The Role of Growth Hormone In Improving Oocyte Quality In IVF Cycles

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Background: Growth hormone has been used as an adjunct in ovarian stimulation of IVF cycles for the past three decades. However, the exact mechanism of its role in improving oocyte quality has not been thoroughly investigated. Although a clear indication for GH co-treatment is in infertile women with GH deficiency, GH has been given mostly to poor ovarian responders.
Method: This paper is a review of the most recent published data on the role of GH supplementation in improving oocyte quality in younger women who are suboptimal or unexpected poor responders to standard ovarian stimulation.
Results: Retrospective cohort and randomized trials demonstrated an improvement in oocyte quality through morphological parameters, mitochondrial function, biomarkers, GH receptors, insulin growth factor, markers of oxidative stress, mature oocytes, good quality embryos, implantation rate, clinical pregnancy and live birth.
Conclusion: Current data suggest GH supplementation may improve oocyte and embryo qualities, endometrial receptivity, clinical pregnancy and live birth. However, better quality evidence is needed before a recommendation can be made for GH supplementation to be given to infertile women who are suboptimal or poor ovarian responders.

Key words: growth hormone, hormone receptor, IGF-1, IVF, oocyte quality, suboptimal responder

Introduction

A successful in vitro fertilization (IVF) outcome is largely dependent on the patient's response to ovarian stimulation, since it reflects both the number and quality of oocytes collected during the stimulation cycle. Despite advancement and innovations in assisted reproductive techniques (ART), approximately 9 to 24% of in vitro fertilization (IVF) cycles fail to respond to standard ovarian stimulation regimens due to poor ovarian response[1,2,3] which may be associated with advanced maternal age, previous ovarian surgery, and high body mass index.[4] However, in as much as 21% of women younger than 35 years with normal ovarian reserve function, unexpected poor ovarian response may occur.[5] Factors that may reduce oocyte developmental competence include advanced maternal age[6], an immature cytoplasm[7], abnormal maternal protein expression , and reduced mitochondrial content.[9]

Oocyte quality is usually evaluated using morphological assessment.[10] The most common morphological parameters assessed in the oocytes (Table 1) include the meiotic spindle, cumulus complex, zona pellucida, perivitelline space, vacuoles or refractile bodies, shape, granulation, and viscosity of ooplasm.[11] However, data are conflicting regarding the ability of these morphological features to accurately predict oocytes with high quality. Thus, metabolic and mitochondrial molecular evaluations have been used to assess more specifically oocyte competence.

Mitochondria are vital in oocyte maturation[12] fertilization, and early embryonic development.[13,14] Studies in humans reported that oocytes of women with ovarian insufficiency have lower mitochondrial (mtDNA) content compared to women with a normal ovarian profile[15], and unfertilized oocytes have lower mtDNA copy numbers than fertilized oocytes.[13] Since the mtDNA content of cumulus granulosa cells (CGCs) correlates with embryo quality and implantation in IVF procedures, it has been used to assess oocyte quality and developmental competence.[16,17]

Growth hormone (GH) is an anabolic peptide hormone secreted mainly by the anterior part of the pituitary gland and plays a vital role in cell growth, development, and metabolism.[18] It exerts a direct action on human oocytes and cumulus cells, and also indirectly improves oocyte quality by activating synthesis of insulin-like growth factor-I or promoting follicle-stimulating hormone-induced ovarian steroidogenesis.[19,20] It is an important regulator of ovarian steroidogenesis[21], follicular development[22] and oocyte maturation.[23] In addition to GH's effects on ovarian function, recent studies also discussed positive effect of GH administration on endometrial receptivity, suggesting an additional potential benefit at the uterine level, especially in cases of thin endometrium, recurrent implantation failure, and older normal responders.[24,25]

Since GH has been demonstrated to play a critical role in folliculogenesis, it has been used to assist in ovulation induction and IVF for the past three decades.[26] It is clearly indicated for treatment of infertility in women with GH deficiency or panhypopituitarism.[27,28] However, indications for use of GH as co-treatment in IVF other than GH deficiency are still not well-established. Multiple studies have been done to explore the benefits of human growth hormone (GH) as an adjunct to stimulation protocols for IVF in normal responders[29,30] women with polycystic ovarian syndrome (PCOS)[31,32] poor ovarian responders[33,34] older women[35], sub-optimal responders to ovarian stimulation in an IVF cycle[36], and women with perceived poor oocyte or embryo quality.[37]

This paper reviewed and summarized the most recent data on the proposed mechanisms of GH and the role of GH co-treatment in improving oocyte quality among younger infertile women undergoing IVF. Weighing the benefits and the cost of GH supplementation, hopefully, an objective conclusion will be made regarding the adjunctive use of GH therapy in improving oocyte competence.

Methods

A literature search was performed using MEDLINE and Scopus databases for identification of relevant articles published from January 2016 to December 2021 (five years). Search terms and search strategies were used including "growth hormone", "oocyte quality", "embryo quality", and "blastocyst". Boolean operators, AND and OR, were used in the search. They were saved using the Mendeley Desktop (2021 version) reference manager. Discussion focused more on the evidence of the role of GH in improving oocyte quality other than those caused by poor ovarian response due to advanced maternal age. Included studies for discussion described the effects of GH cotreatment in IVF cycles in terms of specific morphologic, biochemical, metabolic parameters, and clinical outcomes of oocyte and/or embryo competence.

Discussion

Oocyte Quality

Definition of Oocyte Quality

Oocyte quality (or developmental competence) refers to the ability of the oocyte to complete meiotic maturation (including proper segregation of chromosomes) as well as support fertilization and embryonic development, ultimately leading to a successful live birth.[38,39] To put it more simply, oocyte quality refers to the potential of a fertilized oocyte to result in a live-born infant. It is different from ovarian reserve, or oocyte quantity, which pertains to oocyte number.[40] Fertilization, early embryonic development, and pregnancy maintenance are all dependent on oocyte quality. The most difficult yet important part before applying ART procedures such as ICSI and in vitro fertilization (IVF) is to choose the best oocytes capable of producing high quality embryos. But the ultimate clinical outcome is to achieve an increase in live birth rate.

