~~The~~ Effect of luteal phase gonadotropin-releasing hormone antagonist administration on in vitro fertilization ~~IVF~~ outcomes in women at risk of ovarian hyperstimulation syndrome: A retrospective study ~~OHSS~~

Short title: Luteal phase GnRH antagonist effects on IVF outcomes in women with OHSS risk

Authors:

Affiliations:

***Corresponding Author:**

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Thank you for this opportunity to assist you on your manuscript. I have checked the manuscript for language, grammar, readability, consistency, and flow, and formatting per the target journal guidelines (https://journals.plos.org/plosone/s/submission-guidelines). Please let me know in case you have further queries or doubts regarding the revisions. Do go through my comments in the manuscript and in the attached letter for more detail on my work and the changes I have made.

All my best wishes!!

Commented [_2]: The title may be up to 250 characters. Your current title is well within this limit. I have added the study design to the title in line with journal preferences

Please use this title at all instances going forward.

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Author names will be published exactly as they appear in the accepted manuscript. Indicate affiliations by number only. Affiliation footnotes should appear in numerical order at first mention. Please use the symbols provided in this document for other designations. Numbers and symbols should be in superscript.

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Abstract

Background: Gonadotropin-releasing hormone ~~(GnRH)~~ plays an essential role ~~s~~ in embryo implantation, invasion of trophoblastic tissue, and steroid synthesis in the placenta. However, evidence on the effects of gonadotropin-releasing hormone antagonists on pregnancy outcomes is limited.

Objective: The aim of this study was to evaluate the effect of ~~GnRH~~ gonadotropin-releasing hormone ~~GnRH~~ antagonist administration during the early implantation period on pregnancy outcomes ~~in early implantation period~~.

Materials and Methods: ~~In t~~This retrospective study included ~~,~~ 94 infertile women at risk of ovarian hyperstimulation syndrome undergoing gonadotropin-releasing hormone ~~GnRH~~ antagonist protocol ~~who were at risk of ovarian hyperstimulation syndrome (OHSS) were included~~. Sixty-seven patients (group I) received Cetrorelix 0.25 mg/da~~il~~y ~~in the luteal phase~~during the luteal phase for 3 days, while ~~in~~ 27 ~~participant~~patients (group II) did not receive it~~, it was not administered~~. Pregnancy outcomes were assessed based on chemical and clinical pregnancy rates.

Results: The pregnancy outcomes were not significantly different between the two groups (p=0.224).

Conclusion: Thi~~s~~e ~~present~~ study proposed that ~~luteal phase~~ gonadotropin-releasing hormone ~~GnRH~~ antagonist administration during the luteal phase does not affect~~influence~~ the ~~chance~~probability of successful pregnancy outcomes.

Key Words: *Gonadotropin-releasing hormone antagonist*, *Pregnancy outcome*, *In vitro fertilization*

Introduction

Commented [_8]: The headings have been reformatted per the following journal guideline:
Use Level 1 heading for all major sections (Abstract, Introduction, Materials and methods, Results, Discussion, etc.). • Bold type, 18 pt font. • Only use italics and text formatting where needed (e.g. genus and species names, genes, etc.). • Headings should be written in sentence case (capitalize only the first word of the heading, the first word of the subheading, and any proper nouns and genus names).

Commented [_9]: Please note that the abstract, per the guidelines should be less than 300 words, and should not include abbreviations, if possible. The word count is well within 300 words.

Accordingly, I have removed the abbreviations from the abstract.

Since there is no specific guideline regarding the structure of the abstract, I have retained your original style.

Commented [A10]: I have added a sentence describing the gap in the current literature with respect to the topic of study. Please consider adding a statement that provides some background information on GnRH antagonists.

Commented [_11]: Consider adding a sentence on the statistical tests used to analyze data.

Commented [_12]: Please consider providing more details regarding the critical results of the study. This might be considered a little too abrupt and readers may not want to read the entire study if they get the impression that this was the only point worth noting. Patient mean age and implantation rates can be mentioned, for example.

Commented [A13]: Please considering discussing the implications and applications of these findings in the clinical field at large.

Commented [_14]: There is no specific guideline regarding the key words. Pease verify with the journal whether these are required and remove them if not required.

Female genital ~~tissues~~ organs ~~like~~ such as the ovaries~~y~~, endometrium, and placenta are ~~extra~~ extra-pituitary tissues ~~which~~ that express ~~G~~gonadotropin-releasing hormone (GnRH) receptors ~~([~~1~~)~~]. GnRH plays essential roles in embryo implantation, invasion of trophoblastic tissue, and steroid synthesis in the placenta [~~(~~2~~)~~]. ~~There are l~~Limited ~~evidences~~ evidence is available regarding ~~of~~ the effects of GnRH antagonists~~'~~ ~~effects~~ on pregnancy outcomes.

