Experimed 2024; 14(1): 21-25

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1401479

Efficacy of the GnRH Agonist Trigger in Oocyte and Embryo Quality Through Mitochondrial Unfolded Protein Response

Murat Basar[1,2]

[1]Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA.
[2]Yale Fertility Center, 200 West Campus Drive, Orange, CT, USA

ORCID ID: M.B. 0000-0001-7766-409X

Cite this article as: Basar M. Efficacy of the GnRH agonist trigger in oocyte and embryo quality through mitochondrial unfolded protein response. Experimed 2024; 14(1): 21-25.

ABSTRACT

Objective: Gonadotropin-releasing hormone agonist (GnRHa) trigger induces both Luteinizing hormone (LH) and follicle stimulating hormone (FSH) surges, impacting oocyte maturation, and mitochondrial dysfunction, is responsible for chromosomal anomalies during meiotic divisions. This study aimed to investigate the effect of GnRHa instead of human chorionic gonadotropin (hCG) triggers on unfolded protein responses against embryonic stress in oocytes and embryos.

Materials and Methods: Female mice were divided into control, hCG-triggered, and GnRHa-triggered groups. Superovulation was performed. Oocytes were retrieved 13h after hCG or GnRHa injection, and two pronuclei (2PN) oocytes were retrieved 24h after the appearance of a vaginal plug. ATF5, GRP78, and HSP60 protein levels were analyzed by Western blot. One-way ANOVA and Students' t-test were used for statistical analysis.

Results: When comparing the GnRHa group to the hCG group, their respective oocyte maturation rates (79.8% vs. 75.9%), oocyte areas (10198 μm^2 and 9474 μm^2), 2PN rates (78% vs. 72%), and blastocyst formation rates (82% vs. 77%) were significantly higher ($p < 0.05$). The HSP60 protein level was significantly lower in the GnRHa group compared to the hCG group (22% vs. 55%, $p < 0.05$). Additionally, the ATF5 protein level was significantly lower in the hCG group compared to the GnRHa group ($p < 0.0001$).

Conclusion: GnRHa trigger improves oocyte nuclear and cytoplasmic maturation, as well as blastocyst formation rates. The underlying mechanism for this effect is the downregulation of HSP60 and upregulation of ATF5 levels.

Keywords: mtUPR, GnRHa, mitochondrial stress, hCG

INTRODUCTION

The accurate timing of ovulation must be successfully detected and then controlled if assisted reproductive technologies are to be effective. In controlled ovarian stimulation treatments used to treat infertility, injecting 5,000–10,000 IUs of human chorionic gonadotropin (hCG) used to be considered the gold standard for inducing granulosa cell luteinization, oocyte maturation, and follicle rupture (1). Gonadotropin-releasing hormone agonist (GnRHa) has also been proven to effectively trigger egg maturation mid-cycle by promoting the rise of endogenous gonadotropins. Still, this came to be a valuable alternative to hCG only at the development of the short protocol, where spontaneous ovulation is prevented by the GnRHa, leaving room for the agonist to be successfully used for triggering.

Despite all its advantages, triggering with an agonist results in a defective luteal phase, which among other things reduces implantation and raises the risk of spontaneous pregnancy loss in fresh embryo transfers, thus leading to the freeze-all era (2, 3).

Even though many studies have assessed whether the usage of GnRHa and hCG affects stress response, it remains unclear. During embryo development, increased

Corresponding Author: Murat Basar **E-mail:** murat.basar@yale.edu
Submitted: 07.12.2023 **Revision Requested:** 09.01.2024 **Last Revision Received:** 19.02.2024 **Accepted:** 20.02.2024 **Published Online:** 25.03.2024

endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR), leading to reduced blastocyst formation rates (4-6).

Among the molecules that regulate the mitochondrial unfolded protein response (mtUPR), heat shock protein 60 (HSP60) and mitochondrial HSP70 (mtHSP70) facilitate the protein folding function in the mitochondrial matrix (7), activating transcription factor 5 (ATF5) maintains mitochondrial activity during mitochondrial stress and promotes organelle recovery, and glucose-regulated protein 78 (GRP78) dissociation triggers the UPR endoplasmic reticulum (UPRer) that restores protein homeostasis.

