Increasing dominant follicular proportion negatively associated with good clinical outcomes in GnRH-a prolonged protocol: a large-sample retrospective analysis

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Research

Keywords: IVF/ICSI, controlled ovarian hyperstimulation, HCG trigger time, GnRH-a prolonged protocol, dominant follicular proportion, clinical outcomes

DOI: https://doi.org/10.21203/rs.3.rs-870140/v1

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Increasing dominant follicular proportion negatively associated with good clinical outcomes in GnRH-a prolonged protocol: a large-sample retrospective analysis

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Abstract

Background: Nowadays, there is no universal criteria for trigger time during controlled ovarian hyperstimulation (COH). Particularly, in the so-called GnRH-a prolonged protocol, widely used in China, the ideal time to trigger ovulation is not yet well defined.

Methods: This was a large-sample retrospective analysis. Between January 2016 and January 2020, 1,925 young patients who underwent their first in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles with normal ovarian response were divided into three groups based on their dominant follicular proportions (DFP, defined as $\geq 18$ mm follicles/$\geq 14$ mm follicles; Group A: < 30%; Group B: 30% - 60%; Group C: $\geq 60$%). Binary logistic regression and multivariate linear regression were used to assessed whether DFP levels were related to clinical pregnancy, number of blastocysts frozen, blastocyst formation rate and low blastocysts frozen.
Results: Binary logistics regression analysis showed that compared with Group A, the OR for clinical pregnancy was 1.345 in Group B ($P = 0.023$); however, there was no statistical difference between Group C and Group A ($P = 0.216$). On one hand, multivariate linear regression analysis indicated that DFP was negatively associated with number of blastocysts frozen ($\beta \pm SE$: Group B vs Group A = -0.319 $\pm$ 0.115, $P = 0.006$; Group C vs Group A = -0.432 $\pm$ 0.154, $P = 0.005$) as well as blastocyst formation rate ($\beta \pm SE$: Group B vs Group A = -0.035 $\pm$ 0.016, $P = 0.031$; Group C vs Group A = -0.039 $\pm$ 0.021, $P = 0.067$). On the other hand, compared with group A, the OR for low blastocyst frozen was 1.312 in Group B ($P = 0.039$) and was 1.417 in Group C ($P = 0.041$).

Conclusions: Excessive delay of trigger in GnRH-a prolonged protocol might reduce the developmental potential of oocytes and reduce the number of available blastocysts, which might result in a lower cumulative pregnancy rate. But further confirmation by strict prospective randomized controlled study should be needed.

Trial registration: https://clinicaltrials.gov/; NCT03305510; Registered 08 October 2017 - Retrospectively registered.

Keywords: IVF/ICSI, controlled ovarian hyperstimulation, HCG trigger time, GnRH-a prolonged protocol, dominant follicular proportion, clinical outcomes.

Background

COH is considered a key factor in the success of IVF/ICSI [1, 2], because it induces the development of multiple follicles and obtains as many high-quality oocytes as possible, thereby increasing the numbers of available embryos for transfer and increasing pregnancy rates. A crucial step in improving clinical pregnancy outcomes, however, is identifying an appropriate time for human chorionic gonadotropin (HCG) trigger [2, 3].
To date, there has been no universal criteria for the optimal HCG trigger time for IVF/ICSI cycles in a variety of COH protocols [1, 2, 5]. Traditionally, the majority of reproductive centers administer the trigger when at least three leading follicles have reached $\geq 17$ mm, or at least two leading follicles have reached $\geq 18$ mm [1, 2, 6 - 8]. Ectors et al. proposed to postpone HCG trigger in short GnRH-a protocol. They suggested that it may yield relatively more large follicles, including competent oocytes with mature cytoplasm, and may exert positive effects on clinical outcomes [9]. On the contrary, some studies suggested that delaying oocyte retrieval in short GnRH-a long protocol increased neither the number of mature oocytes retrieved nor the pregnancy rate and may, in fact, impair embryo quality [8]. However, other studies did not find differences in fertilization rate or oocyte maturation rate between large and small follicles (10 - 14 mm; 15 - 19 mm; 20+ mm) [10]; (<16 mm; $\geq 16$ mm) [11]. These discordant findings might be attributed to differences in COH protocols, definition of follicle size, patient characteristics, methods of oocyte insemination (IVF or ICSI), and analysis of endpoints [1].