Oocyte Quality Assessment

Defining the quality and developmental potential of oocytes is typically evaluated through morphological assessment. Morphological grading is mainly based on cytoplasmic and extra-cytoplasmic features, which may confer the state of nuclear and cytoplasmic maturity.[41] In a recent review by Ozturk (2020)[11], morphological evaluation of cumulus complex (presence of blood clots, expansion grade, apoptotic index); thickness of the zona pellucida (ZP), perivitelline space (size, presence of granules), volume and shape of oocytes, first polar body (integrity, size, fragmented), cytoplasmic changes (vacuoles, granulation, viscosity) and meiotic spindle, were thoroughly analyzed with regards to their contribution in selecting high-quality oocytes. Tilia, et al. (2020)[42] showed that a normal oocyte meiotic spindle morphology (OMS) was associated with the adequate stages of embryo development and a good predictor of blastocyst euploidy. However, oocyte selection based on these morphological criteria may have subjective variation and imprecision. Since there is no consensus on which morphologic parameter is best for oocyte selection, a combination of these multiple morphologies can be used to predict the oocytes with the highest potential to produce competent early embryos.[11]

In addition to morphological analysis, molecular biological techniques such as proteomic and genomic applications, may help assess more accurately the developmental competence of the oocyte. Proteomic analysis of follicular fluid (FF) is a non-invasive biochemical method of assessing follicular development and oocyte quality.[43] Follicular fluid (FF) contains proteins, hormones, polysaccharides, metabolites, reactive oxygen species, and antioxidants that are involved in oocyte maturation and competence.[44] Sirait, et al. (2021)[39] discussed eight biomarkers in the cumulus-oocyte-complex (COC) that regulate vital processes involved in oocyte maturation and could serve as predictors of oocyte competence and eventual IVF prognosis (Figure 1).

Evidence has demonstrated mitochondria as vital indicator of oocyte quality.[45] Mitochondria-derived energy and functions are important for early embryo development and implantation, including meiotic spindle formation and the maintenance of the metaphase II (MII) spindle before fertilization to support oocyte maturation, fertilization, and early embryonic development.[46] Mitochondria are exclusively maternal in origin, and emerging data emphasize mitochondrial dysfunction as a pivotal factor to oocyte ageing.[47] Oocytes of women with ovarian insufficiency were also found to have lower

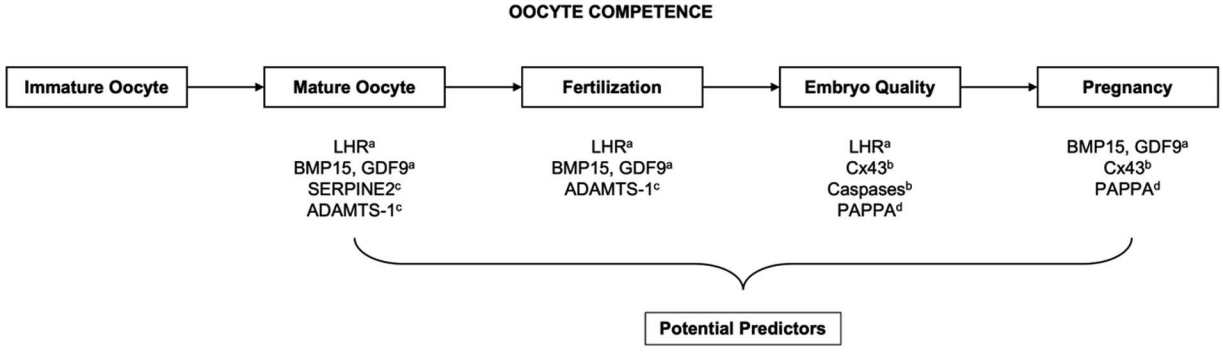

Figure 1. Oocyte competence biomarkers. Expression of the COC-derived genes discussed in this study have exhibited significant predictive values toward the respective IVF stages.[a,b,c,d] refers to the occyte maturation process that the gene regulate: [a]Nuclear maturation, [b]Apoptosis, [c]Extracellular matrix remodeling, [d]Steroid metabolism. Adapted from Sirait et al. Oocyte Competence Biomarkers Associated with Oocyte Maturation: A Review. Front Cell Dev Biol 2021; 9:710292.

LHR: Luteinizing hormone receptor, BMP15: Bone morphogenic protein 15, GDF9: Growth differentiation factor 9, SERPINE2: Serine protease inhibitor-E2, ADAMTS-1: A Disintegrin and Metalloproteinase with Thrombospondin motifs-1, Cx43: Connexin 43, PAPPA: Pregnancy-associated plasma protein-A or pappalysin 1

mtDNA copy numbers than women with a normal ovarian profile15, and unfertilized oocytes have lower mtDNA copy numbers compared to fertilized oocytes.[13] Since direct assessment of mtDNA number destroys the oocyte, evaluation of the cumulus granulosa cells (CGCs) surrounding the oocyte has been used instead of direct oocyte evaluation, to assess oocyte developmental competence.[48] Hence, it is not only the mitochondrial quantity that should be investigated, but the functional capacity as well.

Factors Associated with Poor Oocyte Quality

Advanced maternal age is the most common cause of poor oocyte quality, and is related to reduced oocyte mitochondrial efficiency and increased oxidative stress.[49] Preimplantation genetic testing of 15,000 blastocysts showed aneuploidy rates of 20 to 30% up to 36 years of age, but rose abruptly from 37 years onward, reaching approximately 50% and 70% by 38 and 41 years, respectively.[50] This proposes that female age, especially above the age of 37 years, is the critical age whereby reduced oocyte quality becomes more pronounced. Older age markedly increases the risk of oocyte aneuploidy, leading to an abrupt rise in the incidence of infertility, miscarriage, and birth defects. The main cause of failed IVF in older women is due to oocyte aneuploidy, attributed mainly to premature loss of centromeric cohesion between sister chromatids.[51]

However, there is another proportion of women with normal ovarian reserve function in whom oocyte quality is unexpectedly poor at a young age (< 35 years), and manifest as unexpected poor ovarian responders during stimulation cycles. In a study investigating the incidence of unexpected poor ovarian responders, results showed that among Chinese women < 35 years with normal ovarian reserve function who underwent standard stimulation IVF protocol, 21.16% had below par treatment outcome due to too few retrieved oocytes, and 5.99% had an extremely poor treatment outcome.[2] The exact cause for this unexpected poor/suboptimal ovarian response is not known, although polymorphisms in the receptor or genes of gonadotropins have been identified.[52,53,54,55]

