Women's Health & Gynecology

ISSN: 2369-307X

Research Article

A Sustained Elevated Estradiol is not the Trigger for the Preovulatory Luteinizing Hormone Surge

This article was published in the following Scient Open Access Journal:
Women's Health & Gynecology
Received August 24, 2015; **Accepted** September 23, 2015; **Published** September 29, 2015

Bradley S Hurst*, Kathryn S Merriam, Mollie Elliot, Michelle L Matthews, Paul B Marshburn and Rebecca S Usadi
Carolinas Medical Center, Department of Obstetrics and Gynecology, Charlotte NC, USA

Abstract

Objective: To determine if sustained elevated estradiol (E_2) is the primary trigger for the LH surge and subsequent ovulation.

Methods: This was a prospective randomized controlled study at an academic fertility center of ten healthy ovulatory volunteers with regular menstrual cycles 26-30 days. The first cycle was a monitored natural control cycle, and in the second cycle women took letrozole 5 mg daily from cycle day 1-3 through day 22. Both cycles were monitored with daily urinary LH days 10-18, one ultrasound days 12-14, and serum E_2 and progesterone days 12, 14, 16, 18, 20, and 22. The normal cycle was assessed first to avoid a potential effect of letrozole in a subsequent cycle. **Results:** All 10 women ovulated during the daily letrozole cycle, even though serum E_2 was significantly lower than the natural control cycle. Nine of 10 ovulated in the natural cycle. **Conclusions:** A sustained physiologic E_2 elevation is not the trigger for ovulation, since all women ovulated in cycles when E_2 was continuously suppressed by an aromatase inhibitor. Although E_2 and progesterone may serve permissive roles, we hypothesize that there is another signal for the LH surge which has not been elucidated. Clinical Trials Registry Number: NCT01999569 (clinicaltrial.gov)

Keywords: Luteinizing hormone, Estradiol, Ovulation, Progesterone, Letrozole, Aromatase inhibitor

Introduction

Classic teaching of the hormonal events which trigger female ovulation stresses the importance of the hypothalamic-pituitary-ovarian axis [1,2]. Estradiol produced from the dominant follicle rises rapidly by cycle day 7 and continue to increase through the mid-follicular phase [1,3]. The mid-follicular peak in estradiol exerts a positive feedback influence on gonadotropin releasing hormone (GnRH) which leads to a surge of LH release and subsequent ovulation [1,2].

Estradiol is synthesized from androgen by aromatase enzyme activity in granulosa cells [4,5]. Letrozoleis a nonsteroidal third generation aromatase inhibitor (AI) which results in low serum estrogen levelsduring ovulation induction [6].

Since the common understanding of ovulation in a natural cycle suggests that a sustained, elevated estradiol level is required to trigger the LH surge, administration of letrozole throughout the cycle should lower estradiol levels and prevent the LH surge from occurring. We sought to determine if the LH surge, ovulation, and subsequent progesterone levels increase in spite of maintaining low estradiol levels by daily administration of letrozole in normal ovulatory volunteers in a prospective study.

Materials and Methods

After IRB approval and informed consent were obtained, ten willing volunteerswho met inclusion criteria (no hormonal contraception within 3 months, regular menstrual cycles 26-30 days, normal thyroid function and normal prolactin, and no pregnancy currently or within 3 months) were monitored for one month without treatment for evaluation of normal ovulation.

Natural control cycle

The subjects used home urine LH tests (Clearblue® Easy, SPD Swiss Precision Diagnostics, Switzerland) on days 10-18 to monitor for the LH surge in both the initial

***Corresponding author:** Bradley S. Hurst, 1025 Medical Center Plaza, Suite 500, Charlotte NC 28204, USA, Tel: 704-355-1747, Fax: 704-355-8798, Email: Brad.Hurst@carolinashealthcare.org

Cycle Day	Natural Cycle	Letrozole Cycle
1, 2, or 3 (1st day menses = day 1)		Serum HCG (day 1-3). Begin letrozole if negative.
Day 1-3 to day 22		Letrozole 5 mg/day
10	Begin daily urine LH testing until day 18	Begin daily urine LH testing until day 18
11	Continue daily LH testing	Continue daily LH testing
12-22	Serum E2 and P every other day	Serum E2 and P every other day
12-14	Vaginal ultrasound 1x to measure lead follicle	Vaginal ultrasound 1x to measure lead follicle
22	Last Serum E2 and P	Last Serum E2 and P Discontinue letrozole

Table 1: Protocol Summary.

natural cycle and the letrozole cycle. In the natural cycle and in the letrozole cycle, blood was drawn every other day starting on day 12 of the cycle through day 22 to measure estradiol and progesterone levels, the LH surge was monitored with home urine ovulation tests on days 10-18, and follicular development was monitored one time using transvaginal ultrasound on cycle day 12-14.

