Cycle day 2 insulin-like growth factor-1 serum levels as a prognostic tool to predict controlled ovarian hyperstimulation outcomes in poor responders

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Objective: To study whether patients exhibiting poor ovarian response have abnormal levels of serum insulin-like growth factor (IGF)-1 on cycle day 2 when compared with age-matched normal and high responders.
Design: Retrospective cohort.
Setting: University-based practice.
Patient(s): All women between the ages of 21 and 42 years who underwent in vitro fertilization treatment cycle without estrogen pretreatment at our institution between 2013 and 2015.
Intervention(s): Patients were separated into three groups: poor responders ($\leq$4 oocytes retrieved/cycle cancellation), normal responders (8–12 oocytes), and high responders ($\geq$18 oocytes). Subanalysis focused on the next cycle for poor responders adjacent to the nonpretreated index cycle, in which estrogen pretreatment was implemented.
Main Outcome Measure(s): Serum cycle day 2: IGF-1, insulin-like growth factor-binding protein (IGFBP)-3 levels, and IGF-1:IGFBP3 ratio, number of eggs retrieved, number of two pronuclei embryos, cumulative pregnancy rate, and live birth.
Result(s): A total of 184 patients met the inclusion criteria. The poor responder group exhibited a more than twofold increase in the cycle day IGF-1 serum levels when compared with normal responders and a threefold increase when compared with the high responders. Cycle day 2 IGF-1 level >72 ng/mL in poor responders had 70% sensitivity and 78% specificity for a negative controlled ovarian hyperstimulation cycle outcome with an area under the curve of 0.83. Luteal estrogen pretreatment in the poor responder group was associated with a significant reduction in IGF-1 levels. Significantly, more retrieved and mature oocytes, as well as two pronuclei embryos, were achieved in the pretreated poor responder group when compared with the yield from their adjacent nonpretreated index cycles. Furthermore, cumulative rates were higher for intrauterine pregnancies, and lower for negative pregnancy outcome.
Conclusion(s): Patients who respond poorly to controlled ovarian stimulation, despite normal cycle day 2 follicle-stimulating hormone levels, have significantly higher serum cycle day 2 IGF-1 levels when compared with age-matched normal and high responders. Cycle day 2 IGF-1 level >72 ng/mL in poor responders was predictive of a negative cycle outcome. Luteal estrogen pretreatment in the poor responder group was associated with a significant reduction in IGF-1 levels, improved response to stimulation, and higher cumulative rates for intrauterine pregnancies, and lower for negative pregnancy outcome. (Fertil Steril® 2020;113:1205–14. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Insulin-like growth factor 1, estrogen pretreatment, poor ovarian response, luteal phase, prognostic tool, improved outcome

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Poor responders, representing about 10% of women undergoing in vitro fertilization (IVF) (1), are among the most challenging patient groups to treat. Patients with a poor ovarian response have been defined by the European Society of Human Reproduction and Embryology consensus as having at least two of the following three features: [1] advanced maternal age, [2] a previous cycle with poor ovarian response, and [3] an abnormal ovarian reserve test (2). Limited treatment options can be offered to poor responders and the most effective means of optimizing ovarian stimulation are still a matter of debate. Estrogen pretreatment has been implemented in many clinics as a means of coordinating the follicular cohort; however, there is no broad consensus on the efficacy of this approach and little understanding of ancillary effects that it may have on follicular growth. In addition, no good prognostic tools are in place, and reproductive endocrinologists lack useful tools for the optimization and management of cycles.

De Ziegler et al. (3) first showed that exogenous estradiol (E_2) can delay the intercycle increase in plasma follicle-stimulating hormone (FSH). They ultimately demonstrated that this approach can be used safely for the synchronization of endogenous and exogenous FSH stimuli. Based on this concept, many groups of investigators have attempted to implement estrogen pretreatment protocols in the clinic (4), but a prospective randomized trial found that luteal estradiol (LE) treatment does not affect cycle outcome. Therefore it concluded that LE treatment should be used in clinical practice for programming IVF retrievals to accommodate scheduling considerations only (5). A review and meta-analysis by Griesinger et al. (6) struck a more cautious tone, suggesting that LE treatment with oral contraceptive pills (OCPs) for cycle planning has a significant detrimental effect in the form of the reduction in ongoing pregnancy rate. Although the analyses of estrogen pretreatment have been conducted in the general patient population undergoing IVF, a meta-analysis by Reynolds et al. (7) suggests a potential benefit to poor responders. Their systematic review indicated that the addition of E_2 in the luteal phase decreases the risk of cycle cancellation and increases the chance of a clinical pregnancy in poor responders. The mechanism thought to mediate this improvement was the synchronization of the pool of follicles available to controlled ovarian hyperstimulation (COH) (4).