Several protocols such as GnRH antagonist protocol ~~were~~ have been used for in vitro fertilization (IVF) [~~(~~3, 4~~)~~]. Previous studies have evaluated the role of GnRH antagonists in women with poor response to ovulation stimulation. Some studies have ~~were~~ demonstrated GnRH antagonists ~~is~~ to be comparable ~~with~~to GnRH agonists [~~(~~5, 6~~)~~]. The most important GnRH antagonist benefits ~~are including~~include~~:~~ ~~decrease the~~ reduced ~~need of~~requirement for exogenous gonadotropin, shorter time for stimulation, and a cost~~-~~effective protocol ~~([~~3, 4, 7-9~~)~~].

~~Also~~ Further, GnRH antagonists ~~cause~~s ~~the~~ regression of established severe ovarian hyper~~-~~stimulation syndrome (OHSS) by luteolysis as ~~a~~ the key mechanism in the prevention of OHSS [~~(~~2~~)~~]. ~~After~~ The introduction~~i~~ ~~-~~of GnRH antagonists ~~into~~ in clinical practice led to ~~, it~~ reduced OHSS rates in IVF/intracytoplasmic sperm injection (ICSI) cycles [~~(~~9~~)~~]. GnRH antagonists can alleviate~~improve the~~ poor responses to ovulation stimulation ~~([~~6, 10, 11~~)~~]. ~~Although M~~many ~~m~~studies ~~showed~~ have reported the benefits of GnRH antagonists on IVF/ICSI cycle outcomes; however, ~~but~~ their~~its~~ effect ~~is~~ remains controversial. The use of GnRH antagonists is generally limited to the last few days of ovulation in IVF/ICSI cycles.

The aim of this study was to evaluate the effects of GnRH antagonist administration ~~effects~~ at pharmacological doses ~~given in~~ during the early implantation period on pregnancy outcomes.

Materials and Methods

In this retrospective study, medical records of 94 women ~~that~~ who were at risk of OHSS in IVF/ICSI cycles and ~~has been~~ were ref~~f~~erred ~~to~~ the Research and Clinical Center for Infertility, Yazd, Iran, between October 2014 and February 2015 were reviewed. The study protocol was approved by the

Commented [_15]: In academic writing, ideas need to be conveyed using formal language. I have revised this term to a better academic term alternative.

Commented [_16]: In American English, the pronoun 'which' is usually used to introduce nonessential information, and the pronoun 'that' is used to include essential information. For example, 'The windows, which have red crosses, are sealed' implies that the windows are sealed, and they incidentally have red crosses. However, 'The windows that have red crosses are sealed' implies that only the windows with red crosses are sealed.

Commented [_17]: Intext citations have been reformatted per the preferred journal style.

Commented [A18]: Please consider adding a sentence or two providing background information on GnRH antagonists before talking about the limited evidence on their effects on pregnancy.

Commented [_19]: Avoid beginning sentences and clauses with the overly general "There is," "There are," or "There were" in academic writing. These are just empty filler words.

Commented [A20]: A new paragraph has been started here for better flow.

Commented [_21]: Were these comparable in terms of their effects? Please provide the missing information.

Commented [_22]: It may be a good idea to explain this term and its application briefly for the benefit of the readers.

Commented [_23]: A compound adjective is formed by two words that jointly describe a noun (e.g., 'water' and 'soluble' in 'water-soluble compound'). Such compound adjectives are usually hyphenated to indicate that they form a single unit. The use of the hyphen also aids clarity. For example, in the sentence 'I saw a man-eating alligator,' it is clear that the alligator eats humans. Without the hyphen, the sentence will read as 'I saw a man eating alligator' (i.e., the man was eating an alligator).
Here, "cost effective" is the compound adjective to the noun "protocol"; therefore, it needs to be hyphenated.

Commented [_24]: I suggest moving this part of the text to after the second reference citation in the previous paragraph for better flow. Please let me know if you agree with this change, and we can do it for you.

Please note that the reference number order will change if this change is made.

Commented [_25]: In academic writing, it is customary to provide an expansion of the abbreviation when first used. I have provided the expansion here.

Commented [A26]: While the rationale for the study is provided, its novelty is not clearly stated. Have no previous studies studied the effects of GnRH antagonist administration during the early implantation period on pregnancy outcomes.

ethics committee of the Research and Clinical Center for Infertility, Yazd, Iran, and oral consent was obtained from all participantpatients.