In recent years, IVF and ICSI have developed rapidly, and the available COH protocols are flexible and diverse. For example, the GnRH-a prolonged protocol is an emerging protocol that is widely used in clinical practice (especially in China). Nevertheless, the ideal time to trigger ovulation has not yet been well defined for this protocol. Some reproductive centers simply follow the criteria of trigger time of short GnRH-a long protocol when using this protocol. However, the mechanism of action, endocrine hormones, and medication regimen differ greatly between different COH protocols [4]. It's controversial to choose the same trigger time for different COH protocols [1, 19]. Evidence is still urgently needed to provide a reference for clinical decision-making regarding the trigger time of GnRH-a prolonged protocol.
Folliculogenesis may be asynchronous during ovarian hyperstimulation. We need to focus on the
global development state of the growing follicle group [8]. The dominant follicular proportion (DFP)
was first proposed in the GnRH antagonist protocol, which can reflect the overall development of
follicles. And it was considered to be a more effective and objective indicator to determine the ideal
HCG trigger timing [5]. In this study, we compared the effects of different DFP (≥18 mm follicles /
≥14 mm follicles) on clinical outcomes in a large group of people who underwent IVF or ICSI
treatment with GnRH-a prolonged protocol, including oocyte maturation rate, normal fertilization
rate, clinical pregnancy rate, implantation rate, number of blastocysts frozen, blastocyst formation
rate, and low blastocyst frozen.

Methods

Patient selection

This is a retrospective study. All eligible patients who underwent and completed their first IVF/ICSI
cycle with GnRH-a prolonged protocol from January 2016 to January 2020 at the Reproductive
Medicine Center of Tongji Hospital, Affiliated to Huazhong University of Science and Technology,
were recruited and analyzed retrospectively.

The inclusion criteria were as follows: (1) the first IVF/ICSI fresh embryo transfer cycle, (2) female
with age < 35 years, (3) body mass index (BMI) ≤ 30 kg/m2, (4) antral follicle counting (AFC) ≥ 5,
(5) anti-Müllerian hormone (AMH) ≥ 1.2 IU/L, (6) basal serum follicle-stimulating hormone (FSH)
< 12 IU/L, and (7) 6 - 18 retrieved oocytes.

The exclusion criteria were as follows: (1) donor oocyte cycles/oocyte cryopreservation cycles, (2)
female who had previously undergone an IVF/ICSI cycle either in our center or elsewhere.
Controlled ovarian stimulation protocols

For GnRH-a prolonged protocol, 3.75 mg of long-acting GnRH-a (Decapeptyl; Ferring, Saint-Prex, Switzerland) was administered subcutaneously on day 2 of the menstrual cycle. Pituitary suppression was evaluated 28 days after pituitary downregulation. The criteria for confirming the success of downregulation were as follows: follicle diameter < 5 mm, serum luteinizing hormone (LH) < 5 mIU/ml, serum estradiol (E2) < 50 pg/mL, and endometrial thickness < 5 mm. Then, daily injection of rhFSH (Gonal-F; Merck-Serono, Geneva, Switzerland) ranging from 75 to 300 IU was given for about 10 days. When at least three leading follicles ≥ 17 mm or two leading follicles ≥ 18 mm were observed via transvaginal ultrasound, a dose of 0.25mg rHCG (Ovidrel; Merck-Serono, Geneva, Switzerland) was administered to trigger ovulation. After 36 - 37 hours, transvaginal ultrasound-guided oocyte retrieval was conducted. Fertilization was accomplished by standard IVF or ICSI. If serum progesterone < 1.5 ng/mL [17], the number of oocytes retrieved were < 20, and serum E2 were < 7,000 pg/mL, the one or two best-quality Day 3 embryos were transferred; and the remaining embryos were cultured to Day 5/6 until the blastocyst formed and then frozen. The luteal phase was supported if embryo transfer was performed.

Definition of DFP Levels and Groups

Dominant follicular proportion (DFP) is defined as ≥ 18 mm follicles / ≥ 14 mm follicles. DFP < 30% corresponds to at least two follicles ≥ 18 mm on HCG day, which is the most common trigger time. Then, we set the groups as DFP < 30% (Group A), 30% - 60% (Group B), ≥ 60% (Group C) corresponding to follicles ≥ 18 mm increasing gradually (2, 4, 6 follicles respectively).