The underlying hypothesis of this study is that, because of the high demand for mitochondrial homeostasis in preimplantation development (from oocyte to blastocyst), GnRHa triggers support for oocyte and blastocyst formation by suppressing mitochondrial stress.

MATERIALS AND METHODS

Mice were maintained according to Yale University's requirements for animal research, and all procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 2022-11300). Mouse embryos were collected using standard protocols under the guidelines approved by the Yale Institutional Animal Care and Use Committee. In short, 5-week-old C57BL/6 female mice (Charles River Labs) were super-ovulated by intraperitoneal (IP) injection of 5 Units of pregnant mare serum gonadotropin (PMSG; Folligon, Sigma-Aldrich). An additional injection of 5 Units of hCG (Chorulon, Sigma-Aldrich) was given 48 hours after the PMSG injection. To obtain two-cell embryos, females were placed individually with 12-week-old C57BL/6 males immediately after the hCG injection. The following morning, the effectiveness of mating was confirmed by the presence of a vaginal plug (day 1; D1). Two-cell embryos were collected from the oviducts at 44–48 hours after hCG injection. Two-cell embryos were obtained by puncturing the ampulla portion of the oviduct with a needle in the HEPES-buffered media under the stereomicroscope.

Animals

All mice care, breeding, and experimental protocols were conducted according to the Yale University School of Medicine Animal Research Requirements. The protocols used were approved by the Institutional Animal Care and Use Committee (2022-11300). 3-month-old female Balb/c mice (25-30 g) were used in 3 groups: control group, triggered with hCG, and triggered with GnRHa (n = 30 per group).

Oocyte and Embryo Collection

To collect the metaphase II (MII) stage mature oocytes, 10 IUs of hCG (Sigma, St. Louis, MO) or GnRHa were injected 48 h after the PMSG (Sigma, St. Louis, MO) injection. Control group mice were injected with 0.09% NaCl. Unfertilized MII oocytes were collected from oviducts 14-16 h after the hCG injection (8). After oocytes were retrieved, their diameter was measured from two different positions (Research Instrument, Cronus 3, Video Capture, and Embryo Analysis Software) to calculate the mean oocyte area (n = 15 per group).

To collect the fertilized (2PN) oocytes (n = 15 per group), the female Balb/c mice were mated with males after receiving the hCG or GnRHa injection. The following morning, after the effectiveness of mating was confirmed by the presence of a vaginal plug, female mice were sacrificed by cervical dislocation. The 2PN oocytes were obtained by puncturing the ampulla portion of the oviduct with a needle in the HEPES-buffered media under the stereomicroscope and cultured until day 5 (D5) of embryonic development. Fertilization and blastocyst formation rates were calculated accordingly.

All embryos were cultured in groups of 12 in 50-µL medium drops at 37°C in 6% CO_2, 5% O_2, and 89% N_2 for 96 h without

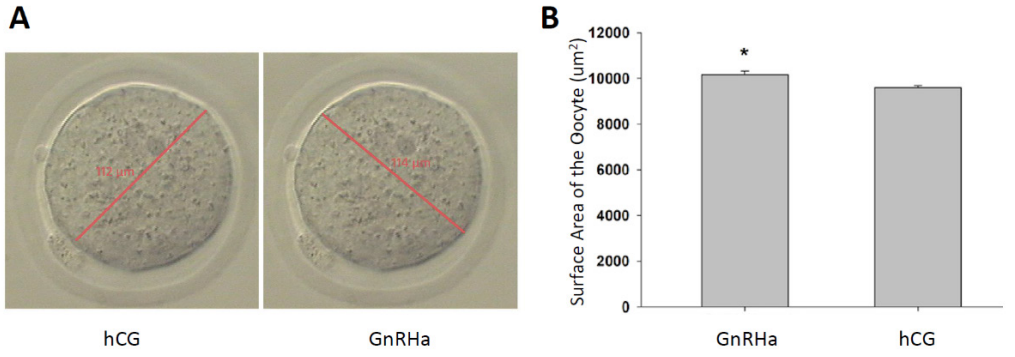

Figure 1. Morphological evaluation. Triggering with GnRHa improves blastocyst formation rate as well as oocyte cytoplasmic and nuclear maturation.

changeovers. An inverted microscope was used to examine the embryos at ×200 magnification at the end of D5.

