% confidence interval 0.45–0.78; $P = 0.43$). Sensitivity and specificity of BMP 4 with a 0.14 cut-off level were 0.52 and 0.67, respectively.

Emmprin was not found to be associated with the probability of pregnancy ($P = 0.15$). However, when transformed levels of BMP 4 and Emmprin were included together in logistic regression, AUC was found to be 0.70 (95% confidence interval 0.55, 0.84; $P = 0.03$). A threshold of 0.14 had a sensitivity of 67% and specificity of 67% (Fig. 1; receiver operating characteristic (ROC) curve of combined BMP 4 and Emmprin levels for prediction of pregnancy).

Pearson correlation analysis was performed to assess correlations between BMP 2, BMP 4, BMP 7, GDF 9, and Emmprin levels and age as well as BMI. Neither age nor BMI showed a correlation with any of these markers (correlation coefficient for age = − 0.04, 0.03, 0.03, 0.02, and 0.24, respectively, $P > 0.05$ for all; correlation coefficient for BMI = − 0.01, 0.02, − 0.14, 0.02, and − 0.10, respectively, $P > 0.05$ for all).

Table 3 shows the comparison of the number and the maturity of the oocytes between the groups. The number of oocytes, metaphase II (MII) oocytes, and MII oocyte rate was significantly higher in the pregnancy group ($P < 0.05$); however, the number of transferred embryos and fertilization rate were comparable between groups ($P > 0.05$).

We also compared the proteins' levels between those with a tubal factor and those with unexplained infertility. No differences were observed between them ($P > 0.05$ for all).

**Table 1** Characteristics of the patients

| | Pregnant ($n = 23$) (mean ± std) | Non-pregnant ($n = 56$) (Mean ± std) | $P$ value |
|---|---|---|---|
| Age (years) | 31.55 ± 4.76 | 33.12 ± 4.67 | 0.21 |
| BMI (kg/m$^2$) | 25.1 ± 3.2 | 25.6 ± 2.3 | 0.76 |
| Period of infertility(months) | 87.90 ± 47.69 | 85.18 ± 60.58 | 0.85 |
| Day-3 serum FSH(mIU/mL) | 7.64 ± 4.55 | 9.11 ± 4.87 | 0.27 |
| Day-3 serum E2 (pg/mL) | 46.84 ± 20.91 | 68.67 ± 89.14 | 0.28 |
| Gonadotropin doses (IU) | 2175 ± 192.3 | 2250 ± 268.32 | 0.86 |
| Number of transferred embryos (grade 1/2) | 1.56 ± 1.15 | 1.46 ± 1.71 | 0.18 |
| Day of Embryo Transfer | | | 0.92 |
| Day 2 | 10 (43.5) | 34 (60.7) | |
| Day 3 | 13 (57.1) | 22 (39.3) | |

## Discussion

In this study, we found that the follicular fluid samples of women who achieved clinical pregnancy had a higher BMP 4 level and a lower Emmprin level when compared with those who did not achieve clinical pregnancy. The combination of BMP 4 with Emmprin predicted clinical pregnancy with a sensitivity of 67% and a specificity of 67% at a cut-off level of 0.14.

BMPs and GDF are members of the TGF-beta superfamily [3, 4]. BMPs are usually found in heterodimer forms: BMP 2/BMP 7, BMP 2/BMP 6, BMP 4/BMP 7, and BMP 15/GDF 9 [8, 9, 16, 17]. These forms are biologically more active than their respective homodimers in the regulation of cellular functions [8, 9, 17].