Other gynecologic conditions that are common causes of infertility, such as pelvic endometriosis and polycystic ovarian syndrome, may have diminished oocyte quality as a confounding factor for poor ovarian response to IVF cycles. Based on a systematic review, oocytes retrieved from women with endometriosis were more likely to fail in vitro maturation, and had altered morphology and lower cytoplasmic mitochondrial content compared to women with other causes of infertility.[56] In addition, results from meta-analyses showed that women with endometriosis had less mature oocytes, and lower fertilization rate was associated with minimal/mild rather than with moderate/severe disease.[57] Results of a recent study evaluating oocyte quality in PCOS women undergoing IVF cycles showed that despite the higher number of retrieved oocytes in PCOS women, the number of high-quality oocytes and embryos and the pregnancy rate did not differ significantly compared to non-PCOS women.[58]

Obesity can change mitochondrial distribution, hyperpolarization of mitochondrial membrane, oxidized redox, and oxidative stress in both oocytes and zygotes, thus affecting oocyte quality. It is reported that 46% of obese mothers experienced the failure of embryo development at the blastocyst stage due to mitochondrial oxidative stress.[59]

Growth Hormone

Growth Hormone: Effects on the Ovary

Growth hormone (GH) is an anabolic peptide hormone secreted mainly by the anterior pituitary gland, and plays a vital role in cell growth, development and metabolism throughout the body.[24] It is secreted in a pulsatile manner in response to growth hormone releasing hormone (GHRH), and inhibited by growth hormone inhibiting hormone (somatostatin) released from the hypothalamus.[23] GH was proven to have both direct and indirect effects on ovarian function, with direct effects mediated by GH-GHR interactions, and indirect effects through the synthesis of Insulin-like growth factor (IGF). Human oocytes and cumulus cells have GH receptors (GHRs) that can be directly influenced by GH, by augmenting nuclear maturation of denuded human oocytes. It has been proven that there is an age-dependent decline in oocyte GHRs expression. Oocytes from younger normo-responders (< 35 years of age) were found to have significantly higher GHRs ($p < 0.04$) compared with older normo-responder

(≥ 35 years) women.[61] Co-treatment with GH has been shown to increase granulosa cell receptor density for FSH receptor (FSHR), LH receptor (LHR), bone morphogenetic hormone receptor (BMPR1B) and GH receptor (GHR) compared with the non–GH-treated patients of the same age and ovarian reserve.[35] GH may also have an indirect effect in improving oocyte quality by activating synthesis of insulin-like growth factor 1 (IGF-I) or promoting FSH-induced ovarian steroidogenesis. IGF-1 potentiates the action of FSH in folliculogenesis, and assists with granulosa cell differentiation, with the largest follicles containing the highest concentrations of IGF-1.[63] A recent study analyzed the association between oocyte cohort quality and follicular levels of different hormones including GH and IGF-1. Results showed that follicular levels of GH and IGF1 were significantly higher in a normal oocyte cohort than in an abnormal oocyte cohort; fertilization rate was likewise higher in the normal oocyte cohort, thus further reinforcing that GH/IGF1 system appears to be important for oocyte development and competency.[64]

Growth Hormone: Effects on the Endometrium

Preliminary studies suggest that women with recurrent implantation failure, thin endometrium and older normo-responders may benefit from GH co-treatment when undergoing ART. A recent review by Altmae and Aghajanova (2019)[24] discussed that GH therapy could improve the endometrial thickness and receptivity among infertile women, either as a direct effect of GH or through IGF-1. Proposed mechanisms of action include GH/IGF-1 induced endometrial proliferation, vascularization and expression of receptivity-related genes such as vascular endothelial growth factor (VEGF) and integrin beta 3 (ITGB3).[65] Ovarian GH may also have an indirect effect on endometrial function, through the action and maintenance of the corpus luteum.[66,67] The exact mechanism and benefits need further investigation.

GH administration may improve IVF outcomes in some, but not all, cases. Whether this effect is mainly due to an increase in the number of mature oocytes, improvement in oocyte quality or uterine receptivity, is still inconclusive. While the main cause of IVF failure in older women is related with oocyte aneuploidy, the etiology is less clear in younger women with unexpected poor response to ovarian stimulation.[68]

Use of Growth Hormone in IVF Cycles to Improve Oocyte Quality

Majority of the published data on the use of growth hormone (GH) supplementation in ovarian stimulation protocols focus on improving IVF outcomes among poor ovarian responders due to advanced maternal age, but the effect of GH on patients with poor oocyte competence or embryonic development other than aging, is still obscure. Given the possible mechanisms by which GH can increase recruitment of mature oocytes, improve oocyte and embryo competence, and eventually increase pregnancy rates, the most recent studies on GH's role in improving oocyte quality will be discussed.

Growth Hormone Co-treatment to Improve Oocyte Quality in Younger Women with Suboptimal Response to IVF

Some studies have focused on the role of GH adjunct to improve IVF outcomes in a subset of women who were not that old (35-39 years), with unexpected or suboptimal response to previous IVF cycles using standard ovarian stimulation regimen, those with repeated implantation failures, or unexplained infertility.

Majority of the published retrospective trials showed positive outcomes for younger (< 39 years) suboptimal responders who were given GH as cotreatment in their succeeding IVF cycle. In a study by Jin, et al. (2018)[69], the effects of GH supplementation on IVF outcomes of 107 Chinese poor responders were analyzed based on oocyte and embryo quality, implantation rate, and pregnancy rate. In a subset of 60 younger POR patients (< 40 years) co-treated with GH, they had significantly higher number of retrieved oocytes, fertilized oocytes, and high-quality embryos compared to age-matched controls. However, the number of transferred embryos, fertilization rate, implantation rate, and clinical pregnancy rate were not different between the GH and non-GH group.[69]