Clinical outcomes
The number of mature oocytes (MII) was measured 3 - 4 hours, and fertilization was assessed 16 - 18 hours after IVF insemination or when ICSI was performed. Oocyte maturation rate was the proportion of MII oocytes to the number of oocytes retrieved. Normal fertilization rate was equal to 2PN/number of oocytes retrieved (IVF) or 2PN/MII (ICSI). The blastocyst formation rate was equal to the number of blastocysts frozen divided by the number of blastocysts continuously cultured. Low blastocysts frozen was defined as the number of blastocysts frozen \( \leq 1 \) (that is, less than half of the average number of blastocysts frozen). Clinical pregnancy was identified with the presence of an intrauterine gestational sac with fetal cardiac activity. The clinical pregnancy rate was the number of clinical pregnancies in a given number of transplant cycles. Implantation rate reflects the number of gestational sacs divided by the number of embryos transferred.

**Statistical analysis**

Pearson chi-square (\( \chi^2 \)) test on categorical variables and analysis of variance (ANOVA) or Kruskall-Wallis H test on continuous variables were performed appropriately. Multivariate linear regression analyses were carried out for the predictive factors of number of blastocysts frozen and blastocyst formation rate. Moreover, binary logistic regression analyze was carried out for the predictive factors of clinical pregnancy and low blastocyst frozen. The results were given in terms of 95% confidence intervals (CI) and P values. A two tailed P-value of < 0.05 indicated statistical significance. All statistical analyses of the data were carried out using the Statistical Package for Social Science version 25.0 (SPSS, Chicago, IL, USA).

**Results**

A total of 1,925 consecutive IVF / ICSI cycles were included. As shown in Table 1, the clinical characteristics, ovarian response characteristics, and reproductive outcomes of cycles were described. The baseline characteristics and ovarian stimulation details were matched evenly in three groups,
including maternal age, BMI, basal FSH, dose of Gn, duration of Gn. Among the DFP groups, there were no significant differences in terms of oocyte maturation rate (IVF/ICSI), normal fertilization rate (IVF/ICSI), and clinical pregnancy rate. Group B had the highest implantation rate (Group A 48.67%, Group B 57.57%, Group C 50.71%, P = 0.031). Interestingly, Group A had the highest number of blastocysts frozen (Group A 3.05±2.35, Group B 2.60±2.16, Group C 2.32±2.12, P < 0.001) and the highest blastocyst formation rate (Group A 42.38% ± 26.59%, Group B 38.69% ± 27.16%, Group C 38.26% ± 28.27%, P = 0.047).

Binary logistic regression analysis was carried out to evaluate the effect of BMI, dose/duration of Gn, number of oocytes retrieved, number of embryo transfer and DFP levels on clinical pregnancy (Table 2). The results showed that compared with Group A, the OR for clinical pregnancy was 1.345 in Group B (P = 0.023); however, there was no statistical difference between Group C and Group A (P = 0.216).

Multivariate linear regression analyses were carried out to evaluate the effect of type of infertility, dose/duration of Gn, number of oocytes retrieved, type of fertilization and DFP levels on the number of blastocysts frozen and blastocyst formation rate, respectively. As shown in Table 3, the results indicated that DFP was negatively associated with number of blastocysts frozen (β ± SE: Group B vs Group A = -0.319 ± 0.115, P = 0.006; Group C vs Group A = -0.432 ± 0.154, P = 0.005). As shown in Table 4, the results indicated that DFP was also negatively associated with blastocyst formation rate (β ± SE: Group B vs Group A = -0.035 ± 0.016, P = 0.031; Group C vs Group A = -0.039 ± 0.021, P = 0.067).

Binary logistic regression analysis was carried out to evaluate the effect of type of infertility, dose/duration of Gn, number of oocytes retrieved, type of fertilization and DFP levels on low blastocysts frozen (Table 5). The results showed that compared with Group A, the OR for low
blastocyst frozen was 1.312 in Group B (95% CI = 1.014 - 1.698, P = 0.039) and was 1.417 in Group C (95% CI = 1.014 - 1.979, P = 0.041).