Treatment options to maximize the outcome of poor responders are limited. Increased knowledge of the molecular mechanisms underlying LE administration may be useful in specifying the patient group that benefits from this treatment modality. Although improvement in outcomes for poor responders is commonly attributed to the suppressive influence of estrogen on the endocrine axis and resultant coordination of the follicular cohort, LE may also affect other aspects of follicular development. Estradiol plays a role in antagonizing growth hormone (GH) receptor function, thus attenuating GH-induced hepatic insulin-like growth factor (IGF)-1 synthesis (8). Friend et al. (9) have shown that transdermal and oral estrogen administration is equally potent in suppressing serum IGF-1 concentrations. This is illustrated by the clinical suppression of GH in patients with acromegaly in response to transdermal estrogen treatment (10).

Insulin-like growth factor 1, a small single-chain polypeptide, is secreted by the liver in response to GH produced by the pituitary and transported to target tissues, where it performs its endocrine actions. The IGF-1 is ubiquitously expressed in most tissues, especially postnatally (11), but has a specific role in the amplification of gonadotropin (GT) hormonal action during follicular growth and development (12). The IGF-1 signaling mediates the anabolic and mitogenic activity of GH (13) and IGF-1 receptor (IGF1R), a tyrosine kinase receptor that is expressed in the ovary (14–17), mediates most of the GH-like actions of IGF-1 and IGF-2 through activation of the Pi3K/Akt pathway. Notably, decreased GH/IGF-1 signaling is characterized by a reproductive phenotype. Patients who suffer from Laron syndrome (characterized by insensitivity to GH) tend to be anovulatory and infertile (18, 19). Female members of the African Pygmy tribe, whose IGF1R mutation renders partial resistance, are subfertile and oligo-ovulatory (20, 21).

On the basis of the demonstrated benefit of luteal E_2 treatment in poor responders and previously described requirement for IGF signaling during folliculogenesis, we sought to investigate whether serum IGF-1 levels differ between patients with various degrees of ovarian response. To test also whether supplementation with luteal E_2 can alter IGF-1 levels and cycle outcomes.

MATERIALS AND METHODS

Patient Inclusion Criteria

All patients who underwent a COH cycle and either IVF or intracytoplasmic sperm injection (ICSI) at our center between January 2013 and January 2015 were analyzed for potential inclusion. Patients were excluded, as follows, if: [1] they had a body mass index (BMI) <18 or >30 kg/m^2; [2] they were aged <21 or >42 years; [3] they received a gonadotropin-releasing hormone (GnRH) agonist protocol for COH; [4] they were pretreated using either OCPs or estrogen patches in preparation for COH; [5] their cycle day 2 FSH level exceeded 15 mIU/mL (22); and [6] the purpose of their COH was elective (social) oocyte cryopreservation. If a patient underwent more than one cycle within this time frame, only the earliest cycle was included in our final analysis. This retrospective cohort study was approved by the Institutional Board Review of Weill Cornell Medicine. After inclusion, patients were separated into three groups based on response to COH. Note: None of the patients in our study had inflammatory liver disease, liver dysfunction, or any liver infections (hepatitis B, hepatitis C).

Poor responder group. The poor responder group included patients who had ≤ 4 large follicles (diameter, >14 mm) on the day of ovulation trigger or ≤ 4 oocytes retrieved, or who had their cycles canceled before oocyte retrieval due to lack of response to COH. This patient group meets the European Society of Human Reproduction and Embryology consensus criteria for the definition of poor responders (2). As this was a retrospective study, patients were designated as poor responders based on their response in their previously completed IVF cycle in which they either developed ≤ 4 dominant follicles, less than four eggs were retrieved, or

were canceled due to inappropriate response to COH. In addition, all of the patients in this group had abnormal ovarian reserve testing (antiMüllerian hormone levels [AMH] and/or antral follicle count [AFC]).

Normal responder group. The normal responder group included patients who had 8–12 large follicles on the day of ovulation trigger or 8–12 oocytes retrieved.

High responder group. The high responder group included patients who had ≥18 large follicles on the day of ovulation trigger or ≥18 oocytes retrieved. In this group, 4 of 59 patients had a diagnosis of polycystic ovary syndrome.

Given the known effect of estrogen (either from estrogen patches or OCPs) on reducing IGF-1 levels (23, 24), we performed a subanalysis by comparing poor responder cycles (women were not pretreated with estrogen patches or OCPs [the initial poor responder group]) with their own cycles, which occurred between 2 and 10 months from the index cycle, but in which they did receive OCP or estrogen patch pretreatment (pretreated poor responder group).

Outcome Measures

Primary outcome measures included, as follows: [1] cycle day 2 IGF-1 serum levels (in nanograms per milliliter); [2] insulin-like growth factor-binding protein (IGFBP)-3 levels (in nanograms per milliliter); [3] IGF-1:IGFBP3 ratio; [4] AMH (in nanograms per milliliter) serum levels measured within 1 year from the index cycle; [5] AFC; and [6] number of retrieved oocytes. Levels of IGF-1 and IGFBP3 were analyzed in serum specimens collected by venipuncture, in the early morning of the second day of a menstrual cycle. These values were determined using Immulite 2000 enzyme-labeled chemiluminescent immunometric assay (Siemens). The lower limit of detection was 13.3 ng/mL. The coefficient of variation was <10% across the standard curve for both intra-assay and interassay variability. Serum AMH levels were determined using Access2 ELISA kit (Beckman Coulter Inc.). A standard curve was generated in parallel to the assay and used to convert the absorbance values to nanograms per milliliters. The lower limit of sensitivity was 0.16 ng/mL. The coefficient of variation was <10% across the standard curve for both intra-assay and interassay variability. This is a retrospective study. Patients were identified and classified based on their response to COH. For research purposes our center routinely stores serum samples for all of the patients for several years after their treatment, thus enabling retrospective assay of IGF-1 and IGFBP3 levels. The AFC represents the sum of antral follicles (diameter, 5–10 mm) in both ovaries as determined by transvaginal ultrasound on cycle day 2.