Another potential indication for GH supplementation would be in young women

with repeated implantation failures after IVF. Recurrent implantation failure (RIF) is defined as failure to achieve a pregnancy after three failed IVF transfers, each with at least one good quality embryo, or failure to achieve pregnancy after transfer of 10 good quality embryos.[70] These women fail to achieve pregnancy after repeated IVF cycles despite adequate endometrial thickness and pattern and high quality embryos. To date, there is no proven effective treatment. Possible causes include impaired endometrial maturation[71], reduced embryo quality and defects in endometrial receptivity.[72,73] The study of Chen, et al. (2018)[74] on young women (< 35 years) diagnosed with RIF who underwent IVF treatment found that GH supplementation increased the endometrial thickness compared to those who did not receive GH ($p< 0.05$), and this may support a more favorable blastocyst implantation. In addition, GH treatment also increased the GHR level in the follicular fluid, which was positively correlated with higher levels of GHR mRNA in granulosa cells ($r=0.460$, $p<0.05$). Consequently, clinical pregnancy and live birth rates were also increased in the GH-treatment RIF group when compared with the non-GH group.[74] On the other hand, Tesarik, et al's. study[75] on a similar population of younger women with repeated implantation failure after IVF, showed no significant difference in endometrial thickness compared with the untreated group. Analysis of the number and morphological characteristics of the oocytes, zygote and embryo qualities showed significantly higher number and percentage of oocytes with the best cumulative morphological quality score, and significantly lower number of oocytes with the worst score in patients treated with GH compared with the control group. Patients treated with GH also had significantly more total zygotes and good-quality zygotes, more cleaving embryos, and more high-quality embryos. Clinically, the GH-treated group had more implantations, pregnancies and live births, compared with the untreated group. Data from these two studies[74,75] show that, independent of the effect on uterine receptivity, GH administration improves the quantity and morphological quality of oocytes, zygotes and cleaving embryos in young women with previous IVF failures. These improvements are manifested clinically by an increase in pregnancy, delivery, implantation and birth rates in this subset of patients.

Two most recent retrospective studies pioneered the investigation of adjuvant GH use in IVF cycles in younger women with suboptimal response to standard ovarian stimulation, where preimplantation genetic testing for aneuploidy (PGT-A) was performed to determine the impact of embryo ploidy. The first published study on GH use in PGT-A cycles in this group of suboptimal responders demonstrated that the total number of biopsied embryos and euploid embryos was significantly higher in the adjuvant GH treatment cycle group compared to the control group. However, the euploid rate did not differ between the two groups.[76] Since the euploidy rate is inherently predetermined based on genetics, woman's age and other factors that remain constant across cycles, it is therefore unlikely to have a significant change in such a short period of two cycles within the same year. Therefore, it can be surmised that the increase in number of euploid blastocysts is due to a greater number of mature oocytes, and eventual increase in the number of blastocysts available for biopsy. This study by Skillern and colleagues[76] demonstrated that the mean number of euploid embryo was 0.8 per cycle in the IVF-PGT-A control group, compared to 2 euploid embryos in the IVF-PGT-A GH cycle. This reflects improved embryo quality, a greater opportunity for a successful embry transfer, and ultimately at pregnancy, which is the more clinically relevant measure of a positive outcome. In a study by Kurtz, et al. (2021)[77], similar to earlier trials, GH co- treatment significantly improved endometrial thickness, and resulted in significantly greater number of retrieved oocytes, mature oocytes, blastocysts, and more usable blastocysts (high enough quality blastocyst suitable for transfer or cryopreservation). Novel information on PGT-A also showed that GH-treated group had a greater number of euploid embryos for transfer, with an overall euploidy rate of 51.3% ($p=0.0158$), versus 35.09% in the non-GH group.[77] These two studies by Skillern, et al. (2021)[76] and Kurtz, et al. (2021)[77] both showed an increase in euploid embryos with GH supplementation in IVF-PGT-A cycles in younger women with suboptimal response to previous IVF cycles which, through improved embryo development, could lead to an improvement in live birth.

Limitations of these studies include the following: retrospective study design; small sample size; different definitions for suboptimal or unexpected responders and recurrent implantation failure; lack of the more clinically relevant outcomes such as clinical pregnancy and live birth rates in some of the trials; varied reporting of morphological parameters; variations in the GH protocol (dose, interval, duration), cost of the drug and overall cost of the research.

Regarding randomized trials discussing the effects of growth hormone use in IVF cycles to improve oocyte or embryo quality in younger women, a recent study by Li, et al. (2020)[78] investigated the role of GH in improving oocyte quality. Subjects included women ≤ 37 years who had at least one failed IVF cycle with no top-quality embryos, had normal hormone levels (FSH, LH, estradiol) during the early follicular phase, or those with unexplained infertility. Results showed a significant increase in the numbers of oocytes retrieved, 2PN fertilized oocytes, and cleaved embryos on day 2 in the GH treated group compared to the control group. Transfer of higher quality embryos in the GH group was made evident by a greater than-twofold increase in the clinical pregnancy and live birth rates versus the control group. In addition, the mtDNA copy number of cumulus granulosa cells (CGCs) of the GH group was significantly higher compared to the mtDNA CGCs of the non-GH group, reinforcing earlier studies that GH co-treatment in IVF cycles may improve oocyte developmental competence. This is a randomized clinical trial with a relatively bigger sample size (107 treatment group, 51 control group) than the previously discussed retrospective trials, with more objective results showing that GH supplementation may have a beneficial effect on IVF outcome, namely, an increase in the numbers of oocytes retrieved and improved clinical pregnancy and live birth rates, in women with poor embryonic development.

Growth Hormone Co-treatment to Improve Oocyte Quality in Young PCOS Women

Current evidence remains inconclusive as to whether women with PCOS are prone to have reduced embryo quality and poor pregnancy outcomes despite producing more oocytes during ovarian stimulation in IVF cycles.[79,80,81] Oxidative stress has been associated with the pathophysiology of PCOS, and earlier studies have shown that increased reactive oxygen species (ROS) in the follicle fluid (FF) and decreased levels of total antioxidant capacity (TAC) and superoxide dismutase correlated with less oocyte maturation and fertilization, poor embryo quality, and lower pregnancy rates.[82,83,84] A recent randomized controlled trial by Gong, et al. (2020)[85] investigated the effects of GH on reducing oxidative stress (OS), and improving oocyte quality and IVF outcomes in women with PCOS. One hundred nine (109) women diagnosed with PCOS were randomly assigned to receive GH co-treatment (PCOS-T) or none (PCOS-C), while 50 women without PCOS served as the control group. Results showed that patients with PCOS treated with GH had significantly higher number of fertilized oocytes, and cleaved embryos. Markers of oxidative stress, such as total oxidant status (TOS) and OS index (OSI), were significantly higher in the PCOS-C group than in the PCOS-T group and non-PCOS group. With regards to assessment of the mitochondrial function of GC, the mitochondrial membrane potential (MMP) was significantly higher and the early and late apoptosis rates were lower in the PCOS GH-treated group than in the PCOS group without GH co-treatment. The MMP was significantly lower and the apoptosis rates were significantly higher in the PCOS-C group than in the group without PCOS. This study demonstrated that GH administration reduced OS by increasing the MMP over three-fold and dropping GC apoptosis by > 50%, while resulting in significantly more fertilized oocytes and cleaved embryos. GH co-treatment with gonadotropin in IVF cycles significantly improved mitochondrial dysfunction in GCs and oocyte quality in women with PCOS.[85] The small sample size and the selective testing of only a few OS markers are main limitations. Since the exact mechanism by which GH improves OS status is still unknown, more studies are warranted to clearly define its role.

