

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Risk factors and clinical features of ovarian pregnancy: a case-control study
AUTHORS	Zhang, Jian; Zhu, Qian; Li, Cheng; Zhao, Weihong; Yuan, Jiangjing; Yan, Mingxing; Qin, Guojuan

VERSION 1 - REVIEW

REVIEWER	Jianzhong Sheng Zhejiang University, China
REVIEW RETURNED	09-Sep-2014

GENERAL COMMENTS	<p>This manuscript by Zhu et al. reports a case-control study to identify risk factors for ovarian pregnancy (OP), and, compare clinical features between OP and tubal pregnancy (TP) patients. They found that OP risk was lower than TP risk in the women with serological evidence of Chlamydia trachomatis infection, previous adnexal surgery, and current levonorgestrel emergency contraceptive use. In vitro fertilization-embryo transfer (IVF-ET) patients had a higher risk of OP than natural conception. When Controlled by IUP women, current users of intrauterine devices (IUDs) had a higher risk of OP than non-users of any contraceptives. OP patients had higher β-Human chorionic gonadotropin (hCG) levels on the day of surgery than TP patients. Patients with OP were less likely to initially present with vaginal bleeding than patients with TP. Moreover, shock, rupture, hemoperitoneum and emergency laparotomy were more common in the OP group than the TP group. The authors concluded that IVF-ET and IUD use may be risk factors for OP, and OP patients tended to have high β-hCG levels and a poor clinical outcome (shock, rupture, hemoperitoneum, and need for emergency laparotomy). The manuscript is well written and provides valuable information on risk factors for ovarian pregnancy. There are many positive attributes of the study, including originality, patient numbers, and beneficial clinical findings.</p> <p>However, I have the following comments: Major comments:</p> <ol style="list-style-type: none">1. The number of OP (n=70) and number of OP in IVF-ET patients (n=7) are small. This may influence the conclusion that IVF-ET might be a risk factor for OP. Authors should discuss the limitation of this study.2. The number of patients in each group is also small, that may result in false positive or negative results. So authors should discuss these.3. Page 7, line 23-25, "However, they were higher than the odds of IUP in women with tubal infertility (OR1: 4.48, 95% CI: 1.44–13.98)". It is confused to understand, please rewrite it for clarity.4. It is well know that IUD can increase the risk of TP. The data in Table 2 showed that the risk of OP and TP increased when exposed
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	<p>to IUD, whereas the rate of OP was higher than that of TP. Consider providing more evidence in detail in the discussion for explanation of the phenomenon.</p> <p>5. Consider determining the time of blood collection for testing the CT IgG. Was it conducted on the day of surgery or before the diagnosing?</p> <p>Minor comments:</p> <ol style="list-style-type: none"> 1. The “odds” should be changed to “odds ratio”. 2. Page 4, line 29: “OP differed form he ...” should be “OP differed from the ...”? 3. Page 5, Line 18: “Two control groups, that is, a TP group and an intrauterine pregnancy (IUP) group” may be changed to “Two control groups including a TP group and an intrauterine pregnancy (IUP) group”.
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REVIEWER	Yong-Bing Xiang
REVIEW RETURNED	<p>Professor Department of Epidemiology Shanghai Cancer Institute Shanghai 200032 P. R. China</p>

