Ovulation Escape in a Gonadotropin-releasing Hormone Antagonist In Vitro Fertilization Cycle is Not an *All or None* Phenomenon: A Case Report

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ABSTRACT: Case presentation of a healthy G2P2 patient in her late 30s, treated with in vitro fertilization–intracytoplasmic sperm injection for severe male factor infertility. The patient was treated with a gonadotropin-releasing hormone antagonist cycle (GnRH-An). GnRH-An (Cetrorelix) daily injections were started on cycle day 7 and switched to a different GnRH-An preparation (Ganirelix) due to an allergic reaction. Serum hormone levels and ultrasound monitoring were uneventful until day 13, when a corpus luteum cyst was detected, in addition to multiple intact follicles. Serum progesterone increased to 45 nmol/L, while serum luteinizing hormone (LH) remained low. Thirty-six hours following a day 13 human chorionic gonadotropin (HCG) triggering, 18 cumulus-oocyte complexes were successfully retrieved, resulting in the development of two blastocysts. This is an example for an isolated single-follicle ovulation without compromising the rest of the cohort. A possible explanation is an increased concentration of LH receptors on a specific follicle or increased sensitivity to endogenous GnRH in GnRH-An cycles. Clinicians facing a similar scenario should consider not cancelling the cycle in case additional intact follicles are present.

KEYWORDS: GnRH receptor antagonist, ovulation inhibition, ovarian follicle, ultrasound, ambulatory monitoring

CITATION: Klement et al. Ovulation Escape in a Gonadotropin-releasing Hormone Antagonist In Vitro Fertilization Cycle is Not an *All or None* Phenomenon: A Case Report. *Reproductive Biology Insights* 2016:9 1–4 doi:10.4137/RBI.S27793.

TYPE: Case Report

RECEIVED: April 16, 2015. **RESUBMITTED:** May 27, 2015. **ACCEPTED FOR PUBLICATION:** May 30, 2015.

ACADEMIC EDITOR: Eliezer Shalev, Editor in Chief

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 538 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: RC is on the scientific advisory boards of Ferring, Merck and EMD Serono, and receives a fellowship training grant from EMD Serono. Other authors disclose no potential conflicts of interest.

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Paper subject to independent expert blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

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Introduction

During recent years, gonadotropin-releasing hormone antagonist (GnRH-An) protocols for in vitro fertilization (IVF) have gained increasing popularity due to shorter treatment duration, reduced amount of gonadotropins required, and a significantly lower incidence of ovarian hyperstimulation syndrome compared to long GnRH agonist protocols.[1–4] Both GnRH-An and GnRH agonist protocols were developed for ovarian stimulation in order to prevent a premature LH surge, thereby allowing timing of oocyte retrieval and reducing the risk for cycle cancellation. However, a sharp rise in serum LH in an antagonist protocol may represent a LH surge escaping the GnRH-An inhibition and may lead to cancellation of the cycle due to possible premature ovulation before oocyte retrieval can be performed. We present an IVF cycle complicated by apparent escape from GnRH-An inhibition leading to a documented ovulation of a single follicle, but not affecting the successful oocyte collection of the rest of the follicular cohort.

Case History

Initial infertility evaluation and previous IVF cycles. Our patient presented as a healthy G2P2 female in her late 30s and her mid 40s male partner, who was diagnosed with acquired obstructive azoospermia. The couple conceived spontaneously in 2008, and the patient had a term normal vaginal delivery with no complications. In 2011, after 2 years of attempts to conceive, they were referred to our clinic. The couple's initial evaluation revealed normal ovulatory cycles, normal uterine cavity, and patent tubes. Semen analyses demonstrated intermittent severe oligospermia, and after the completion of urological assessment, the recommendation was to proceed with percutaneous epididimal sperm aspiration (PESA) and intracytoplasmic sperm injection (ICSI). In June 2011, the patient was treated with a long luteal stimulation protocol with a daily dose of 200 units of recombinant follicle stimulating hormone (rFSH) (Puregon, Merck Canada Inc., Kirkland, QC, Canada). PESA was performed successfully on the day of oocyte retrieval, and the aspirated spermatozoa were used for ICSI. Two vials of sperm were cryopreserved for future use. Eleven oocytes were retrieved and injected of which eight fertilized and after five days of incubation, two blastocysts and three cavitating morulas were formed. The patient failed to conceive after fresh and frozen/thawed embryo transfers, and a second IVF cycle using a frozen PESA sample was performed in December 2011. This was

also along luteal stimulation protocol using 300 units of rFSH (Puregon, Merck Canada Inc.) and 100 IU of hCG (Pregnyl, Merck Canada Inc.) daily. In this cycle, 14 oocytes were retrieved, 13 of them were mature and injected with sperm resulting in nine zygotes. Due to a slow rate of cleavage, four 72-hour embryos were transferred. This cycle resulted in a singleton pregnancy and a term live birth.