Growth Hormone Co-treatment to Improve Oocyte Quality in Poor Ovarian Responders

According to the Bologna criteria, poor ovarian response (POR) is diagnosed by the presence of at least 2 of the following 3 criteria: advanced maternal

age (≥ 40 years) or any other risk factor for POR; a previous POR (≤ 3 oocytes with a conventional stimulation protocol); and an abnormal ovarian reserve test (antral follicle count [AFC] of <5–7 or antimullerian hormone (AMH) level of <0.5–1.1 ng/mL).[86] The Bologna criteria has been criticized due to the lack of homogeneity of the described population of infertile women, and the recognized need to amend the criteria. The current classification is the Poseidon Criteria which is a more homogeneous stratification of the patient subpopulation, taking into account the woman's age, ovarian reserve, and previous response from ovarian stimulation. The Poseidon criteria identified four subgroups of patients (Figure 2). However, published studies, even the more recent ones, still use the Bologna criteria to define POR. For the discussion of growth hormone co-treatment in improving oocyte quality in poor ovarian responders, literature search focused on trials of GH use in POR with specific inclusion of assessment of parameters for oocyte quality as outcome measures.

A most recent prospective randomized trial investigated the effects of different GH treatment regimens on ovarian hormones and insulin-like growth factor (IGF) levels in follicle fluid (FF), hormone receptors in granulosa cells (GCs), and IVF-ET outcomes in poor ovarian responders.[89] The Bologna criteria was used as the definition of POR for the included subjects. Two hundred thirty PORs were randomly assigned to 3 groups: group A, GH pretreatment (4 IU/d) from day 2 of the previous menstrual cycle until the day of trigger; group B, GH treatment (4 IU/d) from day 2 of the menstrual cycle until the trigger day; group C, no GH treatment. Results showed that live birth rate (LBR) per treatment cycle started and per patient randomized was significantly higher in group A than groups B and C ($p<.05$). The doses and duration of gonadotropin stimulation, the E2 level on the day of trigger, the number of transferred embryos, and the multiple pregnancy rate did not differ significantly between 3 groups. Levels of E2, progesterone, testosterone (TT), GH, and IGF-1 in FF were significantly higher in group A than in groups B and C. The levels were significantly higher in group B than in group C ($p<.05$). GH improved the hormone levels in FF, especially with longer GH treatment (group A). FSHR, LHR, and GHR expression was significantly higher in group A than in groups B and C, and significantly higher in group B than in group C. Thus, GH improved hormone receptor expression in GCs, especially with longer GH treatment.

This study demonstrated that GH pretreatment from the previous menstrual cycle improved the oocyte quantity and quality and LBR in poor ovarian responders. GH pretreatment likewise improved IGF-1, GH, E2, progesterone, and TT levels in FF and the expression of GHR, FSHR, and LHR in

POSEIDON GROUP 1 Young patients <35 years with adequate ovarian reserve parameters (AFC ≥ 5; AMH ≥ 1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response. Subgroup 1a: < 4 oocytes retrieved* Subgroup 1b: 4-9 oocytes retrieved* **after standard ovarian stimulation*	**POSEIDON GROUP 2** Older patients ≥ 35 years with adequate ovarian reserve parameters ≥ 5; AMH ≥ 1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response. Subgroup 1a: < 4 oocytes retrieved* Subgroup 1b: 4-9 oocytes retrieved* **after standard ovarian stimulation*
POSEIDON GROUP 3 Young patients <35 years with poor ovarian reserve pre-stimulation parameters (AFC <5; AMH <1.2 ng/ml)	**POSEIDON GROUP 4** Older patients ≥ 35 years with poor ovarian reserve pre-stimulation parameters (AFC < 5; AMH < 1.2 ng/ml)

Figure 2. Poseidon criteria of low prognosis patient with assisted reproductive technology.

GCs, with an obvious increasing trend with longer GH treatment. Longer treatment duration was associated with higher levels of steroid hormones in FF, which indicates that GH's positive effect on poor ovarian responders is through the up-regulation of these hormones in FF. Another possible explanation for long course GH pretreatment (4–6 weeks) is due to findings that GH and IGF-1 improve follicular development from the non-gonadotropin-dependent stage (preantral and early antral follicle) to that of Gn-dependence (antral follicle stage) in poor responders.[90,91] Limitations of the study are its small sample size, and absence of blinding.

Conclusion

Based on the most recent published data consisting mostly of retrospective cohort and a few randomized studies, GH supplementation in IVF cycles may be beneficial in improving oocyte and embryo quality, endometrial receptivity, clinical pregnancy and live birth among younger women with unexpected or suboptimal response to standard ovarian stimulation, and in older women with POR with long course GH pretreatment. However, these findings are barred by the scarcity of well-designed randomized trials. Limitations of the studies such as selection bias, inconsistent definitions for suboptimal or poor ovarian responders, variability in clinical and laboratory techniques assessment, non-standardized dosing of GH protocols, and exorbitant cost, preclude a recommendation for its routine use. Large, well-designed trials with a homogenous set of patients and structured laboratory techniques would be an ideal recommendation, to obtain good quality evidence. However, the cost and difficulty in patient selection and recruitment are realistic deterrents to this recommendation.