GENERAL COMMENTS	<p>Qian Zhu and his colleagues did a hospital-based case-control study which was conducted to explore the risk factors of ovarian pregnancy (OP) among Chinese women. Basically, it is an interesting study, and well conducted with two sets of controls (tubal pregnancy (TP) patients and intrauterine pregnancy (IUP) women) in terms of hospital-based design. As mentioned by authors, it seems very difficult to conduct this kind of case-control study because the OP is an extremely rare type of ectopic pregnancy. The authors also indicated the limitations of the study, such as small sample size in the manuscript.</p> <p>I would like to review this manuscript in terms of design, methods and general results because my major is not in reproductive medicine.</p> <p>Some comments and suggestions:</p> <ol style="list-style-type: none"> 1. Page 1, for part of key words, I may suggest to add more key words, such as “hospital-based case-control study” because this is major characteristic of the study. 2. Page 2, in part of participants in abstract, please re-write this part because the words in some short sentences which are not standard text or paragraph. 3. Page 2, in part of participants in abstract, please add the time interval(s) of the study. 4. Page 5, in part of the design, the authors need to clarify or describe how many amount of blood you collected which was donated by study subjects ? and then clearly clarify it is fasting blood or not. Furthermore, how to process the samples before the assays ? 5. Page 5, in part of the data collection, the authors may add some descriptions on the study variables or main variables you are interested in or want to test in your study, such as the definitions of each variable, or categorical method for the variable if it is continuous variable that was grouped into category. Moreover, normally, it is in two ways to describe the covariates, few are for the
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	<p>hypothesis, and another are the confounding factors which will be adjusted in multivariate analysis.</p> <p>6. Page 6, in part of statistical analysis, line 2, the “OR” and “CI” should be “ORs” and “CIs” which are corresponding to the “odds ratios” and “confidence intervals”.</p> <p>7. Page 6, in part of statistical analysis, second paragraph, I think the method of “the log t–test was used for variables with Poisson distribution (serum β-hCG level and amount of hemoperitoneum)” is not very clear for the readers. How did the authors do it ? Please check the “log t-test” because it seems not a standard statistical term ?</p> <p>8. Page 6, in part of statistical analysis, third paragraph, I think the sentence of “and a p value of less than 0.05 was considered statistically significant” need to be changed as “and were considered statistically significant if it less than 0.05”.</p> <p>9. Page 7, in part of multivariate analysis, I may suggest the authors need to consider the variable of age in the model no matter what it is significant or not both in univariate and multivariate analysis.</p> <p>10. Page 8, in part of discussion, normally, the first paragraph is short summary for the major or main results from the study, not to discuss other issues at the beginning of the discussion. So I suggest the authors can add few words as the first paragraph of the discussion.</p> <p>11. Page 12, before the conclusion, the authors may describe the strengths and limitations of the study although it is in the title page.</p> <p>12. Page 19, in table 1, I may suggest the authors did not list the OR(s) in the table because the major aim is for the comparisons of some characteristics between three groups which the aim is to provide the test of differences, not the estimates of effect. The estimates (ORs) can be in other tables.</p> <p>13. Page 19, in table 1, for the variable of age, I may suggest the authors combine the subjects for two age groups of 35-39 and ≥ 40 because the number of subjects in last group is just for 3 participants.</p> <p>14. Page 19, in table 1, for the variable of smoking, I may suggest the authors just do data analysis for non-smoking women because only 2 subjects are smokers. So the authors may need to do data analysis again.</p> <p>15. Page 19, in table 1, “none smoking” may be changed as “non-smoking” or “never”.</p> <p>16. Page 20 and 21, in table 2 and 3, no descriptions on “OR1” and “OR2”, the authors can add some descriptions in the footnote. I suggest the authors list all variables for the models (OR1 and OR2) in the footnote, respectively.</p> <p>17. Page 21, for table 3, I may suggest the authors did not list the results of “OP vs. IUP” because most of variables are not available in this study.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer 1

Prof. Jianzhong Sheng

We thank the reviewer for acknowledging the value of our work and for the constructive comments, which we believe have greatly helped us to improve our manuscript.

Major comments:

1. The number of OP (n=70) and number of OP in IVF-ET patients (n=7) are small. This may influence the conclusion that IVF-ET might be a risk factor for OP. Authors should discuss the limitation of this study.

Response: Thank you for this suggestion. As a hospital-based study, and the quality of the outcome data may also be biased due to the recall bias and selection bias. Thus, despite the IVF-ET showed to be a high risk for OP, the confidence interval was relative wide. This limitation has been re-discussed in the revision according to your comments (See last paragraph of discussion, line 5-7).

2. The number of patients in each group is also small, that may result in false positive or negative results. So authors should discuss these.

Response: Thank you for this insightful comment. According to your comments, a limitation of this aspect has been discussed in the revision (See last paragraph of discussion, line 4-5).

3. Page 7, line 23-25, "However, they were higher than the odds of IUP in women with tubal infertility (OR1: 4.48, 95% CI: 1.44–13.98)". It is confused to understand, please rewrite it for clarity.

Response: We are sorry for making you confused when reviewing the manuscript. The sentence has been re-written in the revision (see results, univariate analysis, paragraph 3, line 7-9).

