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

Houming Su

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Youhua Lai

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Jie Li

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Tingting Liao

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Licheng Ji

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Xinyao Hu

Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Kun Qian (✉ kunqian1129@yeah.net)

Department of Rehabilitation Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology <https://orcid.org/0000-0001-8063-457X>

Research

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

Posted Date: September 7th, 2021

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

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

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

Houming Su, Youhua Lai, Jie Li, Tingting Liao, Licheng Ji, Xinyao Hu, Kun Qian*

Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

* Correspondence:

Kun Qian

kunqian@tjh.tjmu.edu.cn

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 ≥ 18 mm follicles / ≥ 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 ± 0.115 , $P = 0.006$; Group C vs Group A = -0.432 ± 0.154 , $P = 0.005$) as well as blastocyst formation rate ($\beta \pm SE$: Group B vs Group A = -0.035 ± 0.016 , $P = 0.031$; Group C vs Group A = -0.039 ± 0.021 , $P = 0.067$). 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].

40 To date, there has been no universal criteria for the optimal HCG trigger time for IVF/ICSI cycles in
41 a variety of COH protocols [1, 2, 5]. Traditionally, the majority of reproductive centers administer
42 the trigger when at least three leading follicles have reached ≥ 17 mm, or at least two leading
43 follicles have reached ≥ 18 mm [1, 2, 6 - 8]. Ectors et al. proposed to postpone HCG trigger in short
44 GnRH-a protocol. They suggested that it may yield relatively more large follicles, including
45 competent oocytes with mature cytoplasm, and may exert positive effects on clinical outcomes [9].
46 On the contrary, some studies suggested that delaying oocyte retrieval in short GnRH-a long protocol
47 increased neither the number of mature oocytes retrieved nor the pregnancy rate and may, in fact,
48 impair embryo quality [8]. However, other studies did not find differences in fertilization rate or
49 oocyte maturation rate between large and small follicles (10 - 14 mm; 15 - 19 mm; 20+ mm) [10];
50 (<16 mm; ≥ 16 mm) [11]. These discordant findings might be attributed to differences in COH
51 protocols, definition of follicle size, patient characteristics, methods of oocyte insemination (IVF or
52 ICSI), and analysis of endpoints [1].

53 In recent years, IVF and ICSI have developed rapidly, and the available COH protocols are flexible
54 and diverse. For example, the GnRH-a prolonged protocol is an emerging protocol that is widely
55 used in clinical practice (especially in China). Nevertheless, the ideal time to trigger ovulation has not
56 yet been well defined for this protocol. Some reproductive centers simply follow the criteria of
57 trigger time of short GnRH-a long protocol when using this protocol. However, the mechanism of
58 action, endocrine hormones, and medication regimen differ greatly between different COH protocols
59 [4]. It's controversial to choose the same trigger time for different COH protocols [1, 19]. Evidence is
60 still urgently needed to provide a reference for clinical decision-making regarding the trigger time of
61 GnRH-a prolonged protocol.

62 Folliculogenesis may be asynchronous during ovarian hyperstimulation. We need to focus on the
63 global development state of the growing follicle group [8]. The dominant follicular proportion (DFP)
64 was first proposed in the GnRH antagonist protocol, which can reflect the overall development of
65 follicles. And it was considered to be a more effective and objective indicator to determine the ideal
66 HCG trigger timing [5]. In this study, we compared the effects of different DFP (≥ 18 mm follicles /
67 ≥ 14 mm follicles) on clinical outcomes in a large group of people who underwent IVF or ICSI
68 treatment with GnRH-a prolonged protocol, including oocyte maturation rate, normal fertilization
69 rate, clinical pregnancy rate, implantation rate, number of blastocysts frozen, blastocyst formation
70 rate, and low blastocyst frozen.

71 **Methods**

72 **Patient selection**

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

77 The inclusion criteria were as follows: (1) the first IVF/ICSI fresh embryo transfer cycle, (2) female
78 with age < 35 years, (3) body mass index (BMI) ≤ 30 kg/m², (4) antral follicle counting (AFC) ≥ 5 ,
79 (5) anti-Müllerian hormone (AMH) ≥ 1.2 IU/L, (6) basal serum follicle-stimulating hormone (FSH)
80 < 12 IU/L, and (7) 6 - 18 retrieved oocytes.