References

1. Ben-Rafael Z, Bider D, Dan U, Zolti M, Levran D, Mashiach S. Combined gonadotropin releasing hormone agonist/human menopausal gonadotropin therapy (GnRH-a/hMG) in normal, high, and poor responders to hMG. J In Vitro Fert Embryo Transf 1991; 8(1): 33-6.
2. Jenkins JM, Davies DW, Devonport H, Anthony FW, Gadd SC, Watson RH and Masson GM. Comparison of 'poor' responders with 'good' responders using a standard buserelin/human menopausal gonadotrophin regime for in vitro fertilization. Hum Reprod 1991; 6(7): 918-21.
3. Surrey ES and Schoolcraft WB. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. Fertil Steril 2000; 73(4): 667-76.
4. Kolibianakis EM, Venetis CA, Diedrich K, Tarlatzis BC, Griesinger G. Addition of growth hormone to gonadotrophins in ovarian stimulation of poor responders treated by in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update 2009; 15(6): 613- 22.
5. Zhuang J, Li H, Li X, Tian D, Yang D, Zhu M. The incidence of unexpected poor ovarian response in Chinese young women. Medicine (Baltimore) 2019:98(7):e14379.
6. Faddy MJ. Follicle dynamics during ovarian ageing. Mol Cell Endocrinol 2000; 163(1– 2): 43–8.
7. Watson AJ. Oocyte cytoplasmic maturation: a key mediator of oocyte and embryo developmental competence. J Anim Sci 2007; 85(13 Suppl): E1–3.
8. Wang S, Kou Z, Jing Z, Zhang Y, Guo X, Dong M, Wilmut I, Gao S. Proteome of mouse oocytes at different developmental stages. Proc Natl Acad Sci USA 2010; 107(41): 17639–44.
9. Babayev E, Seli E. Oocyte mitochondrial function and reproduction. Curr Opin Obstet Gynecol 2015:27(3): 175–81.
10. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Hum Reprod Update 2011; 17(1): 34–45.
11. Ozturk S. Selection of competent oocytes by morphological criteria for assisted reproductive technologies. Mol Reprod Dev 2020; 87(10): 1021–36.
12. Bentov Y, Yavorska T, Esfandiari N, Jurisicova A, Casper RF. The contribution of mitochondrial function to reproductive aging. J Assist Reprod Genet 2011; 28(9): 773– 83.
13. Murakoshi Y, Sueoka K, Takahashi K, Sato S, Sakurai T, Tajima H, Yoshimura Y. Embryo developmental capability and pregnancy outcome are related to the mitochondrial DNA copy number and ooplasmic volume. J Assist Reprod Genet 2013; 30(10): 1367-75.
14. Reynier P, May-Panloup P, Chretien MF, Morgan CJ, Jean M, Savagner F, Barrière P, Malthièry Y. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod 2001; 7(5): 425–9.
15. May-Panloup P, Chretien MF, Jacques C, Vasseur C, Malthiery Y, Reynier P. Low oocyte mitochondrial DNA content in ovarian insufficiency. Hum Reprod 2005; 20(3): 593–7.
16. Ogino M, Tsubamoto H, Sakata K, Oohama N, Hayakawa H, Kojima T, Shigeta M, Shibahara H. Mitochondrial DNA copy number in cumulus cells is a strong predictor of obtaining good-quality embryos after IVF. J Assist Reprod Genet 2016: 33(3): 367– 71.
17. Taugourdeau A, Desquiret-Dumas V, Hamel JF, Chupin S, Boucret L, Ferre-L'hotellier V, Bouet PE, Descaps P, Procaccio V, Reynier P, et al. The mitochondrial DNA content of cumulus cells may help predict embryo implantation. J Assist Reprod Genet 2018; 36(2): 223–8.
18. Yovich JL, Regan SLP, Zaidi S, Keane KN. The concept of growth hormone deficiency affecting clinical prognosis in IVF. Front Endocrinol (Lausanne) 2019; 10: 650.