BMP 4 is synthesized by theca cells and acts on the granulosa cells and oocytes in a paracrine manner [18]. Tanwar and McFarlane revealed that BMP 4 protein is detectable in all stages of follicular development and thus may facilitate the transition from primordial to primary follicle [19]. Nilsson and Skinner revealed that the injection of BMP 4 into the ovaries of rats increased the number of developing primary follicles and decreased the number of primordial follicles [20]. They also showed that after adding a neutralizing BMP 4 antibody to the culture medium, the ovaries became smaller in size, the number of oocytes and primordial follicles diminished, and cellular apoptosis increased. In another study, Cunha et al. observed increases in the sizes of primary and secondary follicles following the treatment with 100 ng/mL BMP 4 for a duration of 6 days in in vitro culture medium [7]. On the contrary, some studies on bovine and ovine species claimed that BMP 4 does not affect the primordial primary follicle transition process [7, 21].

The mechanism of action of BMP 4 in follicles is not fully understood. However, Childs et al. suggested that BMP 4 is likely to reduce apoptosis in human granulosa cells, prevent their premature differentiation, and protect their steroidogenic properties [22]. The fact that our study showed higher BMP 4 levels in women who achieved clinical pregnancy compared with those who did not support the existing literature regarding the role of BMP 4 in follicular development. Our study revealed an association between the BMP 4 level and clinical pregnancy ($P = 0.03$); additionally, we found that BMP 4 together with Emmprin predicts clinical pregnancy with a sensitivity of 67% and a specificity of 67% at the cut-off level of 0.14. Accumulating evidence suggests that the amount of BMP 4 in follicular fluid would be a good marker in predicting ICSI outcomes. Although the existing literature suggests the positive effects of those cytokines on follicular maturation and implantation, our study could only point the possibility of predicting clinical pregnancy by using these markers; because of transferring only grade 1 or 2 embryos, we could not speculate on embryo development. Therefore, with our study results, it is not possible to elicit a comment on

**Table 2** Comparison of the follicular levels of the proteins between the groups

| | Non-pregnant ($n = 67$) median (min-max) | Pregnant ($n = 23$) median (min-max) | $P$ value |
|---|---|---|---|
| BMP 2 (ng/mL) | 1.3 (0.6–6.9) | 1.2 (0.8–2.1) | 0.64 |
| BMP 4 (ng/mL) | 41.2 (22.5–186.8) | 47.3 (34.9–205.8) | 0.03* |
| BMP 7 (ng/mL) | 2.9 (1.5–11.9) | 3.1 (1.7–3.8) | 0.96 |
| Emmprin (ng/L) | 183 (16–1329) | 116 (29–778) | 0.00* |
| GDF 9 (ng/L) | 315 (35–2640) | 287 (75–694) | 0.81 |

*$P < 0.05$

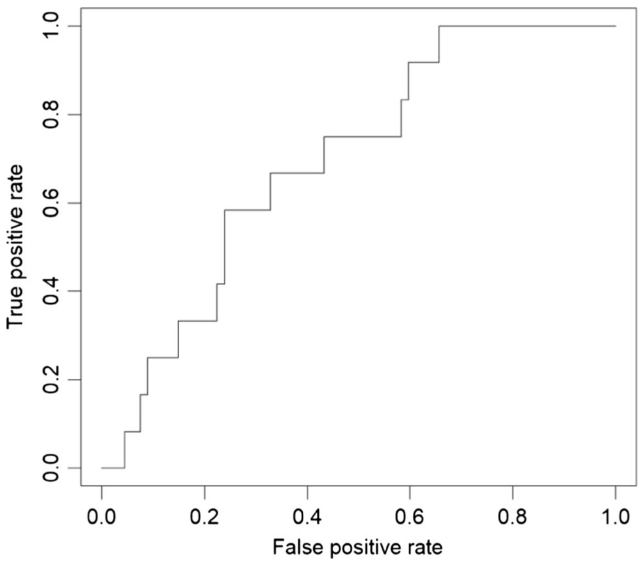


**Fig. 1** ROC curve of BMP 4 + Emmprin for prediction of pregnancy

the relationship between the levels of these proteins and embryo development/embryo quality.

Emmprin (Basigin) is a member of the immunoglobulin superfamily which acts as a cell surface glycoprotein (11). Several studies revealed that Emmprin plays roles in angiogenesis, neuronal signaling, tumorogenesis, and wound healing as well as follicular maturation and embryo implantation [23–26].