4. It is well know that IUD can increase the risk of TP. The data in Table 2 showed that the risk of OP and TP increased when exposed to IUD, whereas the rate of OP was higher than that of TP. Consider providing more evidence in detail in the discussion for explanation of the phenomenon.

Response: Thanks for the reviewer's suggestion. We have revise this part of discussion (see discussion, paragraph 4, line 7-10), and noted in the revision that "as Lehfeldt et al reported, the IUD was effective in preventing intrauterine pregnancy in 99.5% and tubal pregnancy in 95%, but it had little effect in prevent OP". (see reference: Lehfeldt H, Tietze C, Gorstein F: Ovarian pregnancy and the intrauterine device. *Am J Obstet Gynecol* 1970; 108: 1005-1009.) What's more, another 12-year experience on 19 cases of OP also noted that an IUD was present in 13 of 19 (68%) of the patients. Despite this study was limited in sample size, but it can still provide some evidence in the close relation between OP and IUD. (see reference: Raziel A, Schachter M, Mordechai E, Friedler S, Panski M, Ron-El R: Ovarian pregnancy-a 12-year experience of 19 cases in one institution. *Eur J Obstet Gynecol Reprod Biol* 2004; 114 (1): 92-96.)

5. Consider determining the time of blood collection for testing the CT IgG. Was it conducted on the day of surgery or before the diagnosing?

Response: Thank you for this insightful suggestion. As you suggested, we amended the blood sample collection in the methods (See methods, data collection and patient examination, paragraph 2). Five-milliliter blood samples was collected from each subject after recruitment to test for Chlamydia trachomatis (CT) IgG antibody. Another 5 ml blood sample were collected on the day of surgery to test for the serum β -hCG levels.

Minor comments:

1. The "odds" should be changed to "odds ratio".

Response: Thank you for this suggestion. The "Odds" have been changed to "odds ratio (OR)" in the revision.

2. Page 4, line 29: “OP differed form he ...” should be “OP differed from the ...”?

Response: We are sorry for the clerical error which made you difficult in reviewing the manuscript. The mistakes have been corrected in the revision (see introduction, paragraph 2, line 6).

3. Page 5, Line 18: “Two control groups, that is, a TP group and an intrauterine pregnancy (IUP) group” may be changed to “Two control groups including a TP group and an intrauterine pregnancy (IUP) group”.

Response: Thank you for your insightful suggestion. The sentence has been revised as your suggestion in the revision (see methods, study design and subjects, paragraph 2, line 2-3).

Reviewer 2

Prof. Yong-Bing Xiang

Thank you for spending time on reviewing our manuscript and for your good evaluation and suggestions in terms of design, methods and general results.

1. Page 1, for part of key words, I may suggest to add more key words, such as “hospital-based case-control study” because this is major characteristic of the study. Response: Thank you for this suggestion. As your suggestion, we have added the key words “hospital-based case-control study” in the revision.

2. Page 2, in part of participants in abstract, please re-write this part because the words in some short sentences which are not standard text or paragraph.

Response: Thank you for this insightful comment. The part of participant in abstract has been revised in standard epidemiology terms.

3. Page 2, in part of participants in abstract, please add the time interval(s) of the study.

Response: This study was started from January 2005 to May 2014 as mentioned in the methods. According to your suggestion, the time interval of the study has been added in the abstract.

4. Page 5, in part of the design, the authors need to clarify or describe how many amount of blood you collected which was donated by study subjects? And then clearly clarify it is fasting blood or not. Furthermore, how to process the samples before the assays?

Response: Thanks to reviewer for pointing out this important point. We amended the blood sample collection in the methods (see methods, data collection and patient examination, paragraph 2). Five-milliliter blood samples was collected from each subject after recruitment to test for Chlamydia trachomatis (CT) IgG antibody. Another 5 ml blood sample were collected on the day of surgery to test for the serum β -hCG levels. Serum were used for assay following centrifuging the blood samples, and all assays were conducted within 24 hours.

5. Page 5, in part of the data collection, the authors may add some descriptions on the study variables or main variables you are interested in or want to test in your study, such as the definitions of each variable, or categorical method for the variable if it is continuous variable that was grouped into category. Moreover, normally, it is in two ways to describe the covariates, few are for the hypothesis, and another are the confounding factors which will be adjusted in multivariate analysis.