Secondary outcome measures included, as follows: [1] intrauterine pregnancy (defined as the presence of a yolk sac and/or fetal pole within the uterine cavity as determined by transvaginal ultrasound between 5 and 7 weeks of gestation); [2] live birth; [3] negative pregnancy outcome (defined as serum β-human chorionic gonadotropin [hCG] level <5 mIU/mL 11 days after day 3 embryo transfer, or 9 days after blastocyst transfer); [4] maturation rate of oocyte: number of meiosis II out of total harvested; and [5] fertilization rate: number of 2 pronuclei out of total meiosis II.

Clinical Protocols

Protocols for COH, oocyte retrieval, IVF, and embryo transfer were conducted according to the previously outlined practice (25). Briefly, the patients were treated with gonadotropins (Follistim, Merck; Gonal-F, EMD-Serono; and/or Menopur, Ferring) until criteria for pituitary suppression with a GnRH antagonist (0.25mg Ganirelix acetate, Organon) were met (26). The hCG (Pregnyl, Merck), GnRH agonist trigger (leuprolide), or dual trigger (a combination of hCG and GnRH agonist, depending on physicians' preference) were used for final oocyte maturation when the two lead follicles reached a mean diameter >17 mm. Ultrasound-guided transvaginal oocyte retrieval after 35 hours after final oocyte maturation was performed based on our standard practice (26). One day after retrieval, luteal progesterone supplementation with intramuscular progesterone commenced. Fresh embryo transfer was performed on day 3 or day 5 using a Wallace catheter (Marlow/Cooper Surgical). The number of embryos transferred was based on the patient's age, her previous cycles, and clinical criteria (27).

Statistical Analysis

GraphPad Prism 8 was used for data analysis. Continuous variables were analyzed using Student's *t*-test as the data was normally distributed. Categorical variables were analyzed by χ^2 and Fisher's exact tests. A P value $<.05$ was considered statistically significant for all tests.

RESULTS

A total of 184 patients were included in the final analysis. Divided into previously outlined groups, there were 67 poor responders, 58 normal responders, and 59 high responders (Supplemental Fig. 1, available online).

Baseline Characteristics and Primary outcomes of Patients

To have comparable primary outcomes we matched the patients' age, BMI, and gravidity between groups (Table 1). As expected from their inclusion criteria, poor responders had significantly higher cycle day 2 FSH levels when compared with each of the other two groups. Poor responders also demonstrated significantly lower AMH levels, AFC measures, and number of retrieved oocytes when compared with normal or high responders (Table 1). From the 67 poor responder group, 19 patients were excluded from the analysis of "number of retrieved oocytes" due to cycle cancelation because of poor response to stimulation. The remaining 48 patients who proceeded to oocyte retrieval were included in that analysis.

Cycle day 2 Serum Levels of IGF-1, IGFBP3, and their Ratios

Interestingly, the poor responder group demonstrated more than twofold increase in the cycle day 2 mean IGF-1 serum levels (107.4 ± 60.9 ng/mL) when compared with normal

TABLE 1

Baseline characteristics of patients.

Characteristics	Poor responders (N = 67)	Normal responders (N = 58)	*P* value[a]	High responders (N = 59)	*P* value[b]	*P* value[c]
Age (y)	35.9 ± 5	35.9 ± 4	NS	35.4 ± 4.4	NS	NS
Body mass index (kg/m^2)	22.9 ± 2.5	22.7 ± 2.8	NS	22.7 ± 3	NS	NS
Gravidity	1 (0–1)	1 (0–1)	NS	1 (0–1)	NS	NS
CD2 FSH (mIU/mL)	8.8 ± 3.4	6.9 ± 2.4	.0015	5.6 ± 1.6	<.0001	.0009
AMH (ng/mL)	1.0 ± 0.8	2.2 ± 2.9	.0044	4.3 ± 3.1	<.0001	.001
AFC	7.3 ± 3.1	10.6 ± 4.1	<.0001	13.3 ± 4.3	<.0001	.0009
Number of retrieved oocytes	3.4 ± 1.2[d]	10.3 ± 2.6	<.0001	24.8 ± 5.4	<.0001	<.0001

Note: AFC = antral follicle count; AMH = antimüllerian hormone; CD2 = cycle day 2; FSH = follicle-stimulating hormone; NS = not significant.
[a] Poor responders versus normal responders.
[b] Poor responders versus high responders.
[c] Normal responders versus high responders.
[d] N = 48 after excluding 19 poor responders whose in vitro fertilization cycle got canceled before oocyte retrieval due to poor response.