19. Xu YM, Hao GM, Gao BL. Application of growth hormone in in vitro fertilization. Front Endocrinol 2019;10: 502.
20. Hart RJ. Use of growth hormone in the IVF treatment of women with poor ovarian reserve. Front Endocrinol 2019; 10: 500.
21. Nakamura E, Otsuka F, Inagaki K, Miyoshi T, Matsumoto Y, Ogura K, Tsukamoto N, Takeda M, Makino H. Mutual regulation of growth hormone and bone morphogenetic protein system in steroidogenesis by rat granulosa cells. Endocrinology 2012; 153(1): 469–80.
22. Bachelot A, Monget P, Imbert-Bollore P, Coshigano K, Kopchick JJ, Kelly PA, Binart N. Growth hormone is required for ovarian follicular growth. Endocrinology 2002; 143(10): 4104–12.
23. Bevers MM, Izadyar F. Role of growth hormone and growth hormone receptor in oocyte maturation. Mol Cell Endocrinol 2002;197(1–2): 173–8.
24. Altmäe S, Aghajanova L. Growth hormone and endometrial receptivity. Front Endocrinol 2019; 10: 653.
25. Liu FT, Wu Z, Yan J, Norman RJ, Li R. The potential role of growth hormone on the endometrium in assisted reproductive technology. Front Endocrinol 2020; 11: 49.
26. Homburg R, Eshel A, Abdalla HI, Jacobs HS. Growth hormone facilitates ovulation induction by gonadotrophins. Clin Endocrinol (Oxf) 1988; 29(1): 113– 7.
27. Spiliotis BE. Growth hormone insufficiency and its impact on ovarian function. Ann N Y Acad Sci 2003; 997: 77–84.
28. Park JK, Murphy AA, Bordeaux BL, Dominguez CE, Session DR. Ovulation induction in a poor responder with panhypopituitarism: a case report and review of the literature. Gynecol Endocrinol 2007; 23(2): 82– 6.
29. Tapanainen J, Martikainen H, Voutilainen R, Orava M, Ruokonen A, Ronnberg L. Effect of growth hormone administration on human ovarian function and steroidogenic gene expression in granulosa-luteal cells. Fertil Steril 1992; 58(4): 726–32.
30. Younis JS, Simon A, Koren R, Dorembus D, Schenker JG, Laufer N. The effect of growth hormone supplementation on in vitro fertilization outcome: a prospective randomized placebo-controlled double-blind study. Fertil Steril 1992; 58(3): 575–80.
31. Huang ZH, Baxter RC, Hughes SM, Matson PL, Lieberman BA, Morris ID. Supplementary growth hormone treatment of women with poor ovarian response to exogenous gonadotrophins: changes in serum and follicular fluid insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3). Hum Reprod 1993; 8: 850-7.
32. Homburg R, Levy T, Ben-Rafael Z. Adjuvant growth hormone for induction of ovulation with gonadotrophin-releasing hormone agonist and gonadotrophins in polycystic ovary syndrome: a randomized, double-blind, placebo controlled trial. Hum Reprod 1995; 10: 2550–3.
33. Cozzolino M, Cecchino GN, Troiano G, Romanelli C. Growth hormone cotreatment for poor responders undergoing in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril 2020; 114: 97-109.
34. Yang P, Wu R, Zhang H. The effect of growth hormone supplementation in poor ovarian responders undergoing IVF or ICSI: a meta-analysis of randomized controlled trials. Reprod Biol Endocrinol 2020;18: 76.
35. Regan SLP, Knight PG, Yovich JL, Arfuso F, Dharmarajan A. Growth hormone during in vitro fertilization in older women modulates the density of receptors in granulosa cells, with improved pregnancy outcomes. Fertil Steril 2018; 110: 1298–310.
36. Duffy JM, Ahmad G, Mohiyiddeen L, Nardo LG, Watson A. Growth hormone for in vitro fertilization. Cochrane Database Syst Rev 2010:CD000099.
37. Hazout A, Junca A, Menezo Y, Demouzon J, Cohen-Bacrie P. Effect of growth hormone on oocyte competence in patients with multiple IVF failures. Reprod Biomed Online 2009; 18: 664–70.
38. Greaney J, Wei Z, Homer H. Regulation of chromosome segregation in oocytes and the cellular basis for female meiotic errors. Hum Reprod Update 2018; 24(02): 135–61.
39. Sirait B, Wiweko B, Jusuf AA, Iftitah D, Muharam R. Oocyte competence biomarkers associated with oocyte maturation: A review. Front Cell Dev Biol 2021; 9: 710.
40. American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Practice Committee of the American Society for Reproductive Medicine. Fertil Steril 2020; 114: 1151–7.
41. Balaban B, Barut T, Urman B. Assessment of oocyte quality. Practical Manual of in Vitro Fertilization. 2012. New York, NY: Springer, pp.105–19.
42. Tilia L, Chapman M, Kilani S, Cooke S, Venetis C. Oocyte meiotic spindle morphology is a predictive marker of blastocyst ploidy- a prospective cohort study. Fertil Steril 2020; 113: 105–13.
43. Mariani G, Bellver J. Proteomics and metabolomics studies and clinical outcomes. Reproducomics 2018; 147-70.
44. Bianchi L, Gagliardi A, Landi C, Focarelli R, De Leo V, Luddi A, Bini L, Piomboni P. Protein pathways working in human follicular fluid: the future for tailored IVF? Expert Rev Mol Med 2016; 18: 9.
45. Qi L, Chen X, Wang J, Lv B, Zhang J, Ni B, Xue Z. Mitochondria: the panacea to improve oocyte quality? Ann Transl Med 2019; 7(23): 789.
46. Cummins JM. The role of mitochondria in the establishment of oocyte functional competence. Eur J Obstet Gynecol and Reprod Biol 2004; 115(Suppl 1): S23–S29.
47. Van Der Reest J, Cecchino GN, Haigis MC, Kordowitzki P. Mitochondria: Their relevance during oocyte ageing. Ageing Res Rev 2021; 70: 101-378.
48. Taugourdeau A, Desquiret-Dumas V, Hamel JF, Chupin S, Boucret L, Ferre- L'hotellier V, et al. The mitochondrial DNA content of cumulus cells may help predict embryo implantation. J Assist Reprod Genet 2018; 36: 223–8.
49. Homer HA. The role of oocyte quality in explaining "Unexplained" infertility. Semin Reprod Med 2020; 38(1): 21-8.
50. Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014; 101(03): 656–63.
51. Herbert M, Kalleas D, Cooney D, Lamb M, Lister L. Meiois and maternal aging: insights from aneuploid oocytes and trisomy births. Cold Spring Harb Perspect Biol 2015; 7.