Western Blot

Embryos from each group were collected and incubated on ice for 30 minutes in a 5 µL phosphate-buffered saline (PBS), and 5 µL lysis buffer. The samples were mixed with 10 mL of Laemmli buffer, boiled for 5 minutes before being cooled on ice, and centrifuged at 2,000 rpm for 5 minutes at 4°C. GRP78 protein levels were determined using rabbit monoclonal anti-GRP78 (Cell Signaling Technology, Cat. No. 3117), anti-ATF5 (Sigma Aldrich, Cat. No. SAB4500895), and anti-HSP60 antibodies (Cell Signaling Technology, Cat. No. 12165) in the previously described standard Western Blot protocol (4).

Equal loading of proteins (10 µg) in each lane was confirmed by staining the membrane with Ponceau 2S (Sigma, St. Louis, MO). Ponceau red signals for anti-GRP78, anti-HSP60, and anti-ATF5 were quantified using a digital imaging and analysis system (AlphaEase, α Innotech Corp., San Leandro, CA), as well as a laser densitometer (Molecular Dynamics, Inc., Sunnyvale, CA) for the auto radiographic bands. Anti-GRP78, anti-HSP60, and anti-ATF5 expressions were normalized by dividing the arbitrary densitometry units for anti-GRP78, anti-HSP60, and anti-ATF5 by the amount of Ponceau red staining for each band.

Statistical Analyses

All experiments were repeated at least three times. Statistical analysis of the data was performed with Student's t-test and one-way analysis of variance (ANOVA), with a $p<0.05$ being considered statistically significant.

RESULTS

Oocyte Diameter as an Indicator of Cytoplasmic Maturity

Oocytes have two types of maturity. The first one is nuclear maturity, which can be noticed by assessing the polar body in the oocyte (metaphase II). The second is cytoplasmic maturity. The only indicator for this parameter is the oocyte area.

The mean oocyte area is significantly higher in the GnRHa-triggered group than in the hCG-triggered one ($p<0.05$, 10198 µm^2 and 9474 µm^2, respectively; Figures 1A-1B). Additionally, nuclear maturation has been assessed, revealing the maturation rate in the GnRHa-triggered group to be significantly higher than in the hCG-triggered group ($p<0.05$, 79.8% vs. 75.9%, respectively).

The fertilization and blastocyst formation rates have also been checked due to the oocyte cytoplasmic and nuclear maturation being significantly higher in the GnRHa-triggered group. Both fertilization rates ($p<0.05$, 78% vs. 72%, respectively) and blastocyst formation rate ($p<0.01$, 82% vs. 77%, respectively) are significantly higher in the GnRHa-triggered group compared to the hCG-triggered group.

mtUPR is altered in GnRH groups

To examine the mtUPR activity in the mice, the oocytes were treated with ethidium bromide (EB, 0.4 µg/mL) for three hours to stimulate the mtUPR, as described previously (9), after which the HSP60 protein levels were evaluated. The HSP60 levels in the EB-treated group were significantly increased compared