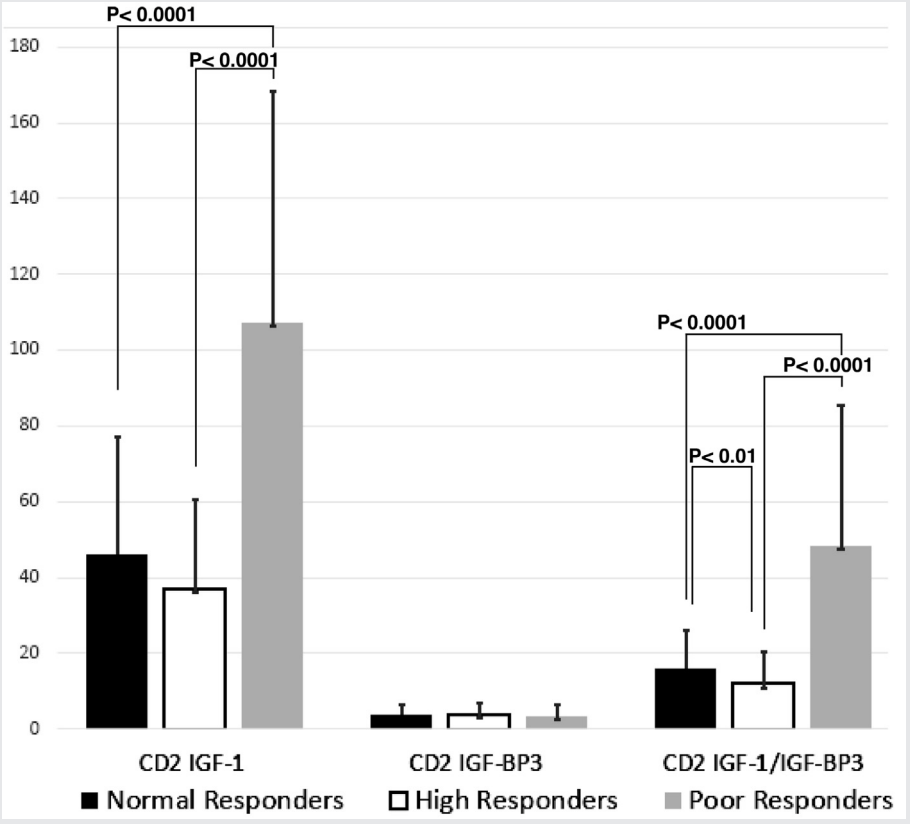
Man. IGF-1 levels predict poor responder outcome. Fertil Steril 2020.

responders (46.1 ± 30.9 ng/mL) and a threefold increase when compared with the high responder group (37.1 ± 23.5 ng/mL) (Fig. 1). Mean cycle day 2 IGF-1 serum levels were the highest among 19 poor responders whose cycle was canceled due to lack of response to COH (121.6 ± 74.4 ng/mL), but this was not significantly different from the poor responders who proceeded to ovum pick-up (101.8 ± 54.5 ng/mL). No difference was found when comparing levels of IGFBP3 between the three groups. Due to increased IGF-1 levels among poor responders, this group had an increased IGF-1:IGFBP3 ratio when compared with high and normal responder groups, suggesting increased free fraction of IGF-1. There was a significant difference in the IGF-1:IGFBP3 ratio between high and normal responders as well (Fig. 1).

Cycle day 2 IGF-1 Serum Levels are Predictive for a Negative outcome to COH in the Poor Responder Group

To determine the sensitivity and specificity of cycle day 2 IGF-1 serum levels in regard to negative outcome to COH

FIGURE 1



Comparison of primary outcomes between the normal, high, and poor responder groups.

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TABLE 2

Baseline characteristics of patients and comparison of outcomes between the nonpretreated, pretreated poor responders, and normal responders.