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

83 **Controlled ovarian stimulation protocols**

84 For GnRH-a prolonged protocol, 3.75 mg of long-acting GnRH-a (Decapeptyl; Ferring, Saint-Prex,
85 Switzerland) was administered subcutaneously on day 2 of the menstrual cycle. Pituitary suppression
86 was evaluated 28 days after pituitary downregulation. The criteria for confirming the success of
87 downregulation were as follows: follicle diameter <5 mm, serum luteinizing hormone (LH) <5
88 mIU/ml, serum estradiol (E2) <50 pg/mL, and endometrial thickness <5 mm. Then, daily injection
89 of rhFSH (Gonal-F; Merck-Serono, Geneva, Switzerland) ranging from 75 to 300 IU was given for
90 about 10 days. When at least three leading follicles ≥ 17 mm or two leading follicles ≥ 18 mm were
91 observed via transvaginal ultrasound, a dose of 0.25mg rHCG (Ovidrel; Merck-Serono, Geneva,
92 Switzerland) was administered to trigger ovulation. After 36 - 37 hours, transvaginal ultrasound-
93 guided oocyte retrieval was conducted. Fertilization was accomplished by standard IVF or ICSI. If
94 serum progesterone <1.5 ng/mL [17], the number of oocytes retrieved were <20 , and serum E2 were
95 $<7,000$ pg/mL, the one or two best-quality Day 3 embryos were transferred; and the remaining
96 embryos were cultured to Day 5/6 until the blastocyst formed and then frozen. The luteal phase was
97 supported if embryo transfer was performed.

98 **Definition of DFP Levels and Groups**

99 Dominant follicular proportion (DFP) is defined as ≥ 18 mm follicles / ≥ 14 mm follicles. DFP $<$
100 30% corresponds to at least two follicles ≥ 18 mm on HCG day, which is the most common trigger
101 time. Then, we set the groups as DFP $<30\%$ (Group A), $30\% - 60\%$ (Group B), $\geq 60\%$ (Group C)
102 corresponding to follicles ≥ 18 mm increasing gradually (2, 4, 6 follicles respectively).

103 **Clinical outcomes**

104 The number of mature oocytes (MII) was measured 3 - 4 hours, and fertilization was assessed 16 - 18
105 hours after IVF insemination or when ICSI was performed. Oocyte maturation rate was the
106 proportion of MII oocytes to the number of oocytes retrieved. Normal fertilization rate was equal to
107 2PN/number of oocytes retrieved (IVF) or 2PN/MII (ICSI). The blastocyst formation rate was equal
108 to the number of blastocysts frozen divided by the number of blastocysts continuously cultured. Low
109 blastocysts frozen was defined as the number of blastocysts frozen ≤ 1 (that is, less than half of the
110 average number of blastocysts frozen). Clinical pregnancy was identified with the presence of an
111 intrauterine gestational sac with fetal cardiac activity. The clinical pregnancy rate was the number of
112 clinical pregnancies in a given number of transplant cycles. Implantation rate reflects the number of
113 gestational sacs divided by the number of embryos transferred.

114 **Statistical analysis**

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

123 **Results**

124 A total of 1,925 consecutive IVF / ICSI cycles were included. As shown in **Table 1**, the clinical
125 characteristics, ovarian response characteristics, and reproductive outcomes of cycles were described.
126 The baseline characteristics and ovarian stimulation details were matched evenly in three groups,

127 including maternal age, BMI, basal FSH, dose of Gn, duration of Gn. Among the DFP groups, there
128 were no significant differences in terms of oocyte maturation rate (IVF/ICSI), normal fertilization
129 rate (IVF/ICSI), and clinical pregnancy rate. Group B had the highest implantation rate (Group A
130 48.67%, Group B 57.57%, Group C 50.71%, $P = 0.031$). Interestingly, Group A had the highest
131 number of blastocysts frozen (Group A 3.05 ± 2.35 , Group B 2.60 ± 2.16 , Group C 2.32 ± 2.12 , $P <$
132 0.001) and the highest blastocyst formation rate (Group A $42.38\% \pm 26.59\%$, Group B $38.69\% \pm$
133 27.16% , Group C $38.26\% \pm 28.27\%$, $P = 0.047$).

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

139 Multivariate linear regression analyses were carried out to evaluate the effect of type of infertility,
140 dose/duration of Gn, number of oocytes retrieved, type of fertilization and DFP levels on the number
141 of blastocysts frozen and blastocyst formation rate, respectively. As shown in **Table 3**, the results
142 indicated that DFP was negatively associated with number of blastocysts frozen ($\beta \pm SE$: Group B vs
143 Group A = -0.319 ± 0.115 , $P = 0.006$; Group C vs Group A = -0.432 ± 0.154 , $P = 0.005$). As shown
144 in **Table 4**, the results indicated that DFP was also negatively associated with blastocyst formation
145 rate ($\beta \pm SE$: Group B vs Group A = -0.035 ± 0.016 , $P = 0.031$; Group C vs Group A = $-0.039 \pm$
146 0.021 , $P = 0.067$).