Response: Thanks for this helpful suggestion. More descriptions on the study variables have been added following each variables in the revision (See methods, data collection patient examination, paragraph 1). What's more, variables including hypothesized factors and confounding factors adjusted in the multivariate analysis have been noted in the footnote of table 3.

6. Page 6, in part of statistical analysis, line 2, the “OR” and “CI” should be “ORs” and “CIs” which are corresponding to the “odd ratios” and “confidence intervals”.

Response: Thanks for this suggestion. The words “ORs” and “CIs” have been corrected (See methods, statistical analysis, paragraph 1, line 2).

7. Page 6, in part of statistical analysis, second paragraph, I think the method of “the log t-test was used for variables with Poisson distribution (serum β -hCG level and amount of hemoperitoneum)” is not very clear for the readers. How did the authors do it? Please check the “log t-test” because it seems not a standard statistical term?

Response: Thanks for this comment, and the statistical analysis of t-test has been re-written (See methods, statistical analysis, paragraph 2, line 2-3).

8. Page 6, in part of statistical analysis, third paragraph, I think the sentence of “and a p value of less than 0.05 was considered statistically significant” need to be changed as “and were considered statistically significant if it less than 0.05”.

Response: Thanks for the comments. The sentence has been changed as your suggestion in the revision (see methods, statistical analysis, paragraph 3, line 2-3).

9. Page 7, in part of multivariate analysis, I may suggest the authors need to consider the variable of age in the model no matter what it is significant or not both in univariate and multivariate analysis.

Response: Thank you for your helpful comment. We have added the age into the multivariate analysis and re-analyzed. Relevant data have been updated in the table, abstract, and the part of result (see result, multivariate analysis, paragraph 2). Variables adjusted in the multivariate analysis, including hypothesized factors and confounding factors, have been noted in the footnote of table 3.

10. Page 8, in part of discussion, normally, the first paragraph is short summary for the major or main results from the study, not to discuss other issues at the beginning of the discussion. So I suggest the authors can add few words as the first paragraph of the discussion.

Response: Thanks for this comment. We have now added a new paragraph to summarize the main results from the study (see discussion, paragraph 1).

11. Page 12, before the conclusion, the authors may describe the strengths and limitations of the study although it is in the title page.

Response: Thanks for this constructive comment. The strengths and limitations of the study has been added in the last paragraph of discussion. As we noted in the title page, the initial of the study is the strengths, whereas limited sample size and some bias of the study may result in some false positive or negative results. Thus, we thought that sample size should be enlarged and a prospective cohort study should be further designed to validate the results of this study.

12. Page 19, in table 1, I may suggest the authors did not list the OR(s) in the table because the major aim is for the comparisons of some characteristics between three groups which the aim is to provide the test of differences, not the estimates of effect. The estimates (ORs) can be in other tables.

Response: Thank you for this suggestion. The OR(s) in the table 1 have been removed.

13. Page 19, in table 1, for the variable of age, I may suggest the authors combine the subjects for two age groups of 35-39 and ≥ 40 because the number of subjects in last group is just for 3 participants.

Response: Thank you for this valuable suggestion. The number of subjects of age groups of 35-39 and ≥ 40 were really small, and we have now combined them into one group named “age of more than 35”.

14. Page 19, in table 1, for the variable of smoking, I may suggest the authors just do data analysis for non-smoking women because only 2 subjects are smokers. So the authors may need to do data analysis again.

Response: Thank you for this suggestion. Although smokers in this study were really few, we still thought it necessary for analyzing for the reason that smoking is an acknowledged risk factor for ectopic pregnancy. So we just combined the occasional smokers and regular smokers into the same group named smokers.

15. Page 19, in table 1, “none smoking” may be changed as “non-smoking” or “never”.

Response: Thank you for this insightful comment. The words “none smoking” has been changed into “non-smoking”.

16. Page 20 and 21, in table 2 and 3, no descriptions on “OR1” and “OR2”, the authors can add some descriptions in the footnote. I suggest the authors list all variables for the models (OR1 and OR2) in the footnote, respectively.

Response: We are sorry for not making the statistical analysis stated clear. “OR1” and “OR2” in the table 2 and 3 were just the crude ORs of the variables. OR1 referred to the odds ratio for OP when using TP patients as control, and OR2 referred to the odds ratio for OP when using IUP women as control. These descriptions have now been added in the footnote of the tables.