Characteristics	Nonpretreated poor responders (N = 67)	Pretreated poor responders (N = 21)	*P* value[a]	Normal responders (N = 58)	*P* value[b]	*P* value[c]
Age (y)	35.9 ± 5	35.5 ± 4.1	NS	35.9 ± 4.0	NS	NS
BMI (kg/m^2)	22.9 ± 2.5	22.9 ± 3.2	NS	22.7 ± 2.8	NS	NS
Gravidity	1 (0–1)	1 (0–1)	NS	1 (0–1)	NS	NS
FSH on CD2 (mIU/mL)	8.8 ± 3.4	3.4 ± 2.1	< .0001	6.9 ± 2.4	.0015	< .0001
AMH (ng/mL)	1.0 ± 0.8	0.9 ± 0.6	NS	2.2 ± 2.9	.0043	NS
AFC	7.3 ± 3.1	6.9 ± 3.6	NS (.07)	10.6 ± 4.1	< .0001	< .0005
IGF-1 on CD2 (ng/mL)	107.4 + 60.9	52.6 ± 33.9	.002	46.1 ± 30.9	< .0001	NS
CD2 IGFBP3 (ng/mL)	3.4 ± 2.8	3.3 ± 1.7	NS	3.9 ± 2.9	NS	NS
CD2 IGF-1:IGFBP3 ratio	48.5 ± 46.3	24.0 ± 40.2	.0321	16.1 ± 9.9	< .0001	NS
Number of retrieved oocytes	3.4 ± 1.2[d]	8.1 ± 4.6[e]	< .0001	10.3 ± 2.6	< .0001	.0096
Total gonadotropins (IU)	3,418.8 ± 1,292.1[d]	4,787.5 ± 1,383.6[e]	.0003	2,512.6 ± 1,134.8	.0002	< .0001
Duration of stimulation (d)	8.5 ± 2[d]	10.8 ± 1.8[e]	< .0001	8.4 ± 1.4	NS	< .0001
Gonadotropin IU per day of stimulation	400.2 ± 115.5[d]	459.7 ± 164.3[e]	NS	294.2 ± 109.9	< .0001	< .0001
Intrauterine pregnancy per ET	30.3% (10)	29.4% (5)	NS	52.6% (30)	.0491	NS
Live birth per ET	21.2% (7)	11.8% (2)	NS	35.1% (20)	NS	NS
Negative pregnancy outcome per ET	66.7% (22)	58.8% (10)	NS	29.8% (17)	.0004	.0439

Note: AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; CD2 = cycle day 2; ET = embryo transfer; FSH = follicle-stimulating hormone; IGF-1 = insulin-like growth factor 1; IGFBP3 = insulin-like growth factor-binding protein; IU = international unit; NS = not significant.

[a] Nonpretreated poor responders versus pretreated poor responders.

[b] Nonpretreated poor responders versus normal responders.

[c] Pretreated poor responders versus normal responders.

[d] N = 48 after excluding 19 poor responders whose in vitro fertilization cycle got canceled before oocyte retrieval due to poor response.

[e] N = 19 after excluding 2 poor responders whose in vitro fertilization cycle got canceled before oocyte retrieval due to poor response.

Man. IGF-1 levels predict poor responder outcome. Fertil Steril 2020.

we used a receiver operating characteristic (ROC) curve. It revealed that cycle day 2 IGF-1 level >72 ng/mL in the poor responder group had 70% sensitivity and 78% specificity, for a negative outcome with an area under the concentration curve (AUC) of 0.83 (95% confidence interval [CI] 0.76–0.89, $P<.0001$) (Supplemental Fig. 2A, available online). In the high responders we found an 89% sensitivity but only 22.4% specificity, with AUC of 0.58 (95% CI 0.48–0.68, $P = .14$) (Supplemental Fig. 2B).

Cycle day 2 IGF-1 Serum Levels Normalize at Early luteal Phase

To determine whether the IGF-1 levels observed on cycle day 2 were maintained at different points in the cycle, we measured serum IGF-1 levels on the day of trigger and the subsequent day in a subset of 6 poor responders (nonpretreated) and 10 normal responders. There were no significant differences at the luteal phase between groups, either on trigger day (56.38 ± 44.04 ng/mL vs. 44.67 ± 28.75 ng/mL) or on the following day (37.82 ± 30.21 ng/mL vs. 41.89 ± 26.56 ng/mL) (Supplemental Fig. 3, available online). Given the increase in systemic E_2 with cycle progression, the decrease in IGF-1 levels between the early follicular and early luteal phase in poor responders was not unexpected.

Baseline Characteristics and Outcomes of Poor Responders with or without Pretreatment

To test the effect that pretreatment has on IGF-1 serum levels we compared 67 nonpretreated poor responders (19 whose cycle got canceled prior to oocyte retrieval and 48 who proceeded with oocyte retrieval) with 21 patients who underwent an additional antagonist IVF cycle at our center. This includes E_2 pretreatment using either an estrogen patch (17 patients) or OCP (remaining 4 patients). These groups were age, BMI, and gravidity matched (Table 2). As we would expect, the FSH levels on cycle day 2 decreased dramatically, more than twofold, with the pretreatment with LE, to a level that was even significantly lower than in the normal responder group. A similar decrease was noted between nonpretreated and pretreated patients when comparing cycle day 2 IGF-1 levels and the ratio of IGF-1:IGFBP3. Comparing the three groups of responders, there was no difference in the cycle day 2 IGFBP3 levels. But we noticed a more than twofold increase with the number of retrieved oocytes with pretreatment compared with the nonpretreated poor responders (Table 2). When comparing the IGF-1 serum levels between the 21 patients who proceeded with another IVF cycle to the remaining 46 patients who did not proceed with another IVF cycle, the cycle day 2 IGF-1 levels were significantly different (144.9 ± 67.8 ng/mL vs. 90.3 ± 49.4 ng/mL; $P = .0004$). The reasons for not perusing an adjacent cycle were diverse, some women conceived and gave birth, some froze their eggs for future preimplantation genetic diagnosis and did not use those cryopreserved oocytes by the time we were performing our analysis, a few women used donor eggs, and of course, it is possible that some of the patients were looking for care elsewhere, outside of our institute.

