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Research Article

Growth Differentiation Factor-9 and Bone Morphogenic Protein-15 as Predictors of Oocyte and Embryo Quality in Sub-Fertile Women Undergoing Assisted Reproduction

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Abstract

Background: Oocyte-secreted proteins can provide evidence about folliculogenesis and express the quality of oocytes and the quality of the resulting embryos. ***Objective***: To evaluate the ability of serum and follicular fluid growth differentiation factor 9 (GDF-9) and bone morphogenic protein 15 (BMP-15) in predicting oocyte and embryonic quality, subsequent embryonic development and pregnancy rate. ***Methods***: A prospective cohort study involved 114 sub-fertile females who sought intracytoplasmic sperm injection (ICSI) to treat infertility. They are 18 to 43 years old, and their body mass index (BMI) ranged from 19 to 30 kg/m^2. Before ICSI, there was controlled ovarian stimulation and pituitary down-regulation. Following oocyte collection, microscopic assessment of oocyte and embryo quality was done. Serum was collected on the second day of the menstrual cycle, while follicular fluid was collected on the day of oocyte collection, and GDF-9 and BMP-15 were measured in both using a special kit by ELIZA. ***Results***: The pregnancy rate was 35.2%. Follicular fluid GDF-9, serum and follicular fluid BMP-15 showed significant positive correlations with the total number of mature oocytes. Follicular fluid BMP-15 showed significant positive correlations with total oocyte count and fertilization rate. Follicular fluid BMP-15 showed a significant and positive correlation to the total embryo quality count. ***Conclusions***: Serum and follicular fluid BMP-15 are good predictors of oocyte number and quality but have no role in predicting embryonic quality, blastocyst count or pregnancy rate.

Keywords: Oocyte-secreted factors, GDF-9, BMP-15, ICSI, Oocytes and embryos quality.

عامل تمايز النمو-9 والبروتين المورفجيني للعظام-15 كمنبئين بجودة البويضة والجنين لدى النساء دون الخصوبة اللائي يخضعن للتقنيات المساعدة على الإنجاب

الخلاصة

الخلفية: يمكن أن توفر البروتينات التي تفرزها البويضات دليلا على تكوين الجريبات وتعبر عن جودة البويضات وجودة الأجنة الناتجة. **الهدف**: تقييم تأثير مستوى المصل والسائل الجريبي من عامل تمايز النمو-9 والبروتين المورفجيني للعظام-15في التنبؤ بجودة البويضات والأجنة والتطور الجنيني اللاحق ومعدل الحمل. **الطريقة**: شملت دراسة أترابية مستقبلية على 114 أنثى دون الخصوبة سعين إلى حقن الحيامن داخل الهيولى لعلاج العقم. تتراوح أعمارهم بين 18 و 43 عاما، ويتراوح مؤشر كتلة الجسم من 19 إلى 30 كجم/م 2. بعد الحقن المجهري، كان هناك تحفيز متحكم فيه للمبيض وتنظيم الغدة النخامية. بعد جمع البويضات ، تم إجراء تقييم مجهري لجودة البويضة والأجنة. تم جمع المصل في اليوم الثاني من الدورة الشهرية، بينما تم جمع السائل الجريبي في يوم جمع البويضات ، وتم قياس GDF-9 و BMP-15 في كليهما باستخدام ELIZA. **النتائج**: كان معدل الحمل 35.2٪. أظهر السائل الجريبي GDF-9 والمصل والسائل الجريبي BMP-15 ارتباطات إيجابية كبيرة مع العدد الإجمالي للبويضات الناضجة. أظهر السائل الجريبي BMP-15 ارتباطات إيجابية كبيرة مع إجمالي عدد البويضات ومعدل الإخصاب. أظهر السائل الجريبي BMP-15 ارتباطا كبيرا وإيجابيا بإجمالي عدد الأجنة. **الاستنتاجات**: يعتبر المصل والسائل الجريبي من BMP-15 من المؤشرات الجيدة لعدد البويضات وجودتها ولكن ليس لهما دور في التنبؤ بالجودة الجنينية أو عدد الكيسات الأريمية أو معدل الحمل.