Letrozole cycle

In the next cycle, all subjects were administered oral letrozole 5 mg daily (Femara®, Novartis Pharmaceuticals Corporation, East Hanover, NJ) starting on cycle day 1-3 and continuing through cycle day 22. Table 1 illustrates protocols for both the natural control cycle and the letrozole study cycle.

The primary outcome, assessment of ovulation in letrozole cycles, was determined by the presence or absence of progesterone elevation (>1.5 ng/mL) and the presence or absence of a positive urinary LH test. The bioequivalence evaluation of two cycles (before and after letrozole administration) was based on pharmacokinetic parameters such as area under the serum concentration–time curve (AUC), the peak serum concentration (C_{max}) and the time of peak serum concentration (T_{max}). C_{max} and T_{max} were determined by visual inspection from each volunteer's serum concentration–time curve for estradiol and progesterone. AUC was calculated by the linear trapezoidal method from day 12 through day 22 in both the initial natural cycle and the letrozole cycle.

Paired t-tests, or Wilcoxon Signed Rank tests if non-normally distributed, were used to evaluate the statistical significance of the mean values of the pharmacokinetic parameters. The McNemar test was used to assess the difference in LH surge and follicular development before and after letrozole administration. A standard of statistical significance (alpha) of 0.05 was used in all cases. The SAS System (SAS Institute, Cary, NC) was used for all analyses.

Results

As expected, the peak serum E_2 levels were significantly lower in the letrozole cycle than in the natural control cycle (118 ± 53 pg/mL vs. 232 ± 95 pg/mL; $p<0.05$). Additionally, the E_2AUC was significantly lower in the letrozole cycle than in the natural cycle (478 ± 275 vs. 1410 ± 347; $p<0.05$). One subject ovulated with a peak estradiol level less than 100 pg/mL.

Despite significantly lower E_2 concentrations, all volunteers ovulated during the letrozole cycle, while 9/10 ovulated during the natural cycle.

In spite of continuing letrozole, the peak progesterone was significantly higher during the letrozole cycle compared to the normal cycle (42.4 ± 27.6 ng/mL vs. 18.4 ± 5.3 ng/mL; $p<0.05$). Additionally, progesterone AUC was significantly increased in the letrozole cycle compared to the natural cycle (207 ± 38 vs. 93 ± 39; $p<0.05$). Other results include a trend toward larger lead follicular diameter in the letrozole cycle (diameter 20.8 ± 7.4 mm vs 17.2 ± 5.8 mm) and slightly decreased endometrial thickness during the letrozole cycle (8.1 ± 1.5 mm vs. 8.4 ± 2.5 mm) compared to the control natural cycle.Results are depicted in Table 2 and Figure 1.

The demographic data are presented in Table 3.

	Natural Cycle	Letrozole Cycle
Peak E2 (pg/mL) mean	232 ± 95	118 ± 53*
E2 AUC	1410 ± 347	478 ± 275*
Peak Prog (ng/mL) mean	18.4 ± 5.3	42.4 ± 27.6*
Prog AUC	93 ± 39	207 ± 38*
Lead Follicle Diameter (mm) mean	17.2 ± 5.8	20.8 ± 7.4
Endometrial thickness (mm) mean	8.4 ± 2.5	8.1 ± 1.5

Table 2: Natural Cycle vs. Continuous Letrozole*

	Mean (Range)
Age	30.5 (23-40)
BMI	23.8 (19.8-36.9)
Ethnicity	**N (Percentage)**
Caucasian	6 (60%)
African American	3 (30%)
Hispanic	1 (10%)

Table 3. Baseline Characteristics of Study Participants.

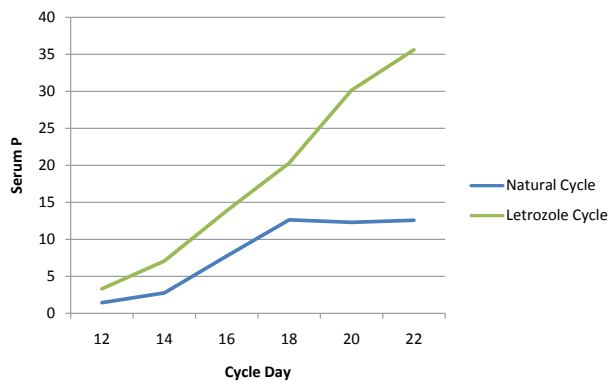

Figure 1: Mean serum estradiol (E_2) during cycle days 12-22 for the natural and Letrozole study cycles.