17. Page 21, for table 3, I may suggest the authors did not list the results of “OP vs. IUP” because most of variables are not available in this study.

Response: We appreciate this helpful comment on our manuscript. The results of “OP vs. IUP” in the table 3 have been removed, and the removed results in the manuscript have been noted that “data not shown on table” in parentheses following the result description.

VERSION 2 – REVIEW

REVIEWER	Yong-Bing Xiang Department of Epidemiology Shanghai Cancer Institute Shanghai 200032 P. R. China
REVIEW RETURNED	06-Oct-2014

GENERAL COMMENTS	<p>Minor corrections or suggestions:</p> <ol style="list-style-type: none"> 1. Page 30 of 62, lines 1-5, "Log processing" may be changed as "with 10 at the bottom of logarithmic transformation", or "natural logarithm transformation" ? 2. Page 30 of 62, lines 9-10, to delete the word of "were" in sentence of ".....two-tailed tests, and were.....". 3. Page 30 of 62, lines 42-43, please check the words of "had had" in sentence of "In women who had had no" ? It seems duplicate ? 4. Page 30 of 62, lines 44-45, please also check the words of "had had" in sentence of ".....OP among women who had had two abortions were lower than....." ? 5. Page 30 of 62, lines 44-45, please change the word of "were" as "was" in sentence of ".....OP among women who had had two abortions were lower than.....". 6. Page 30 of 62, lines 57-58, please change the word of "were" as "was" in sentence of ".....the OR of OP were higher in women.....". 7. Page 31 of 62, lines 1-2, please change the word of "were" as "was" in sentence of ".....The OR of OP among current IUD users were significantly.....".
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REVIEWER	Jianzhong Sheng Zhejiang university, China
REVIEW RETURNED	07-Oct-2014

GENERAL COMMENTS	Authors answered all quesiton. I have no more question.
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VERSION 2 – AUTHOR RESPONSE

Reviewer 1

Prof. Jianzhong Sheng

Thank you for spending time on reviewing our revision and response, and for your recommendation of acceptance.

Reviewer 2

Prof. Yong-Bing Xiang

Thank you for reviewing our revision, and your help for the language editing.

1. Page 30 of 62, lines 1-5, "Log processing" may be changed as "with 10 at the bottom of logarithmic transformation", or "natural logarithm transformation" ?

Response: Thank you for providing professional terms. As your suggestion, "with 10 at the bottom of logarithmic transformation" has been revised in the revision (see methods, statistical analysis, paragraph 2, line 3-4) and the table footnote (see table 4, footnote).

2. Page 30 of 62, lines 9-10, to delete the word of "were" in sentence of ".....two-tailed tests, and were.....".

Response: Thank you for the insightful suggestion. As your suggestion, grammar error has been corrected in the revision (see methods, statistical analysis, paragraph 3, line 2-3).

3. Page 30 of 62, lines 42-43, please check the words of "had had" in sentence of "In women who had had no" ? It seems duplicate ?

Response: Thank you for the insightful suggestion. As your suggestion, additional word "had" has been deleted in the revision (see results, univariate analysis, paragraph 3, line 2-3).

4. Page 30 of 62, lines 44-45, please also check the words of "had had" in sentence of ".....OP among women who had had two abortions were lower than....." ?

Response: Please see question 4.

5. Page 30 of 62, lines 44-45, please change the word of "were" as "was" in sentence of ".....OP among women who had had two abortions were lower than.....".

Response: Thank you for this suggestion. The word of "were" has been changed into "was" in the revision (see results, univariate analysis, paragraph 3, line 3).

6. Page 30 of 62, lines 57-58, please change the word of "were" as "was" in sentence of ".....the OR of OP were higher in women.....".

Response: Thank you for this suggestion. The word of "were" has been changed into "was" in the revision (see results, univariate analysis, paragraph 3, line 8).

7. Page 31 of 62, lines 1-2, please change the word of "were" as "was" in sentence of ".....The OR of OP among current IUD users were significantly.....".

Response: Thank you for this suggestion. The word of "were" has been changed into "was" in the revision (see results, univariate analysis, paragraph 3, line 9).