The complete physiologic mechanism of Emmprin in follicular growth and corpus luteum formation is not clear. However, it was hypothesized that Emmprin inducts matrix metalloproteinases which can facilitate the process of follicular development, ovulatory rupture, and luteinization [27]. Kuno et al. reported that Emmprin null mutant oocytes have significantly reduced fertilization [11]. And also, Smedts and Curry showed an ovarian Emmprin increase during luteal formation and function in rats [28].

In our knowledge, this is the first study that evaluates the human follicular fluid levels of Emmprin. Although several studies revealed an Emmprin increase during follicular growth and corpus luteum in animal models, we found a lower level of Emmprin in the follicular fluid samples from women who achieved clinical pregnancy after ICSI-ET. We may explain this difference in two ways. First, this may be related to the use of different methods in the measurement of Emmprin. In previous studies, Emmprin was evaluated with immunohistologic staining; however, we measured the levels of the Emmprin in the follicular fluid samples. Second, this difference may have resulted from the relation between Emmprin and luteinization. In a rat model, Smedts and Curry revealed that the Emmprin mRNA levels increase in post ovulatory luteinization [28]. And also, Chang et al. reported high Emmprin expression in early CL (day 1–3) in the mouse ovary; however, 6–15 days after hCG stimulation, Emmprin expression decreases [13]. In our study, follicular fluid samples were obtained during oocyte retrieval. If the luteinization of the follicles is the reason of the higher Emmprin levels, follicular Emmprin levels can be used for assessing the early luteinization of the patients who undergo IVF treatment.

Decreased fertility in Emmprin null mutant oocytes and Emmprin localization in granulosa and theca cells in preovulatory stage showed that Emmprin is effective in folliculogenesis and oocyte maturation [11, 28]. As to BMP 4, increasing primordial primary follicle transition and reducing apoptosis in granulosa cells indicate the positive effect of BMP 4 on oocyte maturation [4, 22, 29]. In our study, higher rates of MII oocytes in the pregnancy group supported the hypothesis that BMP 4 and Emmprin might be effective in folliculogenesis and oocyte maturation.

Beside BMP 4 and Emmprin, we also studied BMP 2-7-9 and GDF 9. Some of the previous studies showed the association of follicular development and fertilization capacity with those proteins (8, 9, 21, 22). Our study is the first study that evaluated those proteins in human follicular fluid, and no significant differences were determined for clinical pregnancy rates. Additionally, some studies revealed that BMP 2/BMP 7 and GDF 9/BMP 15 heterodimers are more active and more potent in follicular development [8, 9]. Thus, evaluating BMP 2/BMP 7 and GDF 9/BMP 15 heterodimers can be more reasonable instead of evaluating homodimers. However, Peng et al. showed that the levels of heterodimers in follicular fluid were too low to be detectable [9]. Thus, we did not evaluate the levels of heterodimers in our study samples.

The main limitation of our study is that since only day-2 or day-3 embryos were transferred in the study groups, we did not have data regarding day-5 embryos.

In conclusion, our findings suggested that the levels of Emmprin and BMP 4 in follicular fluid may be used as

**Table 3** Comparison of oocytes in pregnant and non-pregnant patients

| | Pregnant ($n = 23$) | Non-pregnant ($n = 56$) | $P$ |
|---|---|---|---|
| $N$ of oocytes | 10.00 ± 6.85 | 5.96 ± 3.94 | 0.002 |
| $N$ of MII oocytes | 8.65 ± 5.63 | 4.58 ± 3.36 | 0.001 |
| MII oocyte rate (%) | 88.98 ± 10.19 | 78.14 ± 23.30 | 0.04 |
| $N$ of transferred embryos (grade 1/2) | 1.56 1.15 | 1.46 ± 1.71 | > 0.05 |
| Fertilization rate (%) | 81 ± 18.39 | 76.25 ± 21.79 | > 0.05 |

markers in predicting ICSI outcomes. Further research studies are needed to better understand the importance of these markers in the prediction of ICSI outcomes.