52. Yan Y, Gong Z, Zhang L, et al. Association of follicle-stimulating hormone receptor polymorphisms with ovarian response in Chinese women: a prospective clinical study. PLoS One 2013; 10.
53. Alviggi C, Conforti A, Caprio F, et al. In estimated good prognosis patients could unexpected "hyporesponse" to controlled ovarian stimulation be related to genetic polymorphisms of FSH receptor? Reprod Sci 2016; 8: 1103–8.
54. Alviggi C, Humaidan P, Ezcurra D. Hormonal, functional and genetic biomarkers in controlled ovarian stimulation: tools for matching patients and protocols. Reprod Biol Endocrinol 2012; 10: 9.
55. De Placido G, Alviggi C, Mollo A, et al. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadequate response to recombinant FSH (rFSH) after pituitary downregulation. Clin Endocrinol (Oxf) 2004; 5: 637–43.
56. Sanchez AM, Vanni VS, Bartiromo L, Papaleo E, Zilberberg E, Candiani M, Orvieto R, Viganò P. Is the oocyte quality affected by endometriosis? A review of the literature. J Ova Res 2017; 10(43): 1-11.
57. Rossi AC, Prefumo F. The effects of surgery for endometriosis on pregnancy outcomes following in vitro fertilization and embryo transfer: a systematic review and meta-analysis. Arch Gynecol Obstet 2016; 294: 647–55.
58. Nikbakht R, Mohammadjafari R, Rajabalipour M, Moghadam MT. Evaluation of oocyte quality in polycystic ovary syndrome patients undergoing ART cycles. Fertil Res Pract 2021; 7: 2.
59. Khan R, Jiang X, Hameed U, Shi Q. Role of lipid metabolism and signaling in mammalian oocyte maturation, quality, and acquisition of competence. Front Cell Dev Biol 2021; 9.
60. Abir R, Garor R, Felz C, Nitke S, Krissi H, Fisch B. Growth hormone and its receptor in human ovaries from fetuses and adults. Fertil Steril 2008; 90: 1333–9.
61. Weall BM, Al-Samerria S, Conceicao J, Yovich JL, Almahbobi G. A direct action for GH in improvement of oocyte quality in poor-responder patients. Reproduction 2015; 149: 147–54.
62. Hull KL, Harvey S. Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions. Int J Endocrinol 2014; 2014: 234014.
63. Zhou P, et al. IGF-I signaling is essential for FSH stimulation of AKT and steroidogenic genes in granulosa cells. Mol Endocrinol 2013; 27: 511–23.
64. Scheffler F, Vandecandelaere A, Soyez M, Bosquet D, Lefranc E, Copin H, Devaux A, Benkhalifa M, Cabry R, Desailloud R. Follicular GH and IGF1 levels are associated with oocyte cohort quality: A pilot study. Front Endocrinol 2021; 12: 793621.
65. Cui N, et al. Effects of growth hormone on pregnancy rates of patients with thin endometrium. J Endocrinol Invest 2019; 42: 27–35.
66. Juengel JL, Nett TM, Anthony R V, Niswender GD. Effects of luteotrophic and luteolytic hormones on expression of mRNA encoding insulin-like growth factor I and growth hormone receptor in the ovine corpus luteum. J Reprod Fertil 1997; 110: 291– 8.
67. Schams D, Berisha B, Kosmann M, Einspanier R, Amselgruber WM. Possible role of growth hormone, IGFs, and IGF-binding proteins in the regulation of ovarian function in large farm animals. Domest Anim Endocrinol 1999; 17: 279–85.
68. Morin SJ, et al. Diminished ovarian reserve and poor response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in performance. Hum Reprod 2018; 33: 1489–98.
69. Jin M, Feng G, Zhu Y, Liu A. Effects of growth hormone supplementation in poor responders undergoing in vitro fertilization/intracytoplasmic sperm injection treatment. Int J Clin Exp Med 2018; 11: 6187–93. Available online at: www.ijcem.com/ISSN:1940- 5901/IJCEM0066673.
70. Koot YEM, Hviid Saxtorph M, Goddijn M, De Bever S, Eijkemans MJC, Wely MV, Van Der Veen F, Fauser B, Macklon, N.S. What is the prognosis for a live birth after unexplained recurrent implantation failure following IVF/ICSI? Hum Reprod 2019;34: 2044–52.
71. Sebastian-León P, Garrido N, Remohí J, Pellicer A, Diaz-Gimeno P. Asynchronous and pathological windows of implantation: Two causes of recurrent implantation failure. Hum Reprod 2018; 33: 626–35.
72. Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod 2006; 21(12): 3036-43.
73. Revel, A. Defective endometrial receptivity. Fertil Steril 2012; 97(5): 1028-32.
74. Chen Y, et al. Clinical efficacy and mechanism of growth hormone action in patients experiencing repeat implantation failure. Can J Physiol Pharmacol 2018; 96: 929–32.
75. Tesarik J, Galán-Lázaro M, Conde-López C, Chiara-Rapisarda AM, Mendoza-Tesarik R. The effect of GH administration on oocyte and zygote quality in young women with repeated implantation failure after IVF. Front Endocrinol 2020; 11: 519572
76. Skillern A, Leonard W, Pike J, Mak W. Growth hormone supplementation during ovarian stimulation improves oocyte and embryo outcomes in IVF/PGT-A cycles of women who are not poor responders. J Assisted Reprod Genetics 2021; 38: 1055– 60.
77. Kurtz J, Clements N, Bloom A, Oris J, Michael J. Glassner MJ. The effect of growth hormone on IVF outcomes during ovarian stimulation: a matched cohort study. Advance access published September 7, 2021, https://doi.org/10.21203/rs.3.rs-700354/v1.
78. Li J, Chen Q, Wang J , Huang G, Ye H. Does growth hormone supplementation improve oocyte competence and IVF outcomes in patients with poor embryonic development? A randomized controlled trial. BMC Pregnancy and Childbirth 2020; 20: 310.
79. Qiao J, Feng H. Extra- and intra-ovarian factors in polycystic ovary syndrome: Impact on oocyte maturation and embryo developmental competence. Hum Reprod Update 2011; 17: 17–33.
80. Sha T, Wang X, Cheng W, Yan Y. A meta-analysis of pregnancy-related outcomes and complications in women with polycystic ovary syndrome undergoing IVF. Reprod Biomed Online 2019; 39: 281–93.

81. De Vos, M. et al. Cumulative live birth rates after IVF in patients with polycystic ovaries: Phenotype matters. Reprod Biomed Online 2018; 37: 163–71.
82. Sabatini L, Shawaf TA, Wilson C, Lower A, Grudzinskas JG. Follicular fluid superoxide dismutase (SOD) activity in women with polycystic ovarian syndrome (PCOS). Fertil Steril 2000; 74(Suppl.): s253–s254.
83. Ruder EH, Hartman TJ, Blumberg J, Goldman MB. Oxidative stress and antioxidants: exposure and impact on female fertility. Hum Reprod Update 2008;14: 345– 57.
84. Chattopadhayay R, Ganesh A, Samanta J, Jana SK, Chakravarty BN, Chaudhury K. Effect of follicular fluid oxidative stress on meiotic spindle formation in infertile women with polycystic ovarian syndrome. Gynecol Obstet Invest 2010; 69: 197– 202.
85. Gong Y, Luo S, Fan P, Jin S, Zhu H, Deng T, Quan Y, Huang W. Growth hormone alleviates oxidative stress and improves oocyte quality in Chinese women with polycystic ovary syndrome: a randomized controlled trial. Scientific Reports 2020; 10: 18769.
86. Ferraretti AP, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011; 26: 1616– 24.
87. Norman RJ, Hart RJ. Human growth hormone use in poor ovarian response – caution and opportunities. Ther Adv Reprod Health 2021; 15: 1-9.
88. Alviggi C, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril 2016; 105: 1452–3.
89. Gong Y, Zhang K, Verwoerd G, Zhang Y, Liu W, Lai W. Growth hormone improves insulin-like growth factor 1 and steroid hormone levels in follicle fluid, expression of hormone receptors in granulosa cells, and in vitro fertilization outcomes of poor ovarian responders. Fertil Steril 2021; 26: S0015-0282(21)00029-7.
90. Xu Y-M, Hao G-M, Gao B-L. Application of growth hormone in in-vitro fertilization. Front Endocrinol (Lausanne) 2019; 10: 502.
91. Dosouto C, Calaf J, Polo A, Haahr T, Humaidan P. Growth hormone and reproduction: lessons learned from animal models and clinical trials. Front Endocrinol 2019; 10: 404.