Inclusion criteria were women age <under 40 years old, having more than 20 follicles (>14 mm) at triggering time, and on being at risk of OHSS during the ICSI IVVF/ICSIF cycle with embryo transfer. Women with a history of endometriosis, history of or more than 2two implantation failures, and those with severe male factor infertility were excluded.

TotallyOverall, 94 eligible women were studied in two groups. All participantpatients were treated with the GnRH antagonist protocol. Patients received recombinant human follicle- stimulating hormone (Gonal-F) (150 IU, subcutaneously) for 5 days. Serial transvaginal-vaginal sonography was performed. When the a mature follicle (≥14 mm) was detected, GnRH antagonist (Cetrorelix (Cetrotide) (0.25 mg/daily, subcutaneously) was injected subcutaneously. Triggering was started initiated with 1500 IU hCG (Pregnyl, Organon, Netherland) and 0.2 mg GnRH-a (Decapeptyl®, ®; 0.1 mg)) (subcutaneously) injection when at least two follicles with a mean diameter of 17 mm was were observed.

Trans-vaginalTransvaginal egg retrieval was performed egg retrieval was done under sedation after 36 hrshours. Sixty-seven67 women received 25 mg CetrorelixCetrotide subcutaneously for 3 days from the day of oocyte retrieval (case group) and 27 participantpatients did not receive CetrorelixCetrotide in luteal phaseduring the luteal phase (control group). 2Two embryos were transferred 48 hours after oocyte retrieval using an embryo transfer Labotect catheter (Labotect Gmbh. Labor-Technik-Göttingen GmbH, GöttingenGottingen, Germany) In in all patients. All transferred embryos were in grade A and B. Progesterone suppositories (Cyclogest®), 400 mg twice in a day, was were used vaginally on the day of oocyte collection for luteal phase support, and it continued until the fetal heart activity was documented viaby ultrasonography. Serum beta-hCG (β-hCG) levels wasere assessed on day 14 after embryo transfer.

PA positive pregnancy test was define defined as β-hCG levels >50 IU/L. Pregnancy outcomes were assessed based on clinical pregnancy rates (observation of fetal heart activity onby transvaginal

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As per the guidelines, All research involving human patients must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki.
You may mention this specifically.

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Further, per the guidelines,
Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

Commented [_29]: When expressing numbers (both cardinal and ordinal) within your text, write numbers one through nine alphabetically and write numbers 10 and above numerically, except when the numerals indicate time periods or are followed by units of measurement.

Commented [_30]: For academic writing, the product name, model number, **manufacturer, and country** should be mentioned within the parenthesis. Please provide the missing information.

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Commented [_32]: Does this apply for both hCG and GnRH-a? I have assumed this and removed the parentheses. If only applicable for GnRH-a, this can be added within the previous set of parentheses as (Decapeptyl, 0.1 mg, subcutaneous administration)

Commented [_33]: Starting a sentence, title, or heading with a numeral is considered slightly informal in academic writing and should be avoided. You could either reconstruct the sentence such that you do not have to start the sentence with a number or you could spell out the numeral.
Avoid: "20 college students participated in this study."
Better: "Twenty college students participated in this study"
OR "The participants were 20 college students."

ultrasonography 2-3 weeks after positive β-hCG). Implantation rate was defined as the ratio of the number of gestational sacs to the number of embryos transferred.

Statistical analysis

All ~~of~~ statistical analysis~~ic~~s were ~~was done~~ performed ~~by~~ using SPSS 20 (SPSS, Chicago, IL). The normal distribution of data was checked. Mean±SD values were calculated for descriptive analysis. The ~~I~~independent ~~-~~t-test and χ^2 test were used. The level of statistical significance~~s considered as~~was set at 0.05. According ~~with~~ to the power analysis, ~~-~~the power of the study was 0.8 and α was 0.05.