Although the length of stimulation after LE pretreatment was longer compared with the nonpretreated poor responders with a higher GT cumulative dose (daily dose, no statistical

TABLE 3

Comparison of stimulation regimen and outcomes between poor nonpretreated poor responders and pretreated poor responders.

Characteristics	Nonpretreated poor responders (N = 21)[a]	Pretreated poor responders (N = 21)[b]	*P* value
Total gonadotropins (IU)	3,237.5 ± 895.3	4,787.5 ± 1,383.6	.0018
Duration of stimulation (d)	8 ± 1.3	10.8 ± 1.8	< .0001
Gonadotropin IU per day of stimulation	405.2 ± 94.5	464.4 ± 115.5	NS
Number of oocytes retrieved	3.5 ± 1.2	8.1 ± 4.6	.0128
Number of mature oocytes	2.4 ± 1.7	5.9 ± 4.2	.0349
Maturity rate (MII/harvested)	71.7%	72%	NS
2PN (mean ± SD)	1.8 ± 1.4	3.9 ± 2.7	.0209
Fertilization rate (2PN/MII)	60.2%	59%	NS
Cumulative intrauterine pregnancy per ET	7.7% (1/13)	40% (8/20)	.0197
Cumulative live birth per ET	0 (0/13)	20% (4/20)	NS
Negative pregnancy outcome per ET	92.3% (12/13)	50% (10/20)	.0216

Note: 2PN = two pronuclei; ET = embryo transfer; IU = international unit; MII = meiosis II; SD = standard deviation.
[a] N = 12 after excluding 9 poor responders whose in vitro fertilization cycle got canceled before oocyte retrieval due to poor response.
[b] N = 19 after excluding 2 poor responders whose in vitro fertilization cycle got canceled before oocyte retrieval due to poor response.

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significance difference) (Table 2). Not surprisingly, the poor responders were treated for a longer duration with a higher dose of gonadotropins. When comparing the groups as a whole there were less intrauterine pregnancies in the nonpretreated poor responders compared with normal responders. Higher negative pregnancy outcome was noted when comparing nonpretreated and pretreated poor responders to normal responders (Table 2).

Subanalysis of Patients who Proceeded with Luteal E_2 Pretreatment

For testing the effect LE pretreatment has on IGF-1 serum levels and cycle outcomes we used the patients as their own controls to compare adjacent IVF cycles. We compared each patient to her index, nonpretreated, cycle (21 patients). Of the 21 patients in the pretreated group, only 2 were canceled. Pretreated poor responders had significantly lower mean cycle day 2 IGF-1 serum levels than their adjacent nonpretreated cycle (52.6 ± 33.9 ng/mL vs. 144.9 ± 67.8 ng/mL; $P<.0001$) (Supplemental Fig. 4, available online) exhibiting levels that were comparable with those of normal responders (Table 2). A significant decrease was found in all but 1 of the 21 patients (blue line, Supplemental Fig. 4) whose IGF-1 levels at the pretreated cycle were higher than in the nonpretreated one.

Importantly, relative to index cycles pretreated poor responders exhibited more than twice as many mean number of retrieved oocytes, mature oocytes, and two pronuclei embryos (Table 3). Notably, no difference was found in maturity or fertilization rates. Although increased oocyte yields in the pretreatment group coincided with increased total dose of gonadotropins, this stemmed from the increased length of stimulation, as opposed to the increased dose per day (Table 3). Although there was no significant benefit to secondary outcomes at the reference cycle (intrauterine pregnancy, live birth, negative pregnancy outcome) (Table 2), there was a significant benefit when cumulative pregnancy rates were calculated (Table 3). In the index, nonpretreated, cycle 11 patients underwent a total of 13 embryo transfers, compared with 17 patients, of the same cohort, who underwent 20 embryo transfers (almost twice as many). That summed up to a significantly higher intrauterine pregnancy rates (nonpretreated: 7.7% vs. pretreated 40%), and a lower cumulative negative pregnancy outcome (nonpretreated: 92.3% vs. pretreated 50%) in favor of the pretreated patients (Table 3).

It is possible that the improved outcomes result from regression to mean values and the relatively low number of patients in this subanalysis. To address this possibility, we also analyzed one primary outcome of the study, the number of retrieved oocytes. We calculated the relative change in retrieved oocytes ([cycle 2 egg number - cycle 1 egg number]/cycle 1 egg number) between our study group and an equivalent control group of poor responders that underwent consecutive cycles, both with LE pretreatment. Patients were aged between 21 and 42 years, with a BMI between 18 and 30 kg/m^2, and underwent a COH antagonist cycle with either IVF or ICSI at our center between January 2013 and January 2015. Patients from control and study groups were matched for age, BMI, cycle day 2 FSH levels, and AMH levels (Supplemental Table 1, available online), and importantly, we ensured that the daily gonadotropin dose was comparable between cycles of each patient. After excluding of all unmatched parameters, we were left with 71 patients who underwent 142 cycles (111 LE pretreated with E_2 patch, 31 with OCP). This comparison revealed a significant (>threefold) increase between control and the study group (33.19 ± 105.36 vs. 113.18 ± 97.96) (Supplemental Fig. 5, available online).