**Acknowledgments** We are thankful for all procedural help of the Infertility Department of Istanbul School of Medicine staff.

**Funding** This study was funded by authors.

## Compliance with ethical standards

Institutional review boards approved the study and informed consent forms had been obtained from all participants included in the study. All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Burdge GC, Hanson MA, Slater-Jefferies JL, Lillycrop KA. Epigenetic regulation of transcription: a mechanism for inducing variations in phenotype (fetal programming) by differences in nutrition during early life? Br J Nutr. 2007;97(6):1036–46. https://doi.org/10.1017/S0007114507682920.
2. Leroy JL, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PE, et al. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. Theriogenology. 2004;62(6):1131–43. https://doi.org/10.1016/j.theriogenology.2003.12.017.
3. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev. 1996;17(2):121–55. https://doi.org/10.1210/edrv-17-2-121.
4. Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. Reproduction. 2006;132(2):191–206. https://doi.org/10.1530/rep.1.01074.
5. Juengel JL, Reader KL, Bibby AH, Lun S, Ross I, Haydon LJ, et al. The role of bone morphogenetic proteins 2, 4, 6 and 7 during ovarian follicular development in sheep: contrast to rat. Reproduction. 2006;131(3):501–13. https://doi.org/10.1530/rep.1.00958.
6. Persani L, Rossetti R, Di Pasquale E, Cacciatore C, Fabre S. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. Hum Reprod Update. 2014;20(6):869–83. https://doi.org/10.1093/humupd/dmu036.
7. da Cunha EV, de Souza GB, Passos JRS, Silva AWB, Dau AM, Saraiva MVA, et al. Effects of bone morphogenetic protein 4 (BMP4) on in vitro development and survival of bovine preantral follicles enclosed in fragments ovarian tissue. Zygote. 2017;25(3):256–64. https://doi.org/10.1017/S0967199417000089.
8. Israel DI, Nove J, Kerns KM, Kaufman RJ, Rosen V, Cox KA, et al. Heterodimeric bone morphogenetic proteins show enhanced activity in vitro and in vivo. Growth Factors. 1996;13(3–4):291–300.
9. Peng J, Li Q, Wigglesworth K, Rangarajan A, Kattamuri C, Peterson RT, et al. Growth differentiation factor 9:bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions. Proc Natl Acad Sci U S A. 2013;110(8):E776–85. https://doi.org/10.1073/pnas.1218020110.
10. Kaivo-Oja N, Mottershead DG, Mazerbourg S, Myllymaa S, Duprat S, Gilchrist RB, et al. Adenoviral gene transfer allows Smad-responsive gene promoter analyses and delineation of type I receptor usage of transforming growth factor-beta family ligands in cultured human granulosa luteal cells. J Clin Endocrinol Metab. 2005;90(1):271–8. https://doi.org/10.1210/jc.2004-1288.
11. Kuno N, Kadomatsu K, Fan QW, Hagihara M, Senda T, Mizutani S, et al. Female sterility in mice lacking the basigin gene, which encodes a transmembrane glycoprotein belonging to the immunoglobulin superfamily. FEBS Lett. 1998;425(2):191–4.
12. Igakura T, Kadomatsu K, Kaname T, Muramatsu H, Fan QW, Miyauchi T, et al. A null mutation in basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. Dev Biol. 1998;194(2):152–65.
13. Chang H, Ni H, Ma XH, Xu LB, Kadomatsu K, Muramatsu T, et al. Basigin expression and regulation in mouse ovary during the sexual maturation and development of corpus luteum. Mol Reprod Dev. 2004;68(2):135–41. https://doi.org/10.1002/mrd.20060.