Results

The mean age of ~~participant~~patients was 28.56±4.03 years in the case group and 28.03±4.8 years in the control group. Basic characteristics of ~~participant~~patients in the groups are ~~shown~~ summarized in Table 1 ~~table I~~. ~~There were not~~ No ~~statistical~~significant ~~ly~~ differences were noted in age~~, ;~~ duration of infertility~~, ; and~~ basal serum follicle-stimulating hormone~~FSH serum~~, progesterone, and estradiol level~~s~~ ~~in~~ on the day of HCG triggering between the groups. ~~While~~ However, the mean number of embryos was different between the groups (Table 1) ~~(Table I)~~. ~~The pregnancy outcome was not significantly different between case and control group (p=0.224). The implantation rate was 14.39% in case group and 9.25% in controls (p=0.089) (Table II).~~

~~I~~

~~**The basic** Data are presented as mean±SD. *Independent Students'Testin s H~~**Table 1: ~~I~~**

Baseline characteristics of patients in the two groups

Variables	Case group (n=67)	Control group (n=27)	p-value *

Commented [_34]: Are you referring to confirmation of a positive pregnancy or β-hCG levels >50 IU/L? If so, I recommend phrasing in either of these ways for clarity. Else, "test results" can be added after "positive β-hCG."

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Level 2 Heading • Use Level 2 headings for sub-sections of major sections. • Bold type, 16 pt font. • Only use italics and text formatting where needed. • Use sentence case.

Commented [_36]: Commas are typically used to separate elements in a series (e.g., "I've visited Japan, the UK, and Egypt"), but semicolons are used if the elements themselves contain commas (e.g., "I've visited Japan, Thailand, and China in Asia; the UK, Belgium, and France in Europe; and Egypt, Morocco, and Kenya in Africa").

Commented [_37]: Was this significantly different? If so, please mention the p-value.

Commented [_38]: As per the guidelines, the tables are inserted immediately after the first paragraph in which they are cited. Hence, I have moved them from the end of the manuscript to within the manuscript close to where they are first cited.

Commented [_39]: I made this change per the preferred journal style.

Variables	Case group (n=67)	Control group (n=27)	p-value *
Age (years)	28.56 ± 4.03	28.03 ± 4.8	0.593
3rd dDay 3 FSH level	5.76 ± 2.73	5.71 ± 3.42	0.942
Infertility duration (years)	6.73 ± 3.85	6.66 ± 4.49	0.942
Serum progesterone** (ng/ml)	1.09 ± 0.55	1.11 ± 0.55	0.924
Serum estradiol** (pg/mL)	3337.07 ± 514.44	3301.63 ± 459.04	0.756

Data are presented as mean±SD.

*Independent Students's t-test.

** on the day of HCG triggering

The pregnancy outcome was not significantly different between the groups (p=0.224). The implantation rate was 14.39% in the case group and 9.25% in the control group (p=0.089) (Table 2).

Table 2H

Commented [A40]: The journal guidelines do not mention anything about the use of abbreviations in tables. Please confirm with the journal whether abbreviations used only once should outright avoided in the tables or whether abbreviations should be defined below each respective table.

Commented [_41]: Please mention the measuring unit for this metric.

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ART outcomes in the two ~~studied~~ study groups

	Case group (n=67)	Control group (n=27)	p-value *
Number of oocytes [a]	19.86 ± 4.94	18.51 ± 2.84	0.023
Number of embryos [a]	6.31 ± 5.21	5.22 ± 4.06	0.332
Clinical pregnancy rate [b]	19 (28.78%)	5 (18.51%)	0.224
Implantation rate	14.39%	9.25%	0.089

[a]Data are presented as mean±SD.

[b]~~:~~ Data are presented as n (%).

* χ^2 test

Discussion

Commented [_43]: Please provide the expanded form for this abbreviation after confirming the guidelines on abbreviation use in tables.

Our results ~~showed~~ indicated that ~~luteal phase~~ GnRH antagonist administration during the luteal phase ~~administration~~ did not ~~influence~~affect the ~~chance~~probability of pregnancy. The clinical pregnancy rate ~~in studied groups~~ was not significantly different between the study groups. Triggering of final oocyte maturation by hCG induces massive luteinization, increase in the secretion of angiogenic factors ~~secretion~~ (such as angiotensin II, interleukins, vascular endothelial growth factor, histamine, prolactin, prostaglandins, endothelin-1, and selectins) from the corpus ~~luteums~~ luteum of hyperstimulated ovaries~~. It leads to~~ development of OHSS ~~by~~ due to increase in vascular permeability, and finally, fluid shift to the third space ~~([~~12-15~~])~~.