**Discussion**

LH plays an essential physiological role in follicle steroidogenesis and development, as well as oocyte maturation [20, 21]. Since the introduction of GnRH-a prolonged protocol, pituitary desensitisation has usually been profound and endogenous LH level has been suppressed to be very low (<1.0 IU/L). There is not any data available in published literature in regards to when to administer HCG trigger in GnRH-a prolonged protocol. Some clinicians believe that the timing could be determined using the same criteria as are used in short GnRH-a long protocol. However, the traditional HCG trigger timing criteria are not strict, and the decision remains controversial [2 - 5]. The previous views were that follicular size was positively related to follicular maturity, fertilisation and subsequent development [1, 2, 5, 12]. Oocytes derived from large follicles (14~21 mm; mean diameter: 19.1 ± 2.1 mm) seem to be more inclined to form high-quality embryos in GnRH antagonist protocol [13]. Therefore, in actual practice, the HCG trigger time is usually delayed [8]. On the contrary, a previous study reported that oocytes in oversized follicles in the same protocol might decrease in quality, and the recovery of oocytes [5]. However, our results showed that the DFP groups did not differ in terms of oocyte maturation rate or normal fertilisation rate. This was consistent with the views of previous studies: enlarging follicle size might not improve oocyte maturation or fertilisation [4, 14 - 16]. In our study, the clinical pregnancy rate and the implantation rate seemed to decrease as DFP increased (Group B vs Group C). This could be explained by the fact that a larger DFP might be negatively associated with satisfactory pregnancy outcomes. Consistent with our results, one study showed that high-quality embryo rate, pregnancy rate, and implantation rate were significantly higher
in the low proportion group (diameter $\geq 18$ mm divided by the total number of follicles, low proportion: $<15\%$; middle proportion: $15 - 27\%$; high proportion: $>27\%$ in short GnRH-a long protocol) [8]. In another two randomised controlled trials of GnRH antagonist cycles, enlarging follicle size by delaying HCG administration by one or two days after the time that three follicles had reached $17$ mm, corresponded with a decrease in ongoing pregnancy rates in the delayed group [4, 14]. Availability of surplus embryos for freezing was lower when delaying two days compared with delaying one day, but this did not reach statistical significance [14]. Interestingly in our study, as DFP increased, the number of blastocysts frozen and blastocysts formation rate decreased significantly. Multiple linear regression results showed that the number of blastocysts frozen and blastocysts formation rate were negatively associated to DFP. Furthermore, increasing DFP is a risk factor for low blastocysts frozen. These results revealed that the overgrowth of dominant follicles might lead to oocyte post-maturity, which in turn could have an inverse impact on the quality of oocytes and ultimately lead to unsatisfying pregnant outcomes [8, 18]. This phenomenon may be related to the increased incidence of ultrastructural abnormalities in the oocytes, for example, the appearance of degenerate organelles-smooth surface endoplasmic reticulum (sER) [22]. Embryos that accumulate the sER may have a low rate of blastocyst formation and poor pregnancy outcomes [23]. It is believed that sER aggregation is related to high E2 levels on HCG day and long-term Gn stimulation [22]. From the results of clinical pregnancy and available blastocysts, it is speculated that excessive delay of the trigger might negatively affect the cumulative pregnancy rate. But further prospective study should be needed.

There are several advantages and limitations in our study. The most important innovation is the discovery that the larger the DFP, the smaller the number of blastocysts frozen and blastocyst formation rate. In other words, increasing DFP is a risk factor for low blastocysts frozen, and is also negatively associated with good clinical outcomes in general. In addition, our study is the first to
analyse the GnRH-a prolonged protocol in terms of HCG trigger time. Finally, for the analysis of the DFP groupings and clinical outcomes, we used a large panel of data, established multiple linear regression and binary logistic regression, and conducted a thorough and comprehensive evaluation of these relationships. To a certain extent, it would provide useful information for clinical decision-making. The limitations are as follows: First, due to its retrospective nature, some confounding bias may exist. Second, we lack cumulative pregnancy rate results. It is hard to query and count the data for such a large sample. In addition, we screened standardised young patients with normal ovarian responses. The results may be not applicable to older patients or patients with low or high ovarian responses.

In all, pursuing more and larger follicles may be of no benefit to clinical outcomes. This practice might increase patient cost and time to a certain extent, without increasing the oocyte maturation rate, normal fertilisation rate, or the number of embryos available for transfer. It might be preferable to trigger as early as possible with patients who have not formed any available blastocysts (surprisingly, these patients account for 1/5 calculated from our data), to avoid diminishing oocyte and embryo quality, and avoid consequently poor IVF/ICSI outcomes.

**Conclusion**

Excessive delay of the HCG trigger during prolonged pituitary downregulation might reduce the developmental potential of oocytes and reduce the number of available blastocysts, which might result in a lower cumulative pregnancy rate. But further confirmation by strict prospective randomized controlled study should be needed.