Current IVF cycle. In October 2014, the patient returned to the clinic for another IVF treatment. There was no change in her medical background. Calculated body mass index was 22.6. By day 3, a transvaginal ultrasound (TVUS) demonstrated a normal-appearing uterus with a 6-mm lining. Serum hormonal concentrations on cycle day 3 were follicle stimulating hormone (FSH) 8 IU/L, LH 5 IU/L, E2 114 pmol/L, and progesterone 2 nmol/L. Both ovaries were of normal size and texture. Antral follicular count was 19 (all had a diameter of <8 mm). Cycle monitoring data are presented in Table 1. The patient was started on a GnRH-An cycle using 300 units of rFSH (Puregon, Merck Canada Inc.) on cycle day 3. An ultrasound on cycle day 7 (after 4 days of FSH stimulation), as routinely performed in our facility, showed a 10-mm uterine lining and eight follicles measuring 11–13 mm in the left ovary and three follicles measuring 11, 12, and 17 mm on the right side. A GnRH-An (Cetrorelix; Cetrotide®, 0.25 mg, EMD Serono, Mississauge, Canada) daily was started on cycle day 7, and the rFSH was kept at the same dose. She was scheduled for another monitoring appointment on the following day. In the morning, on cycle day 8, the patient contacted the clinic complaining of a local skin reaction she noticed after injecting the GnRH-An. The lesions were itchy and included hives. She was instructed to switch to Ganirelix Acetate, 0.25 mg (Orgalutran®, Merck Canada Inc.). However, the patient was unable to obtain the Ganirelix until the next morning and therefore skipped the injection of GnRH-An on day 8 of her cycle. Serum LH concentration on cycle day 8 was low (2 IU/L). Monitoring on cycle day 11 demonstrated follicular growth in both ovaries, blood estradiol results that were consistent with the number and size of follicles, and a serum LH concentration of 1 IU/L. A TVUS on day 13 of her cycle showed a new hyperechoic cyst in the right ovary, compatible with a right ovarian corpus luteum cyst. The size of the cyst was 32 × 33 mm (Fig. 1). Other than the cyst, there were multiple intact follicles on both sides. Serum progesterone levels increased to 45 nmol/L, while serum LH values remained low at 2 IU/L. The patient was triggered with 10,000 units of hCG (Pregnyl, Merck Canada Inc.) on the same day (cycle day 13) at 7 pm, and retrieval took place 36 hours later. Eighteen cumulus-oocyte complexes (COCs) were successfully retrieved. Of the 18 COCs, 15 were mature metaphase II (MII) and injected with thawed PESA sperm while three were empty zonas. The next day, 11 of the 15 oocytes had two pronuclei, and the cycle eventually ended in the development of two blastocysts (3BB, 3AB) that were cryopreserved.

Discussion

We present a case of a single follicle escaping GnRH-An suppression during an IVF cycle, while the rest of the follicular stimulated cohort was not compromised. GnRH-An–assisted reproductive technology (ART) protocols have recently been proven to be safe and effective and are growing in popularity.[1–5] The GnRH-An were introduced in the early 1990s[6,7] and unlike GnRH agonists, they do not produce an initial stimulation of gonadotropin release. Instead, GnRH-An use leads to a rapid and reversible suppression of gonadotropin secretion by directly competing with endogenous GnRH for its receptor binding sites, thereby reducing the risk for a premature gonadotropin surge when combined with controlled ovarian hyperstimulation. The potent action of both GnRH agonists and antagonists is enabled by amino acid substitutions in the native GnRH decapeptide chain.[8] In ART, two GnRH-An are currently available for clinical use: Ganirelix and Cetrorelix, both are subcutaneous injections.

In order to achieve an efficient blockade of the GnRH receptor and to successfully compete with endogenous GnRH,

Table 1. Sonographic and laboratory cycle monitoring data.

DAY OF DALE	ENDOMETRIAL THICKNESS (mm)	OVARY SCANNED	FOLLICLES (mm)	FSH IU/L	LH IU/L	ESTRADIOL pmol/L	PROGESTERONE nmol/L
Day 3	6	Right ovary	7 follicles < 8 mm	8	5	114	2
		Left ovary	12 follicles < 8 mm				
Day 7	10	Right ovary	17,12,11	22	1	2140	1
		Left ovary	13,13,12,12,12,12,12,11				
Day 8	10	Right ovary	16,15,12,10,10	22	2	5920	5
		Left ovary	15,15,13,13,13,12,11,11,11				
Day 11	13	Right ovary	19,16,15,15,14,13,12,12,11	23	1	9163	11
		Left ovary	17,16,15,14,14,14,13,13,13				
Day 13 (HCG day)	13	Right ovary	Corpus luteum cyst 32*33 mm 18,16,15,14,13,13,11,11	16	3	14,216	45
		Left ovary	21,18,18,17,16,16,14,13,12,10				