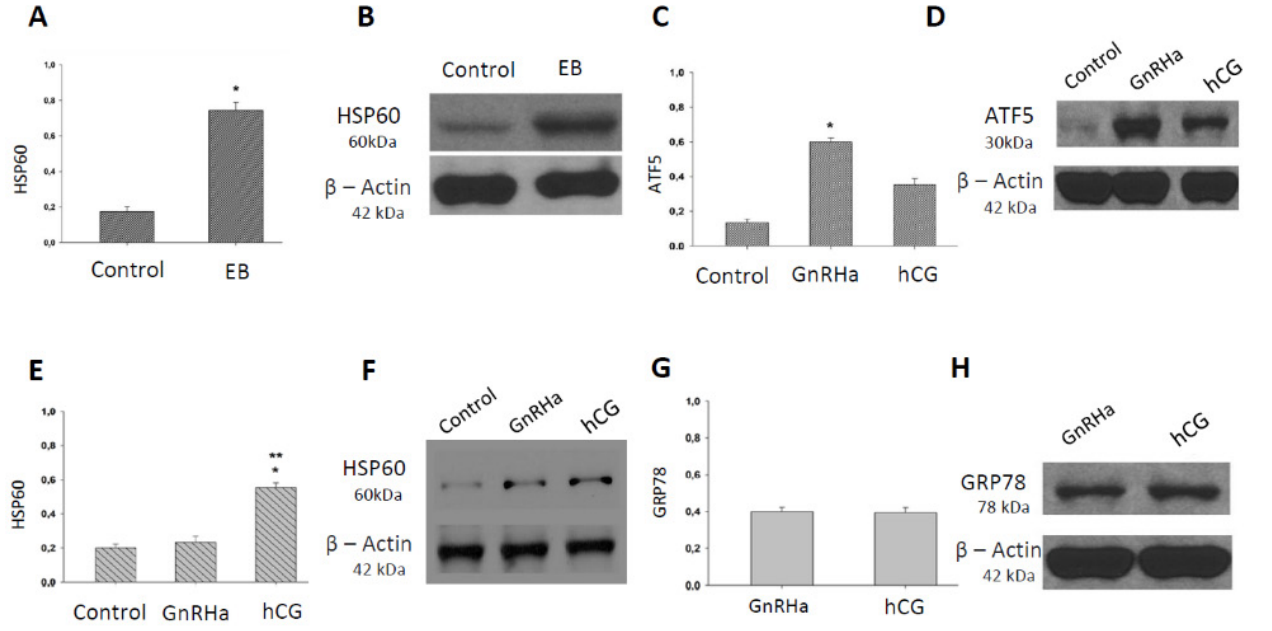

Figure 2. GnRHa-trigger improves oocyte and embryo quality by activating mtUPR.

to the control group (78% vs. 17%, respectively; Figures 2A-2B). Furthermore, the oocytes from the hCG-treated mice have significantly higher levels of HSP60 compared to the control and GnRHa-treated groups (22% for GnRHa, 55% for hCG, and 20% for control; $p<0.05$; Figures 2E-2F).

Additionally, the ATF5 level was assessed as a regulator of mtUPR. The oocytes from the mice triggered with hCG have a 41% ATF5 level, compared to 62% in the mice triggered with GnRHa ($p<0.0001$; Figures 2C-2D).

To ensure that all these effects are due to mtUPR but not UPRer, the study checked the GRP78 level, which is the marker for UPRer. The results show no significant difference in GRP78 levels between the GnRHa-treated and hCG-treated mice (24% and 25%, respectively; $p>0.05$; Figures 2G-2H).

DISCUSSION

Embryo competency is determined by oocyte quality, which is influenced by various parameters including treatment mode (9). Triggering oocyte maturation is the last critical step of ovulation induction. GnRHa has been widely used in ovarian stimulation to prevent endogenous fast augmentation of the luteinizing hormone (LH) surge, which is essential for the development of the corpus luteum. As previously stated, triggering with a GnRH agonist causes a shorter duration of the LH surge than triggering with hCG, resulting in less LH support for the growing corpus luteum and possibly causing early luteolysis (10-12). Luteolysis has been proposed to result in much lower levels of estradiol and progesterone following GnRH agonist triggering than hCG triggering (10).

This study's findings provide valuable insights into the efficacy of GnRHa triggering for enhancing oocyte and embryo quality through mtUPR. Our results align with and sometimes extend the findings reported in the existing literature (11, 12).

The observed increase in oocyte maturation rates and oocyte diameter within the GnRHa group correlates well with the findings of Sukur et al., who reported enhanced cytoplasmic maturity in oocytes following GnRHa triggering (13). However, the current study has extended these findings by quantitatively assessing oocyte diameter, thus providing a more nuanced understanding of cytoplasmic maturity. The improvement in fertilization and blastocyst formation rates in the GnRHa-treated group resonates with the work of Yang et al. (14). However, the current study further elucidates the underlying molecular mechanisms, specifically focusing on the role of mtUPR, a perspective not extensively covered in their research.

The present study's differential expression of HSP60 and ATF5 provides a deeper understanding of mitochondrial stress in oocyte and embryo development. This finding adds to the framework established by Moehle et al. (2019), who first suggested the role of mtUPR in oocyte quality but did not differentiate between the specific impacts of HSP60 and ATF5 (15).

The present research supports the theoretical model proposed by Dumollard et al., which emphasizes the critical role of mitochondrial health in oocyte and embryo viability (16). The current study has provided empirical evidence that strengthens this theoretical model by demonstrating the specific changes in mitochondrial stress markers.