**Declarations**

**Ethics approval and consent to participate**
This study was approved by the Institutional Review Board at Huazhong University of Science and Technology, Wuhan, China. All of the participants provided informed written consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was supported by the National Key Research and Development Project [grant number 2018YFA0108401] and the National Natural Science Foundation [grant number 81571464] of China.

Author Contributions

K.Q. designed the study and revised the manuscript. HM.S. collected data, performed the statistical analyses, and wrote manuscript. YH.L., J.L., TT.L., LC.J., and XY.H. collected the data. All authors contributed to the interpretation of the results and editing of the manuscript.

Acknowledgments
We thank the staff at the Reproductive Medicine Center of Tongji Hospital for their outstanding support.

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Table 1. Baseline Characteristics and Clinical Outcomes.

|                              | Group A <30% | Group B 30%-60% | Group C ≥60% | P   |
|------------------------------|--------------|-----------------|--------------|-----|
| No. of cycles                | 398          | 1230            | 297          | -   |
| Duration of infertility(years)| 3.21±1.98    | 3.35±2.14       | 2.97±2.06    | 0.026|
| Maternal age (years)         | 29.17±2.94   | 29.19±2.84      | 29.13±2.60   | 0.939|
| BMI (kg/m²)                  | 22.24±3.03   | 22.19±3.09      | 22.42±2.94   | 0.500|
| AFC                          | 16.55±6.42   | 16.39±6.15      | 14.79±5.01** | <0.001|
| Basal FSH (mIU/ml)           | 7.27±1.79    | 7.23±1.69       | 7.32±1.94    | 0.685|
| AMH (ng/ml)                  | 6.47±4.20    | 6.59±4.00       | 5.72±3.52**  | 0.003|
| Dose of Gn (IU)              | 2435.31±962.72 | 2401.90±855.15 | 2511.15±811.93 | 0.149|
| Duration of Gn (days)        | 11.31±2.01   | 11.25±2.11      | 10.99±1.84   | 0.087|
| Dose/Duration of Gn (IU/d)   | 214.10±70.19 | 213.27±63.17    | 228.88±64.52** | 0.001|
| E2 (HCG day, pg/ml)          | 2322.80±1083.71 | 2289.81±1047.77 | 2283.90±1065.46 | 0.849|
| P (HCG day, ng/ml)           | 0.72±0.34    | 0.79±0.52*      | 0.82±0.34**  | 0.010|
| No. of ≥14mm follicles       | 11.92±3.27   | 11.21±3.17*     | 9.45±2.95**  | <0.001|
| No. of oocytes retrieved     | 12.74±3.22   | 12.22±3.35*     | 11.59±3.29** | <0.001|
| Oocyte maturation rate (%)   |              |                 |              |     |
| IVF                          | 86.10±14.39  | 88.17±14.22     | 87.31±14.57  | 0.101|
| ICSI                         | 82.27±14.87  | 80.59±14.88     | 81.41±13.05  | 0.553|
| Normal fertilisation rate (%)|              |                 |              |     |
| IVF                          | 62.70±16.99  | 63.26±18.53     | 61.74±19.25  | 0.525|
| ICSI                         | 57.53±19.46  | 57.78±19.08     | 55.16±17.80  | 0.573|
| Endometrial thickness (mm)   | 12.26±2.58   | 12.30±2.58      | 12.01±2.38   | 0.223|
| No. of blastocysts frozen    | 3.05±2.35    | 2.60±2.16*      | 2.32±2.12**  | <0.001|
| Blastocyst formation rate (%)| 42.38±26.59  | 38.69±27.16*    | 38.26±28.27** | 0.047|
|                | ET 1 embryo                                                                 | ET 2 embryos                                                                 |
|----------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------|
|                | Clinical pregnancy rate(%) 58.20(188/323)                                   | Clinical pregnancy rate(%) 68.00(51/75)                                     |
|                | 65.30(510/781)                                                            | 73.94(332/449)                                                            |
|                | 62.82(98/156)                                                             | 67.38(95/141)                                                             |
|                | 0.084                                                                      | 0.232                                                                      |
| Implantation   | 48.67(73/150)                                                             | 57.57(517/898)*                                                           |
| rate (%)       | 50.71(143/282)                                                            | 0.031                                                                      |

BMI = body mass index; AFC = antral follicle counting; FSH = follicle stimulating hormone; IVF = in vitro fertilization; ICSI = intracytoplasmic sperm injection.