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INTRODUCTION

Folliculogenesis is a process of follicular formation and oocyte development that requires a coordinated interaction between the oocyte and the granulosa cells that surround it [1]. Multiple factors tend to regulate folliculogenesis; among these are specific transforming growth factor beta family proteins (TGF-β), growth differentiation factor 9 (GDF-9), and bone morphogenic protein 15 (BMP-15). These are dimeric proteins structurally related to each other with some degree of synergism [2]. Growth factors GDF-9 and BMP-15 (the GDF-9 co-factor) are started to be expressed by the oocyte during the primary follicle stage. Following the activation of certain follicular granulosa cell signaling pathways, GDF-9 supports the growth of the primary follicle to the secondary follicle stage [3]. Together, GDF-9 induces pre-antral follicular granulosa cells to produce hedgehog ligands and thus enhances follicular theca cell formation [4]. These oocyte-secreted factors also support granulosa cell proliferation and survival of the oocyte [5, 6]. Both have a pivotal role, supporting the metabolism and survival of cumulus cells upon reaching the pre-antral stage and promoting the expression of factors that maintain the integrity of cumulus cells and oocyte meiotic arrest [7]. GDF-9 has anti-apoptotic and granulosa cell proliferative effects during the pre-antral and early antral stages of follicle development [8]. BMP-15 has more anti-apoptotic effects and some proliferative effects. BMP-15 stimulates the expression of other proteins that are essential for the expansion of cumulus cells [9]. Studies exhibited that inactivation of GDF-9 and BMP-15 produces insufficient granulosa cell proliferative potential and failure of support follicular growth to the large antral stage. BMP-15 seems to be present throughout folliculogenesis, but its main effect is inhibiting cumulus cell apoptosis and preventing luteinization [10]. In summary, BMP-15 and GDF-9 have a role in the development of a competent oocyte with high fertilization potential [11]. It had been believed that serum BMP-15 is directly proportional to cumulus-granulosa cells and oocyte number [12], and serum GDF-9, especially when conducted with BMP-15, may be a good marker of oocyte quantity and/or quality [13]. So, this study aims to evaluate the ability of serum and follicular fluid levels of both factors to predict oocyte and embryonic quality following assisted reproduction.

METHODS

Study design and participants

A cohort observational prospective study involved 114 sub-fertile females who visited the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies consultation clinic, Al-Nahrain University, Baghdad, Iraq, throughout the period from September 2021 to January 2023 and were subjected to the ICSI program. The age of female partners ranged from 18 to 43 years old, and their body mass index (BMI) ranged from 19 to 30 kg/m^2.

Inclusion criteria

Females aged 18-43 years old, BMI 19-30 kg/m^2, a GnRH antagonist was used for pituitary down regulation, and those who accepted to participate in the study.

Exclusion criteria

Females aged less than 18 and more than 43 years old, BMI > 30 kg/m^2, pituitary down regulation with GnRH agonist, females with diminished ovarian reserve (hypogonadotropic hypogonadism, severe endometriosis, previous ovarian surgery, radiation or chemotherapy, premature ovarian failure), females who take oral contraceptives or GnRH agonists for 1 month before the start of the ICSI program (as both affect ovarian reserve markers, especially AMH) and those whose male partners had severe oligo-astheno-teratozospermia, azoospermia (frozen sperms obtained from the testes surgically), as poor sperm quality affects embryo quality following ICSI. Additionally, any participant who declined to participate in the current study was excluded.

Outcome measurements

Females were evaluated by medical and gynecological history, examination, anthropometric measures (weight, height and BMI), hormonal analysis (LH, FSH, and AMH), and trans-vaginal ultrasound (TVUS) for the assessment of ovarian reserve in the form of antral follicle count (AFC), which is the number of small follicles of 2–6 mm in diameter in both the ovaries and endometrium (endometrial thickness (ET) and endometrial pathology). These investigations were done on the second day of the menstrual cycle. About 6 ml of vein blood was taken from the ante-cubital fossa so that ELIZA could test the serum for GDF-9 and BMP-15 using a GDF-9 and BMP-15 Kit (Elabscience, USA). According to WHO (2010), male partners underwent seminal fluid analysis. Controlled ovarian stimulation was performed by the administration of different types of gonadotropins: human menopausal gonadotropin (HMG) in the form of in vitro fertilization-Menotropin (IVF-M), LG Chem Ltd., Korea, 75–150 IU (75 IU FSH+75 IU LH), or recombinant FSH (r-FSH) in the form of Gonal-F, Merck, 75–300 IU. When follicular recruitments are started and a good number of follicles reach a size of 14 mm, pituitary down-regulation is

