Granulosa Cell Aromatase Activity in Women Undergoing IVF: A Comparison of Good and Poor Responders

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Abstract

Objective: We wished to investigate the aromatase activity (AA) of granulosa cells (GCs) in women undergoing ovarian follicular stimulation for in vitro fertilization (IVF).

Methods: Granulosa cells were harvested from follicular fluid aspirated at the time of oocyte retrieval in women undergoing IVF. Data related to the follicular stimulation and IVF were collected by chart review. We conducted a retrospective analysis of the relation between the response to stimulation and the AA of GCs obtained from IVF patients. We assessed the response to stimulation by calculation of the area under the curve (AUC) of the monitored serum estradiol levels, and divided patients into "poor responders" and "good responders."

Results: There was no difference in AA between women with a poor response to stimulation and women with a good response. Implantation rates and pregnancy rates were significantly lower in poor responders (5.3% and 9.1% respectively) than in good responders (19.7% and 54.8% respectively), even though embryo quality was similar in each group.

Conclusions: Women who have a poor response to ovarian follicular stimulation preceding IVF have lower pregnancy rates than women with a good response. The lower pregnancy rates do not appear to be a consequence of an abnormal follicular environment, because AA and the ratio of serum estradiol AUC to oocytes retrieved was similar in both groups of women.

Key Words: poor responder, granulosa cell, aromatase, in-vitro fertilization, controlled ovarian hyperstimulation.

Competing Interests: None declared.

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Résumé

Objectif : Nous souhaitons explorer l'activité aromatase (AA) des cellules de la granulosa (CG) chez les femmes subissant une stimulation folliculaire ovarienne en vue d'une fécondation *in vitro* (FIV).

Méthodes : Les cellules de la granulosa ont été recueillies à partir du liquide folliculaire aspiré au moment de la récupération de l'ovocyte chez les femmes subissant une FIV. Les données associées à la stimulation folliculaire et à la FIV ont été recueillies au moyen d'une analyse de dossier. Nous avons mené une analyse rétrospective sur la relation entre la réaction à la stimulation et l'AA des CG obtenues chez les patientes FIV. Nous avons évalué la réaction à la stimulation en calculant l'aire sous la courbe (ASC) des taux sériques d'estradiol surveillés; de plus, nous avons réparti les patientes en deux groupes : « faible réaction » et « bonne réaction ».

Résultats : Aucune différence n'a été constatée entre les femmes du groupe « faible réaction » et celles du groupe « bonne réaction » en matière d'AA. Les taux d'implantation et de grossesse étaient significativement plus faibles chez les femmes du groupe « faible réaction » (5,3 % et 9,1 %, respectivement) que chez celles du groupe « bonne réaction » (19,7 % et 54,8 %, respectivement), et ce, même si ces groupes présentaient une qualité d'embryon semblable.

Conclusions : Les femmes qui réagissent faiblement à la stimulation folliculaire ovarienne précédant la FIV présentent des taux de grossesse plus faibles que ceux des femmes qui y réagissent bien. Cette faiblesse des taux de grossesse ne semble pas être attribuable à un milieu folliculaire anormal, puisque l'AA et le rapport « ASC de l'estradiol sérique-ovocytes récupérés » étaient semblables dans les deux groupes de femmes.

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INTRODUCTION

An essential component of treatment in an IVF program is controlled ovarian hyperstimulation, the purpose of which is to recruit a large number of good quality follicles. However, it is estimated that 9% to 24% of infertile women undergoing assisted reproduction have a poor response to COH,[1] and these so-called "poor responders" are known to have a much lower pregnancy success rate.[2] This poor

outcome may be attributed in part to factors related to the poor stimulation response, including a low number of oocytes retrieved, sub-optimal fertilization rates, and poor oocyte and/or embryo quality. Taken together, these factors lead to very low implantation and pregnancy rates in poor responders.