14. Bastu E, Gokulu SG, Dural O, Yasa C, Bulgurcuoglu S, Karamustafaoglu Balci B, et al. The association between follicular fluid levels of cathepsin B, relaxin or AMH with clinical pregnancy rates in infertile patients. Eur J Obstet Gynecol Reprod Biol. 2015;187:30–4. https://doi.org/10.1016/j.ejogrb.2015.02.009.
15. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
16. Little SC, Mullins MC. Bone morphogenetic protein heterodimers assemble heteromeric type I receptor complexes to pattern the dorsoventral axis. Nat Cell Biol. 2009;11(5):637–43. https://doi.org/10.1038/ncb1870.
17. Valera E, Isaacs MJ, Kawakami Y, Izpisua Belmonte JC, Choe S. BMP-2/6 heterodimer is more effective than BMP-2 or BMP-6 homodimers as inductor of differentiation of human embryonic stem cells. PLoS One. 2010;5(6):e11167. https://doi.org/10.1371/journal.pone.0011167.
18. Young JM, McNeilly AS. Theca: the forgotten cell of the ovarian follicle. Reproduction. 2010;140(4):489–504. https://doi.org/10.1530/rep-10-0094.
19. Tanwar PS, McFarlane JR. Dynamic expression of bone morphogenetic protein 4 in reproductive organs of female mice. Reproduction. 2011;142(4):573–9. https://doi.org/10.1530/rep-10-0299.
20. Nilsson EE, Skinner MK. Bone morphogenetic protein-4 acts as an ovarian follicle survival factor and promotes primordial follicle development. Biol Reprod. 2003;69(4):1265–72. https://doi.org/10.1095/biolreprod.103.018671.
21. Bertoldo MJ, Duffard N, Bernard J, Frapsauce C, Calais L, Rico C, et al. Effects of bone morphogenetic protein 4 (BMP4) supplementation during culture of the sheep ovarian cortex. Anim Reprod Sci. 2014;149(3–4):124–34. https://doi.org/10.1016/j.anireprosci.2014.07.010.
22. Childs AJ, Kinnell HL, Collins CS, Hogg K, Bayne RA, Green SJ, et al. BMP signaling in the human fetal ovary is developmentally regulated and promotes primordial germ cell apoptosis. Stem Cells. 2010;28(8):1368–78. https://doi.org/10.1002/stem.440.
23. Davidson B, Goldberg I, Berner A, Kristensen GB, Reich R. EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. Clin Exp Metastasis. 2003;20(2):161–9.
24. Muramatsu T, Miyauchi T. Basigin (CD147): a multifunctional transmembrane protein involved in reproduction, neural function, inflammation and tumor invasion. Histol Histopathol. 2003;18(3):981–7. https://doi.org/10.14670/HH-18.981.
25. Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H, et al. The human tumor cell-derived collagenase stimulatory factor

(renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res. 1995;55(2):434–9.
26. Fan QW, Yuasa S, Kuno N, Senda T, Kobayashi M, Muramatsu T, et al. Expression of basigin, a member of the immunoglobulin superfamily, in the mouse central nervous system. Neurosci Res. 1998;30(1):53–63.
27. Smedts AM, Lele SM, Modesitt SC, Curry TE. Expression of an extracellular matrix metalloproteinase inducer (basigin) in the human ovary and ovarian endometriosis. Fertil Steril. 2006;86(3):535–42. https://doi.org/10.1016/j.fertnstert.2006.01.042.
28. Smedts AM, Curry TE Jr. Expression of basigin, an inducer of matrix metalloproteinases, in the rat ovary. Biol Reprod. 2005;73(1):80–7. https://doi.org/10.1095/biolreprod.104.036145.
29. Shimizu T, Kayamori T, Murayama C, Miyamoto A. Bone morphogenetic protein (BMP)-4 and BMP-7 suppress granulosa cell apoptosis via different pathways: BMP-4 via PI3K/PDK-1/Akt and BMP-7 via PI3K/PDK-1/PKC. Biochem Biophys Res Commun. 2012;417(2):869–73. https://doi.org/10.1016/j.bbrc.2011.12.064.