started by using a gonadotropin-releasing hormone (GnRH) antagonist; Cetrotide 0.25 mg 1*1 S.C. (flexible protocol); ovulation trigger is done by the administration of 500 micrograms of recombinant human chorionic gonadotropin (r-hCG); Ovitrelle S.C. Oocyte collection was performed under general anesthesia (GA) by follicular puncture guided by TVUS for 34–36 hours following the ovulation trigger. A 17-g double-lumen needle of 30 cm length attached to the Cook® suction pump had been utilized, guided by a trans-vaginal U/S probe (5 MHZ). 1 ml of follicular fluid was collected for measuring GDF-9 and BMP-15 using the same serum kit by ELIZA. Microscopic assessment of oocyte maturity and quality was done, followed by ICSI, in which a single sperm was injected inside the oocyte using a fresh semen sample that was collected by masturbation in a special room inside the ICSI lab, and after preparation by swimming up from the pellet. Assessment of embryo quality was also estimated (oocyte and embryo quality assessment was done by the embryologist depending on special grading systems [14,15]. The fertilization rate was calculated by dividing the total number of fertilized oocytes (Zygotes) by the total number of injected mature oocytes by 100%. At least 2–3 good-quality embryos were transferred to the uterus using an embryo transfer catheter (fresh embryo transfer). Luteal phase support using vaginal progesterone suppositories (Cyclogest 200 µg * 3 per day) and injectable progesterone intramuscularly (Primolute Depot 250 µg every third day) was done starting from the evening of the day of oocyte pick-up until the day of the pregnancy test, which was performed by measuring beta-hCG in the serum 14 days following fresh embryo transfer. Pregnancy rates are calculated based on the total number of females with positive pregnancy tests divided by the number of females for whom fresh embryo transfer was done (100%), respectively.

Ethical considerations

The High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, approved the study as partial fulfillment of a Doctor of Philosophy degree in infertility and clinical reproduction. Informed consent was obtained from every participant to be involved in the study.

Statistical analysis

The data were presented in an Excel 2010 sheet and analyzed using SPSS version 26. Number and percentage were used to express qualitative variables, while quantitative variables that are normally distributed were expressed as mean and standard deviation. The chi-square test evaluated the relationship between any two categorical variables. One-way analysis of variance (ANOVA) assessed the difference in mean of numerical variables between more than two groups, provided that these numerical variables had a normal distribution. In order to assess individual differences in mean values between any two groups, a post hoc LSD test came after an ANOVA. The Pearson correlation test was used to study bivariate correlations. The level of significance was considered at a *p*-value of less than 0.05.

RESULTS

The demographic characteristics of the patients enrolled in this study are shown in Table 1.

Table 1: Demographic characteristics of patients enrolled in this study

Characteristic	*n*=114
Age (year)	32.37±6.0
BMI (kg/m^2)	23.47±3.19
Duration of infertility (year)	7.68±4.26
AMH (pg/ml)	2.00±1.85
FSH (miu/ml)	5.37±1.98
LH (miu/ml)	4.61±2.06
Serum GDF-9 (pg/ml)	124.14±59.06
Follicular fluid GDF-9 (pg/ml)	124.29±54.51
Serum BMP-15 (pg/ml)	130.21±53.73
Follicular fluid BMP-15 (pg/ml)	132.74±54.14
Type of infertility n(%)	
Primary	90(78.94)
Secondary	24(21.05)

Values were expressed as mean±SD, numbers, and percentages.

The mean serum and follicular fluid GDF-9 levels were 124.14±59.06 and 124.29±54.51 pg/ml, respectively. While mean serum and follicular fluid BMP-15 levels were 130.21±53.73 and 132.74±54.14 pg/ml, respectively. The causes of infertility were illustrated in Table 2, as shown below: The most common cause is unexplained subfertility, followed by polycystic ovarian syndrome (PCOS), moderate male factor and female age older than 38 years old, and male factor and female age more than 38 years old, respectively. While only four females are due to tubal obstruction, (Only mild-moderate male factor infertility was included; those with severe impairment of semen parameters and frozen sperm obtained by testicular biopsy were excluded in order to not affect embryo quality or pregnancy rate).

Table 2: Causes of infertility among the study population

Cause of infertility	*n*(%)
Unexplained	32(28.07)
Polycystic ovarian syndrome	25(21.92)
Male factor + advanced female age	20(17.54)
Male factor	19(16.66)
Advanced female age	14(12.28)
Tubal factor	4(3.50)

The pregnancy rate in the study group is illustrated in Table 3.

Table 3: Pregnancy rate of patients enrolled in the study (*n*=105).

Pregnancy	*n*(%)
Positive	37(35.23)
Negative	68(64.76)
Total	100(100)

Nine women were excluded from the calculation of the pregnancy rate due to the development of ovarian hyperstimulation syndrome (OHSS), the cancellation of fresh embryo transfer, and the freezing of all embryos. The pregnancy rate was 35.23%. Correlations of serum and follicular fluid GDF-9 and BMP-15 to oocyte characteristics and fertilization rate are shown in Table 4.