There is currently no standardized definition of "poor response." Several authors have defined it as the retrieval of less than three or four oocytes, or having a serum E_2 level of less than 500–1000 pg/mL on the day of administration of hCG.[3–5] Unfortunately, women are identified as poor responders only after they have undergone a costly cycle of COH. To identify women at risk of having a poor response to COH, several tests have been proposed, including markers of ovarian reserve such as serum levels of FSH, E_2, and inhibin B on cycle day three,[6] the ratio of serum FSH to serum LH,[7] and antral follicle count.[8] However, none of these strategies has allowed clinicians to predict accurately who will be a poor responder. This lack of predictive ability underscores the need to elucidate the mechanisms underlying poor responsiveness to COH.

There is a paucity of information on mechanisms that may contribute to a poor response to ovarian stimulation. It has long been noted that poor responders have a lower serum E_2 level in response to gonadotropin administration.[3] Furthermore, poor response is a hallmark of diminished ovarian reserve commonly observed in older women.[4] Estradiol production in the ovary is thought to follow the two-cell two-gonadotropin model, in which ovarian theca cells produce androstenedione in response to LH stimulation, and granulosa cells synthesize estrogen from androgen precursors, including androstenedione, in response to FSH.[9] Within the ovarian granulosa cells, the enzyme responsible for estradiol production is aromatase (P450arom), which is encoded by the CYP19 gene.[9] We have previously shown that granulosa cell aromatase activity is correlated with pregnancy potential.[10] It is proposed that poor responders may have insufficient AA at the ovarian level, thereby limiting estrogen production in follicles and causing a poor response to COH.[11]

If a woman has had a poor response to COH, most clinicians increase the dose of gonadotropins administered in the subsequent cycle in an attempt to improve follicle recruitment and development. However, it is unclear whether or not this strategy is valid in the treatment of poor responders. Therefore, the objectives of this retrospective study were to investigate the relationship between the aromatase activity of granulosa cells and the ovarian response, and to attempt a better understanding of the dynamics of stimulation in poorly responding women.

ABBREVIATIONS

AA	aromatase activity
ANOVA	analysis of variance
AUC	area under the curve
CES	cumulative embryo score
COH	controlled ovarian hyperstimulation
E_2	estradiol
FSH	follicle stimulating hormone
GC	granulosa cell
IVF	in vitro fertilization
hCG	human chorionic gonadotropin
LH	luteinizing hormone

MATERIALS AND METHODS

Ethical approval for this study was obtained from the Centre for Reproductive Care Research Committee and Hamilton Health Sciences / McMaster University Research Ethics Board.

Study Subjects and Collection of Granulosa Cells

Granulosa cells were harvested from the follicular fluid aspirated at the time of oocyte retrieval from 42 patients undergoing IVF. Ovarian follicular stimulation was conducted using a long luteal protocol of leuprolide 0.5 mg subcutaneously per day for 10 to 14 days. Provided that the serum E_2 level was < 150 pmol/L and no ovarian cysts were identified on ultrasound examination, controlled ovarian hyperstimulation was initiated with subcutaneous administration of recombinant FSH. The dose of FSH was adjusted according to changes in serum E_2 levels and follicular growth as seen on ultrasound. When three or more follicles of mean diameter ≥ 18 mm were seen and the serum E_2 level was deemed to be adequate, 10 000 IU of hCG was administered. Oocytes were retrieved approximately 34 to 36 hours after hCG injection, using transvaginal ultrasound-guided aspiration. Cumulus oocyte complexes were identified in the follicular aspirates, placed in HEPES buffered culture medium with modified human tubal fluid (Irvine Scientific, Santa Ana CA), and covered with mineral oil. The remaining follicular aspirate was collected and pooled with other aspirates, and the granulosa cells recovered by centrifugation at 1000 rpm followed by treatment with sterile distilled water, and then 10 × phosphate buffered saline, as previously described.[12]

Determination of Aromatase Activity

GCs were cultured in 48 well plates for 48 hours in defined media (DMEM F12 containing 10% fetal bovine serum, 100 units/mL penicillin, 0.1 mg/mL streptomycin and 2.5 μL amphotericin B). Aromatase activity was measured