Previous studies have reported that GnRH antagonist administration ~~in the luteal phase~~during the luteal phase improves~~d~~ severe OHSS within ~~two~~ 2 days ~~after~~ of the injection of GnRH antagonist by decreasing the ovarian volume, hematocrit, ascites, and ~~oestradiol~~estradiol and progesterone concentrations ~~[(~~3, 4, 8~~])~~. ~~It~~ These findings suggest ~~s~~ a luteolytic effect of the GnRH antagonist that leads ~~d~~ to ~~a decrease of~~ reduced ovarian activity and secretion of angiogenic factors ~~secretion~~, resulting in regression of severe OHSS [~~(~~4, 8~~)~~]. ~~"~~GnRH antagonist inhibits ~~M~~matrix metalloproteinase ~~(MMP)~~ and ~~therefore~~ can, therefore, disrupt embryo ~~the~~ implantation. GnRH antagonist ~~effect on~~ affects the expression of *HOXA10* genes in the endometrium, which is an important regulator of endometrial receptivity~~"~~ ~~([~~16~~]).~~

GnRH antagonist administration during the peri-implantation period may cause some concerns ~~about~~ regarding their potential adverse effects ~~of GnRH antagonist~~ on embryo implantation ~~,~~ and pregnancy and neonatal outcomes ~~([~~11~~])~~. Our findings ~~showed~~ revealed that the pregnancy rate ~~is~~ was similar between the two ~~studied~~ study groups. ~~There are~~ A few studies have ~~about~~reported on the effect of ~~luteal phase~~ GnRH antagonist administration during the ~~administration~~ luteal phase on pregnancy outcomes ~~([~~8, 11, 12~~])~~. Lainas *et al.*~~,~~ in a prospective cohort study on 192 IVF patients ~~,~~who were at risk of OHSS, ~~showed~~ reported that pregnancy and neonatal outcomes did not decrease after ~~luteal~~ GnRH antagonist administration during the luteal phase ~~administration~~ ~~([~~1~~])~~. Some recent studies have documented that GnR~~h~~H antagonist administration is not associated with pregnancy or congenital adverse effects ~~([~~1-4, 8-11, 17-20~~])~~.

Commented [_44]: The original had an awkward and rather long *noun string*. As its name suggests, noun strings are strings containing several nouns in a single sentence, placed one after the other. This makes it difficult to comprehend the information. It is better to break this string down and rephrase the text in a way that improves clarity and enhances understanding. Please check my changes in this regard made here.

Commented [_45]: Some conventions differ between British and American writing. One of these differences can be found in the spelling of certain words. For example, "flavor" in American English is spelt as "flavour" in British English. Some other examples are
American English: color
British English: colour
American English: anemia
British English: anaemia
It's always a good idea to refer to a dictionary to find the most suitable spelling for your document. You can refer to Merriam Webster's for American English and Oxford Dictionary for British English, among others.

Commented [_46]: The original idea was incomplete. Please review my addition.

Commented [_47]: The original statement appeared incomplete as the effect was not described as such. I have rephrased the sentence to ensure completeness of the idea presented. Please review.

Commented [_48]: Even when the original study is cited, quoting directly from a previous study is discouraged. I have, thus, edited the statements for grammar and clarity and removed the quotation marks.

Commented [_49]: Do you mean "successful/positive pregnancy and neonatal outcomes"? Alternatively, do you mean "pregnancy and neonatal outcomes did not worsen"?

Commented [_50]: Twenty references have been cited consecutively in the manuscript.

Commented [_51]: Since several comparable studies are cited, it may be a good idea to elaborate on their findings with respect to those of this study. Were the findings exactly the same? In what ways were these different from the current study?

This study has some limitations. First, ~~As our study limitations,~~ OHSS evolution and ~~, neonate~~ outcomes in neonates and children ~~outcomes~~ were not studied. Furthermore, ~~Also~~ the ~~follow~~ follow-up period was very short.

Conclusion

In conclusion, ~~luteal phase~~ GnRH antagonist administration during the luteal phase does not affect~~influence~~ the ~~chance~~probability of pregnancy after ART. The incidence of chemical and clinical pregnancy ~~in groups~~ was not significantly different among patients who received GnRH antagonist during the luteal phase and those who did not receive it.

Acknowledgements

This study was supported by Yazd Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Conflicts of Interest

The authors declare that there were no conflicts of interests regarding the publication of this article.

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Commented [_52]: There is a potential to improve this subsection of the discussion section. All elements of the limitations need to be highlighted, including the measures taken, if any, to avoid those limitations, and ideas stemming from the limitations for future studies.

Commented [A53]: As flagged in the abstract, your conclusions should be restricted to the antagonist studied.

Commented [_54]: This abbreviation has been only used once in the main text. Per the guidelines, it is recommended to use abbreviation only if used at least thrice in the manuscript.
Accordingly, please replace this abbreviation with its expanded form.

Commented [A55]: Please considering discussing the implications and applications of these findings in the clinical field at large.

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Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588.

Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005.

Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.

Commented [_58]: The citation in PubMed lists the page number as just 69. Please check this.

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Tables

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