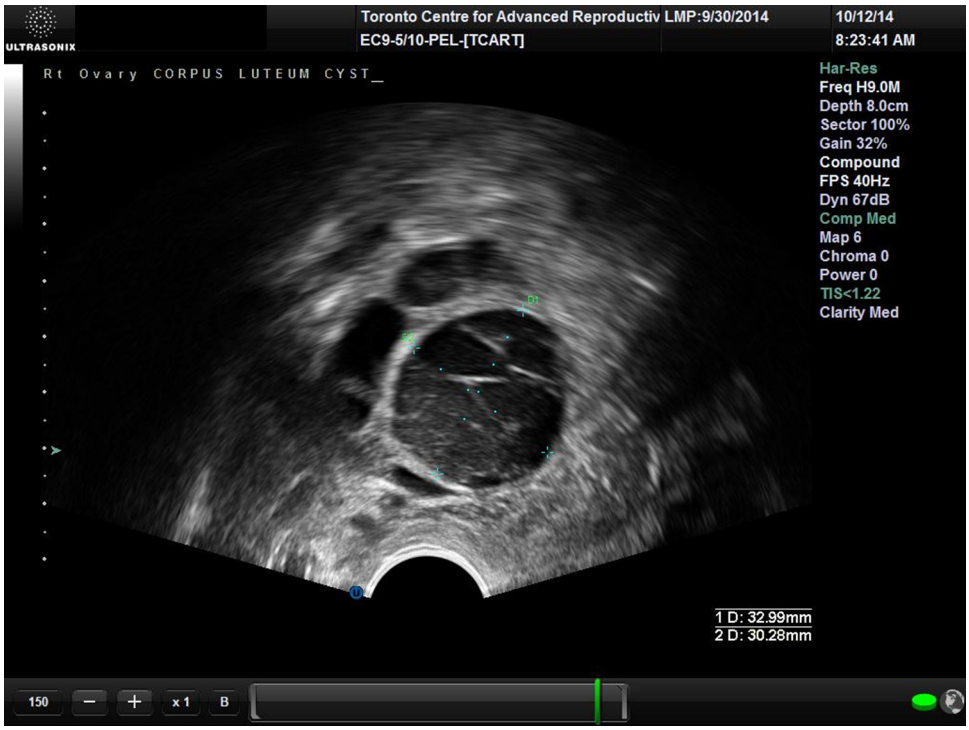

Figure 1. Cycle day 13 ultrasound monitoring: right ovarian corpus luteum cyst.

a constant exposure to GnRH-An should be maintained during the advanced phase of ovarian stimulation protocols. It was demonstrated that an interruption or delay in a repeated GnRH-An injection may result in a rapid rise of LH levels, reflecting the reversibility of the block and the lack of influence over endogenous LH synthesis and release.[9] The first study to address the minimal dose required to achieve sufficient pituitary GnRH receptor antagonism was published in 1997 and tested Cetrorelix in 0.5, 0.25, and 0.1 mg daily doses. This study led to the common use of the 0.25-mg dose for the GnRH-An protocols that are used today. A year after this publication, a double-blind, randomized, multicenter dose study was published, this time testing Ganirelix dosing.[9] Serum levels of Ganirelix showed a linear increase and a steady state was achieved after two days in all the tested doses. When tested for efficiency and outcome, the 0.25-mg dose of Ganirelix was selected as having the best results.

In the present case report, the patient ovulated while being treated with GnRH-An, as proven by a high serum concentration of progesterone and the presence of a corpus luteum. She did not ovulate from at least 15 other follicles that yielded 15 mature oocytes four days after the isolated ovulation took place. The patient's serum LH levels were not detected to be elevated at any point of the monitoring period (≤3 IU/L). However, the patient demonstrated a histamine release reaction to one preparation and was switched to another, resulting in a missed dose of GnRH-An. It is possible that there was a transient rise of LH during the missed dose of GnRH-An, that was blocked by the next dose of the GnRH-An and was not measured. Alternatively, a gradual rise in LH during GnRH-An treatment, as described by the Ganirelix dose finding group,[9] is speculated to reflect a gradual increase in endogenous GnRH secretion displacing the GnRH-An at the receptor site. An increased sensitivity to endogenous GnRH with prolonged GnRH-An has been suggested by a study of postmenopausal women exposed to GnRH-An.[10] LH levels were measured before and after eight days of treatment with GnRH-An, with or without estrogen administration. A clear rise in the serum LH concentration was detected after eight days, but was statistically significant only for the group not exposed to estrogen. This experiment provides support for a possible gonadotropin escape from GnRH-An suppression created by some recovery of pituitary responsiveness to endogenous GnRH.

The unique feature of the present case is the ovulation of a single follicle, while the rest of the cohort remained intact. To the best of our knowledge, this is the first description of this phenomenon. A possible explanation might be an increased concentration of LH receptors on the specific follicle that ovulated making it more sensitive to any exposure to LH. Due to the increased progesterone levels associated with the presence of a corpus luteum, a fresh embryo transfer was avoided and the patient was planned for a frozen embryo transfer (FET). The embryological performance of this cycle did not seem to be compromised by the single follicle escape from suppression when compared to the previous cycle in our clinic.

In summary, we believe this is an unusual case demonstrating that asynchrony in follicle growth may lead to

ovulation without compromising the rest of the cohort. Clinicians facing a similar scenario should consider not cancelling the cycle if ultrasound examination supports the presence of additional intact growing follicles.

Author Contributions

All the signed authors have done a substantial contribution to conception and design, acquisition of data, and interpretation of data; been involved in drafting the article or revising it critically for important intellectual content; and approved the final version.

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