This study's findings being consistent and extending those in the existing literature suggests a potential shift in clinical practice towards the preferential use of GnRHa triggering in assisted reproductive technologies. The results advocate for a more nuanced approach that considers the rates of fertilization and blastocyst formation, as well as the molecular markers that are indicative of oocyte and embryo health.

The study has shown that using GnRHa during superovulation positively affects oocyte development and embryonic growth. These advancements are mediated through the modulation of mitochondrial stress responses, underscoring the vital role of mitochondrial homeostasis in reproductive efficacy. This research could significantly contribute to the optimization of fertilization (IVF) protocols, potentially leading to higher fertility treatment success rates.

Ethics Committee Approval: Mice were maintained according to Yale University requirements for animal research, and all procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 2022-11300).

Peer-review: Externally peer-reviewed.

Conflict of Interest: The author declare that they have no competing interests.

Financial Disclosure: The author declare that this study has received no financial support.

REFERENCES

1. Hu KL, Wang S, Ye X, Zhang D, Hunt S. GnRH agonist and hCG (dual trigger) versus hCG trigger for follicular maturation: a systematic review and meta-analysis of randomized trials. Reprod Biol Endocrinol 2021; 19(1): 78.
2. Gao F, Wang Y, Fu M, Zhang Q, Ren Y, Shen H, Han H. Effect of a "Dual Trigger" using a GnRH agonist and hCG on the cumulative live-birth rate for normal responders in GnRH-antagonist cycles. Front Med (Lausanne) 2021; 8: 683210.
3. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update 2006; 12(2): 159-68.
4. Basar M, Bozkurt I, Guzeloglu-Kayisli O, Sozen B, Tekmen I, Schatz F, et al. Unfolded protein response prevents blastocyst formation during preimplantation embryo development in vitro. Fertil Steril 2014; 102(6): 1777-84.
5. Khatun H, Ihara Y, Takakura K, Egashira J, Wada Y, Konno T, et al. Role of endoplasmic reticulum stress on developmental competency and cryo-tolerance in bovine embryos. Theriogenology 2020; 142: 131-7.

6. Michalak M, Gye MC. Endoplasmic reticulum stress in periimplantation embryos. Clin Exp Reprod Med 2015; 42(1) :1-7.
7. Kumar R, Chaudhary AK, Woytash J, Inigo JR, Gokhale AA, Bshara W, et al. A mitochondrial unfolded protein response inhibitor suppresses prostate cancer growth in mice via HSP60. J Clin Invest 2022; 132(13): e149906.
8. Seli E, Lalioti MD, Flaherty SM, Sakkas D, Terzi N, Steitz JA. An embryonic poly(A)-binding protein (ePAB) is expressed in mouse oocytes and early preimplantation embryos. Proc Natl Acad Sci U S A 2005; 102(2): 367-72.
9. Baart EB, Macklon NS, Fauser BJ. Ovarian stimulation and embryo quality. Reprod Biomed Online 2009; 18 Suppl 2: 45-50.
10. Andersen CY, Humaidan P, Ejdrup HB, Bungum L, Grondahl ML, Westergaard LG. Hormonal characteristics of follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction. Hum Reprod 2006; 21(8): 2126-30.
11. Humaidan P, Alsbjerg B. GnRHa trigger for final oocyte maturation: Is HCG trigger history? Reprod Biomed Online 2014; 29(3): 274-80.
12. Humaidan P, Kol S, Papanikolaou EG, Copenhagen Gn RHATWG. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update 2011; 17(4): 510-24.
13. Sukur YE, Ozmen B, Ozdemir ED, Seval MM, Kalafat E, Sonmezer M, et al. Final oocyte maturation with two different GnRH agonists in antagonist co-treated cycles at risk of ovarian hyperstimulation syndrome. Reprod Biomed Online 2017; 34(1): 5-10.
14. Yang BC, Uemura T, Minaguchi H. Effects of a gonadotropin releasing hormone agonist on oocyte maturation, fertilization, and embryonal development of mice. J Assist Reprod Genet 1995; 12(10): 728-32.
15. Moehle EA, Shen K, Dillin A. Mitochondrial proteostasis in the context of cellular and organismal health and aging. J Biol Chem 2019; 294(14): 5396-407.
16. Dumollard R, Duchen M, Carroll J. The role of mitochondrial function in the oocyte and embryo. Curr Top Dev Biol 2007; 77: 21-49.