DISCUSSION

This retrospective cohort study analysis identifies a link between high serum IGF-1 levels and poor response to COH

when compared with normal and high responders in the presence of normal cycle day 2 FSH levels. Specifically, for the poor responders, a cutoff of 72 ng/mL, as shown by the ROC curve (Supplemental Fig. 2), may be a useful threshold for deciding whether to proceed with a COH cycle or supplementing the luteal phase with E_2 and commencing at the next cycle to yield a better outcome, with a 70% sensitivity, 78% specificity, and an AUC of 0.83.

Follicular fluid level of IGF-1 has been reported as a biomarker of oocyte and embryo quality (28), and several studies (29, 30) have shown a relationship between IGF-1 and gonadotropin responsivity within the follicular cohort. Given the positive correlation of IGF-1 with follicular growth, significantly higher levels of serum IGF-1 at cycle day 2 in poor responders was an unexpected outcome of the present study; however, "desensitization" of follicles in the presence of chronically elevated IGF-1 could account for the observed correlation. Ligand-induced internalization and proteolysis of IGF-1 receptors (e.g., IGF1R) play an important role in regulating downstream signaling and biological response, and numerous mediators of IGF1R ubiquitination (31) have been identified. Interestingly, when comparing measured levels of IGF-1 of a small subgroup of poor to normal responders at the luteal phase, at the day of the trigger and the following day, no difference was found, perhaps due to high E_2 levels at those time points in the cycle. Although the present study did not examine follicular fluid IGF-1 levels or expression of IGF1R in granulosa cells, one possibility is that increased oocyte and embryo yield after LE pretreatment may stem from a recovery of surface IGF1R expression upon systemic normalization of IGF-1 levels.

Subanalysis of poor responders between successive cycles with and without LE pretreatment suggested that normalization of IGF-1 levels may confer a therapeutic benefit. Although the length of stimulation after LE pretreatment was longer, with a higher GT cumulative dose, the daily dose was not statistically different. This phenomenon has been previously shown in poor responders (32) and women with regular ovulatory cycles (5). Unlike previous meta-analyses showing that increased dose or duration of stimulation did not improve outcomes for poor responders, in our study the moderately increased duration of stimulation in LE-treated poor responders resulted in double the number of retrieved and mature oocytes as well as two pronuclei embryos without a difference in maturity or fertilization rates (Table 3). Not surprisingly, patient follow-up to assess cumulative pregnancy rate showed significantly more intrauterine pregnancies, less negative pregnancy outcomes, and a trend ($P = .1$) when comparing cumulative live birth rates (4 in pretreatment arm) with no live births in the index cycles (Table 3). Roughly doubling the number of embryos available for transfer, with excellent freezing techniques for the extra numerous embryos, confers a major benefit in this patient group, for whom the pregnancy and live birth rates are nearly half that of normal responding patients. Notably, supporting results have been shown by Reynolds et al. (7) suggesting that the improved chance of pregnancy among women undergoing an LE stimulation protocol may be attributed, at least in part, to the increased likelihood of these women making it to oocyte retrieval. However, this meta-analysis has been strongly criticized by Polyzos et al. (33), who suggest that these results should be interpreted with caution, with more randomized trials needed before drawing firm conclusions.

At present, variations in stimulation protocols have shown little benefit to poor responders. Using short, ultrashort, mini, or micro-dose flare-up regimens are widely implemented in poor responding patients but do not significantly improve clinical outcomes (34). In addition, an increase of the dose of GT >450 IU daily does not increase oocyte yield, the number of embryos obtained, or pregnancy rate (35). Although alternative protocols have not shown a significant benefit to poor responders (30) or only a slight one, the addition of growth hormone (GH) to stimulation cycles does increase the probability of a clinical pregnancy and live birth in these patients (36). The link between GH and follicular output remains unclear, but studies in mouse (37, 38) and human (39) have identified diminished follicular development as a byproduct of aberrant GH signal integration (40) and GH is thought to modulate the action of FSH on granulosa and theca cells by up-regulating local synthesis of IGF-1 (12). The LE pretreatment, with or without simultaneous use of GnRH antagonist, has also been shown to increase the chance of clinical pregnancy in poor responders (7, 41). Although the benefits of LE are attributed to its suppressive influence on GT and a resultant synchronization of the follicular cohort (4), E_2 has also been linked to IGF-1 signaling when administered to suppress abnormally high IGF-1 levels in the context of acromegaly (10, 42). Similar to the results we obtained in our poor responder subanalysis (Supplemental Fig. 3), it has been shown that OCP can modulate the GH and IGF-1 axis in reproductive aged women, resulting in a reduction of mean IGF-1 concentration by 12%–30% (43). In our dataset, we noted a greater reduction of serum IGF-1 (58%), possibly because most patients were pretreated with E_2 patches and not OCPs, which are metabolized on passing through the liver. Interestingly, it was noted that the extent of individual changes in GH and IGF-1 levels depends on the basal level before pill intake (44). Given the diversity of stimulation protocols and meta-analyses of their efficacy for treatment of poor responders, the uniquely positive influence of GH stimulation and LE treatment on outcomes may suggest an important function for IGF-1 signaling in amplifying ovarian stimulation among poor responders.