Table 4: Correlations of serum and follicular fluid GDF-9 and BMP-15 to oocyte characteristics and fertilization rate

Characteristic	GDF-9 S		GDF-9 F		BMP-15 S		BMP-15 F	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Total oocytes	0.169	0.072	0.176	0.061	0.175	0.062	0.185	0.048
MII (mature) oocytes	0.182	0.053	0.189	0.044	0.188	0.045	0.196	0.036
Fertilization rate	0.173	0.066	0.179	0.057	0.177	0.060	0.185	0.049

Follicular fluid GDF-9, serum and follicular fluid BMP-15 showed significant positive correlations with the total number of mature oocytes. Follicular fluid BMP-15 showed significant positive correlations with total oocyte count and fertilization rate. Correlations of serum and follicular fluid GDF-9 and BMP-15 to embryonic characteristics and pregnancy rate are shown in Table 5.

Table 5: Correlations of serum and follicular fluid GDF-9 and BMP-15 to embryo characteristics and pregnancy rate

Characteristic	GDF-9 S		GDF-9 F		BMP-15 S		BMP-15 F	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Total embryo	0.173	0.066	0.181	0.054	0.180	0.055	0.189	0.044
Good quality embryo (Grade I&II)	0.144	0.128	0.150	0.111	0.149	0.113	0.160	0.090
Blastocyst count	0.075	0.427	0.083	0.378	0.077	0.413	0.087	0.356
Pregnancy rate	0.040	0.671	0.044	0.645	0.049	0.604	0.060	0.528

Serum, follicular fluid GDF-9 and serum BMP-15 showed no significant correlation to total embryo count, embryo quality count, blastocyst development or pregnancy rate. Follicular fluid BMP-15 showed a significant and positive correlation to the total embryo quality count.

DISCUSSION

Growth factors that are specific to oocytes, like GDF-9 and BMP-15, are regulatory proteins that are released from the primordial follicle and primary oocyte. They are very important during folliculogenesis [2]. Both intra-ovarian and extra-ovarian factors regulate the complex process of foliculogeneis [3]. The quality of oocytes (maturity and morphology) is determined during folliculogenesis [5]. Assisted reproduction, in vitro fertilization (IVF), and ICSI can all be used to check the quality of an oocyte by looking at it under a microscope and doing some genetic and biochemical tests. Thus, this study tried to estimate the role of serum and follicular fluid oocyte secretion factors in assessing or expressing oocyte quality. The overall PR of all the females included in the study was 35.3%, which is accepted as global PR following ICSI [16]. Regarding the oocyte characteristics of the included females, the current study showed that follicular fluid GDF-9, serum and follicular fluid BMP-15 showed a significant positive correlation to mature MII oocytes. Follicular fluid BMP-15 showed a significant positive correlation with total oocyte count and fertilization rate. Several studies were in agreement with the current study results, suggesting a significant positive correlation between GDF-9 and BMP-15 in serum and follicular fluid with the number of mature oocytes and oocyte maturation rates [4,17–19]. A study by Pantos et al. showed that serum BMP-15 had a significant positive correlation with the total number of oocytes, MII oocytes, oocyte fertilization rate and even embryo cleavage rate [20]. A study by Sumapradja et al. showed no correlation between serum, follicular fluid GDF-9 and follicular fluid BMP-15 with oocyte maturation and fertilization rate [19]. Regarding embryo characteristics and PR, serum, follicular fluid GDF-9 and serum BMP-15 showed no significant correlation to total embryo count, embryo quality count, blastocyst development or pregnancy rate. Follicular fluid BMP-15 showed a significant and positive correlation to the total embryonic count. Similar results were shown in studies that exhibited a positive correlation between BMP-15 in serum and follicular fluid with embryonic quality and even subsequent embryonic development [18,21,22].

While a study by Gode *et al*. showed a significant correlation between follicular fluid GDF-9 and embryo quality [17], Regarding pregnancy rate, our results were consistent with studies by Gode *et al*. and Hashim *et al*. that exhibited a significant correlation between serum BMP-15 and pregnancy rate [17, 21].

Study limitations

A single center, a small sample size and the method of assessment of oocyte and embryo quality were indirect (microscopic assessment depends on visual assessment under an inverted microscope), so the genetic, biochemical and molecular characteristics of oocytes and embryos were not estimated precisely.

Conclusions

Serum and follicular fluid BMP-15 levels are good predictors of oocyte number and quality, and have no role in prediction of embryos quality, blastocyst count and pregnancy rate.

Conflict of interests

No conflict of interest was declared by the authors

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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