* Group B versus Group A, P < 0.05; ** Group C versus Group A, P < 0.05.
Table 2. Logistics Regression Analysis of Clinical Pregnancy.

| Variable                                      | OR  | 95% CI       | P      |
|-----------------------------------------------|-----|--------------|--------|
| BMI                                           | 0.935 | 0.904, 0.966 | <0.001 |
| Dose/Duration of Gn                           | 0.992 | 0.990, 0.993 | <0.001 |
| No. of oocytes retrieved                      | 0.950 | 0.920, 0.980 | 0.001  |
| No. of embryo transfer                        |      |              |        |
| 2                                             | 1.469 | 1.180, 1.830 | 0.001  |
| 1                                             | REF  |              |        |
| DFP                                           |      |              |        |
| Group A                                       | REF  |              |        |
| Group B                                       | 1.345 | 1.041, 1.738 | 0.023  |
| Group C                                       | 1.242 | 0.881, 1.751 | 0.216  |

REF = reference value. P-value of < 0.05 indicated statistical significance.
Table 3. Multiple linear regression analysis of number of blastocysts frozen.

|                         | β±Standard error | Standardized β | t     | P     |
|-------------------------|------------------|----------------|-------|-------|
| **Infertility**         |                  |                |       |       |
| Secondary              | 0.194±0.097      | 0.042          | 2.010 | 0.045 |
| Primary                | REF              |                |       |       |
| **Dose/Duration of Gn** | -0.003±0.001     | -0.078         | -3.692| <0.001|
| **No. of oocytes retrieved** | 0.252±0.014 | 0.380          | 18.051| <0.001|
| **Fertilization**       |                  |                |       |       |
| ICSI                   | -0.513±0.103     | -0.104         | -4.966| <0.001|
| IVF                    | REF              |                |       |       |
| **DFP**                |                  |                |       |       |
| Group A                | REF              |                |       |       |
| Group B                | -0.319±0.115     | -0.070         | -2.769| 0.006 |
| Group C                | -0.432±0.154     | -0.071         | -2.798| 0.005 |

REF = reference value. P-value of < 0.05 indicated statistical significance.
Table 4. Multiple linear regression analysis of blastocyst formation rate.

|                          | β±Standard error | Standardized β | t     | P    |
|--------------------------|------------------|----------------|-------|------|
| **Infertility**          |                  |                |       |      |
| Secondary                | 0.027±0.012      | 0.055          | 2.330 | 0.020|
| Primary                  | REF              |                |       |      |
| **Dose/Duration of Gn**  | -0.0004±0.0001   | -0.095         | -4.038| <0.001|
| **No. of oocytes retrieved** | 0.006±0.002     | 0.071          | 3.029 | 0.002|
| **Fertilization**        |                  |                |       |      |
| ICSI                     | -0.027±0.014     | -0.045         | -1.914| 0.056|
| IVF                      | REF              |                |       |      |
| **DFP**                  |                  |                |       |      |
| Group A                  | REF              |                |       |      |
| Group B                  | -0.035±0.016     | -0.061         | -2.160| 0.031|
| Group C                  | -0.039±0.021     | -0.052         | -1.833| 0.067|

REF = reference value. P-value of < 0.05 indicated statistical significance.
Table 5. Logistics regression analysis of low blastocysts frozen (≤ 1).

|                          | OR   | 95% CI        | P value |
|--------------------------|------|---------------|---------|
| Infertility              |      |               |         |
| Secondary                | 0.768| 0.621, 0.949  | 0.015   |
| Primary                  | REF  |               |         |
| Dose/Duration of Gn      | 1.003| 1.001, 1.005  | <0.001  |
| No. of oocytes retrieved | 0.832| 0.806, 0.858  | <0.001  |
| Fertilization            |      |               |         |
| ICSI                     | 1.464| 1.175, 1.826  | 0.001   |
| IVF                      | REF  |               |         |
| DFP                      |      |               |         |
| Group A                  | REF  |               |         |
| Group B                  | 1.312| 1.014, 1.698  | 0.039   |
| Group C                  | 1.417| 1.014, 1.979  | 0.041   |

Low Blatocysts Frozen = number of blastocysts frozen ≤ 1; REF = reference value. P-value of < 0.05 indicated statistical significance.