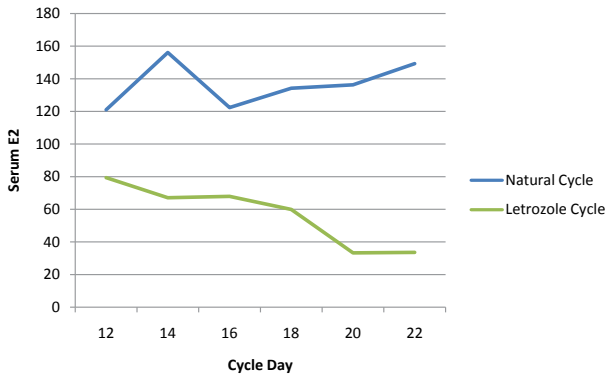

Figure 2: Mean progesterone during cycle days 12-22 for the natural and letrozole study cycles

Discussion

The results of this study found that women taking letrozole ovulated even though estradiol levels were significantly lower than in a natural ovulation cycle. This observation is contrary to the classical teaching that sustained elevated estradiol is the trigger for ovulation [1-6]. These results demonstrate that a high sustained estradiol level is notrequired to trigger ovulation as commonly believed. Perhaps estradiol has a permissive role in ovulation induction, or it may be supplemented by other factors, likely one or more substances produced by the mature preovulatory follicle.

The exact trigger for ovulation induction has been the subject of much investigation, but it remains unclear. One theory suggests that progesterone augments the effects of estradiol to induce the LH surge and subsequent ovulation [1,7]. Estradiol induces expression of progesterone receptors in the hypothalamus, and this increases the hypothalamic sensitivity to circulating progesterone [1,7]. Another theory involving progesterone as an adjuvant trigger is based on the discovery of de novo steroidogenesis in neural tissue [8]. Estradiol binds to estrogen receptor alpha (ERα) receptors on astrocycte membranes and induces de novo progesterone production, and this progesterone augments the peak estradiol to induce a GnRH pulse and the subsequent LH surge [9]. However, this theory is refuted by the observations in the current study, as ovulation occurred even though estradiol levels were low. Finally, kisspeptin is involved in generation of the luteinizing hormone surge and inducing ovulation, and kisspeptin-54 has been used to trigger egg maturation in women undergoing IVF [9]. However, since kisspeptin is at least partially regulated by estrogen, it is surprising that the LH surge and ovulation occurredeven though estradiol levels were low when letrozole was administered.

Strengths include this being a randomized controlled study with patients serving as their own controls. The protocol design is simple; however there are limitations with this work. Only one dosage 5 mg of letrozole was studied. We also chose to use home urine LH tests rather than serum or urine LH levels or serial follicular ultrasounds to determine if ovulation occurred, which was done both for ease of our subjects and because home urine LH tests were thoughts to be accurate to determine ovulation.

The results of this research and clinical observation suggest thatestradiol is not the trigger to induce the LH surge in humans. Supraphysiologic estradiol levels do not induce the LH surge. Estradiol levels may be markedly elevated during ovarian stimulation for in vitro fertilization (IVF), but premature ovulation is rare when the leading follicle diameter is less than 14 mm [10]. Likewise, in our study, we showed that the LH surge occurs in spite of a subphysiologic estradiol levels. It is most likely that the true trigger for the LH surge comes from some other factor secreted by the dominant follicle in humans. The factor that triggers ovulation has yet to be elucidated.

Acknowledgments

None

Conflict of Interest

The authors have no conflicts of interest to report.

References

1. Christensen A, Bentley GE, Cabrera R, Ortega HH, Perfito N, Wu TJ, et al. Hormonal regulation of female reproduction. *Horm Metab Res.* 2012;44(8):587-591.
2. Welt CK. Physiology of the normal menstrual cycle. WF Crowley, Jr., AB Middleman, KA Martin (Eds.). Up to Date. Updated: Apr 08, 2015.
3. Jones RE, Lopez KH. Human Reproductive Biology, 4th Edn. Academic Press. London, UK. 2014, pp. 55-58.
4. Lee VC, Ledger W. Aromatase inhibitors for ovulation induction and ovarian stimulation. *Clin Endocrinol (Oxf).* 2011;74(5):537-546.
5. Laven JS, Fauser BC. What role of estrogens in ovarian stimulation. *Maturitas*. 2006;54(4): 356-362.
6. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med,* 2014;371(2):119-129.
7. Sinchak K, Wagner EJ. Estradiol signaling in the regulation of reproduction and energy balance. *Front Neuroendocrinol,* 2012;33(4):342-363.
8. Micevych P, Sinchak K. The neurosteriod progesterone underlies estrogen positive feedback of the LH surge. *Front Endocrinol (Laussane),* 2011;90:10.3389.
9. Jayasena CN, Abbara A, Comninos AN, Nijher GM, Christopoulos G, Narayanaswamy S, et al. Kisspeptin-54 triggers egg maturation in women undergoing in vitro fertilization. *J Clin Invest,* 2014;124(8):3667-77.
10. Copperman AB, Benadiva C. Optimal usage of the GnRH antagonists: a review of the literature. *Reprod BiolEndocrinol.* 2013;11:20.