One weakness in the subanalysis of this retrospective study is the relatively small group of women who were analyzed. To demonstrate the benefit of LE pretreatment in poor responders did not result from regression to mean values, we included a subanalysis of control patients who underwent successive cycles, both with LE pretreatment. Hypothetically it would be ideal to compare the relative change between two cycles with and then without LE pretreatment. However, we were not able to find sufficient patients of this characteristic to include in this analysis, as our center supports routine pretreatment with E_2, using a protocol in poor responders that incorporates transdermal E_2 and a GnRH antagonist in the preceding luteal phase (41). For this reason, it was impossible

to compare enough patients that were treated with a non-LE protocol after a LE one. Instead, we choose to compare poor responders who had consecutive cycles with LE pretreatment, and tested the relative change between comparable cycles of the same patient. We included 71 patients in that analysis and found a stark difference—33.19% ± 105.36% vs. 113.18% ± 97.96% when comparing patients who underwent consecutive LE pretreatment to those who underwent untreated then pretreated cycles.

In conclusion, elevated serum IGF-1 levels can serve as a biomarker of poor ovarian response among poor responders. The LE pretreatment normalizes serum IGF-1 levels and may provide a benefit in gonadotropin and GnRH antagonist cycles. Hence, measurement of cycle day 2 IGF-1 serum levels may serve as a new tool as it can be easily assayed in serum immediately before stimulation. Although higher power analysis and/or randomized control trials will be necessary to clearly define the relevance of serum IGF-1 levels to follicular growth and maturation, these results put forth a novel hypothesis of the mechanism underlying a commonly used LE priming protocol. Additionally, the data suggest that hyperstimulation in poor responders may be influenced by the relative sensitivity of the follicular cohort to IGF-1.

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Niveles séricos en el día 2 del ciclo del factor 1 de crecimiento similar a la insulina como una herramienta para predecir los resultados de estimulación ovárica controlada en bajas respondedoras

Objetivo: Estudiar si las pacientes que presentan baja respuesta tienen niveles anormales de factor 1 de crecimiento similar a la insulina (IGF)-1 en el día 2 del ciclo cuando se comparan con pacientes normo y altas respondedoras de la misma edad.

Diseño: Cohorte retrospectiva

Escenario: Centro universitario

Paciente(s): Todas las pacientes entre 21 y 42 años que fueron sometidas a un ciclo de fecundación in vitro sin pre-tratamiento con estrógenos en nuestra institución entre 2013 y 2015.

Intervención(es): Las pacientes fueron separadas en tres grupos: bajas respondedoras ($\leq$ 4 ovocitos recuperados/ciclo cancelado), normo reponedoras (8-12 ovocitos) y altas respondedoras ($\geq$ 18 ovocitos). El sub-análisis se enfocó en el ciclo siguiente para las bajas respondedoras adyacente al ciclo índice no pre-tratado, en el cual se implementó el pre-tratamiento con estrógenos.

Medidas de resultado principal: Suero de día 2 del ciclo: niveles de IGF-1, proteína fijadora de factor de crecimiento similar a la insulina (IGFBP)-3 y la relación IGF1:IGFBP3, número de ovocitos recuperados, número de embriones con dos pronúcleos, tasa de gestación acumulada y tasa de recién nacido vivo.

Resultados: Un total de 184 pacientes cumplieron con los criterios de inclusión. El grupo de bajas respondedoras presentó un incremento de más de dos veces en los niveles séricos de IGF-1 cuando se comparó con las normo-respondedoras y un incremento de tres veces cuando se comparó con las altas respondedoras. El nivel de IGF-1 el día 2 del ciclo 72 ng/ml en bajas respondedoras tuvo una sensibilidad de 70% y una especificidad de 78% para un resultado negativo de una estimulación ovárica controlada con un área bajo la curva de 0.83. El pre-tratamiento con estrógenos en fase lútea en el grupo de bajas respondedoras fue asociado con una reducción significativa de los niveles de IGF-1. Más ovocitos recuperados y maduros, así como también embriones con dos pronúcleos se obtuvieron de forma significativa en el grupo pre-tratado de bajas respondedoras cuando se comparó con el resultado de su ciclo índice adyacente no pre-tratado. Además, las tasas acumuladas fueron más altas para gestación intrauterina y más bajas para resultados negativos de gestación.

Conclusión(es): Pacientes que respondieron pobremente a una estimulación ovárica controlada, a pesar de niveles normales de FSH en día dos del ciclo, tuvieron niveles séricos de IGF-1 mayores cuando se compararon con pacientes normo respondedoras y altas respondedoras de la misma edad. El nivel de IGF-1 el día 2 del ciclo 72 ng/ml en bajas respondedoras fue predictivo de un resultado negativo en el ciclo. Pre-tratamiento con estrógenos en la fase lútea en las bajas respondedoras fue asociado con una reducción significativa de los niveles de IGF-1, respuesta mejorada a la estimulación, tasas acumuladas de gestación intrauterina mayores y menores resultados negativos de gestación.