147 Binary logistic regression analysis was carried out to evaluate the effect of type of infertility,
148 dose/duration of Gn, number of oocytes retrieved, type of fertilization and DFP levels on low
149 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

173 in the low proportion group (diameter ≥ 18 mm divided by the total number of follicles, low
174 proportion: <15%; middle proportion: 15 - 27%; high proportion: >27% in short GnRH-a long
175 protocol) [8]. In another two randomised controlled trials of GnRH antagonist cycles, enlarging
176 follicle size by delaying HCG administration by one or two days after the time that three follicles had
177 reached 17 mm, corresponded with a decrease in ongoing pregnancy rates in the delayed group [4,
178 14]. Availability of surplus embryos for freezing was lower when delaying two days compared with
179 delaying one day, but this did not reach statistical significance [14]. Interestingly in our study, as
180 DFP increased, the number of blastocysts frozen and blastocysts formation rate decreased
181 significantly. Multiple linear regression results showed that the number of blastocysts frozen and
182 blastocysts formation rate were negatively associated to DFP. Furthermore, increasing DFP is a risk
183 factor for low blastocysts frozen. These results revealed that the overgrowth of dominant follicles
184 might lead to oocyte post-maturity, which in turn could have an inverse impact on the quality of
185 oocytes and ultimately lead to unsatisfying pregnant outcomes [8, 18]. This phenomenon may be
186 related to the increased incidence of ultrastructural abnormalities in the oocytes, for example, the
187 appearance of degenerate organelles-smooth surface endoplasmic reticulum (sER) [22]. Embryos that
188 accumulate the sER may have a low rate of blastocyst formation and poor pregnancy outcomes [23].
189 It is believed that sER aggregation is related to high E2 levels on HCG day and long-term Gn
190 stimulation [22]. From the results of clinical pregnancy and available blastocysts, it is speculated that
191 excessive delay of the trigger might negatively affect the cumulative pregnancy rate. But further
192 prospective study should be needed.

193 There are several advantages and limitations in our study. The most important innovation is the
194 discovery that the larger the DFP, the smaller the number of blastocysts frozen and blastocyst
195 formation rate. In other words, increasing DFP is a risk factor for low blastocysts frozen, and is also
196 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

219 This study was approved by the Institutional Review Board at Huazhong University of Science and
220 Technology, Wuhan, China. All of the participants provided informed written consent.

221 **Consent for publication**

222 Not applicable.

223 **Availability of data and materials**

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

226 **Conflict of Interest**

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

229 **Funding**

230 This work was supported by the National Key Research and Development Project [grant number
231 2018YFA0108401] and the National Natural Science Foundation [grant number 81571464] of China.

232 **Author Contributions**

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

236 **Acknowledgments**

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

239 **References**

- 240 1. Rosen MP, Shen S, Dobson AT, Rinaudo PF, McCulloch CE, Cedars MI: A quantitative
241 assessment of follicle size on oocyte developmental competence. *Fertil Steril* 2008, 90(3):684-690.
- 242 2. Lin HY, Li Y, Wang WJ, Qiu Q, Zhang QX, Li Y: Role of the proportion of dominant follicles in
243 patients with polycystic ovary syndrome undergoing in vitro fertilization-embryo transfer. *Chin Med*
244 *J (Engl)* 2019, 132(12):1448-1453.
- 245 3. Mochtar MH, Custers IM, Koks CA, Bernardus RE, Verhoeve HR, Mol BW, van Wely M, van der
246 Veen F: Timing oocyte collection in GnRH agonists down-regulated IVF and ICSI cycles: a
247 randomized clinical trial. *Hum Reprod* 2011, 26(5):1091-1096.
- 248 4. Kolibianakis EM, Albano C, Camus M, Tournaye H, Van Steirteghem AC, Devroey P:
249 Prolongation of the follicular phase in in vitro fertilization results in a lower ongoing pregnancy rate
250 in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing
251 hormone antagonists. *Fertil Steril* 2004, 82(1):102-107.
- 252 5. Hu X, Luo Y, Huang K, Li Y, Xu Y, Zhou C, Mai Q: New Perspectives on Criteria for the
253 Determination of HCG Trigger Timing in GnRH Antagonist Cycles. *Medicine (Baltimore)* 2016,
254 95(20):e3691.
- 255 6. Falagario M, Trerotoli P, Chincoli A, Cobuzzi I, Vacca MP, Falagario D, Nardelli C, Depalo R:
256 Dynamics of the development of multiple follicles by early versus late hCG administration in ART
257 program. *Gynecol Endocrinol* 2017, 33(2):105-108.

258 7. European, Middle East Orgalutran Study G: Comparable clinical outcome using the GnRH
 259 antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of
 260 premature LH surges in women undergoing ovarian stimulation. *Hum Reprod* 2001, 16(4):644-651.

261 8. Li Y, Li RQ, Ou SB, Ren L, Zhang NF, Wei LN, Zhang QX, Yang DZ: Association between the
 262 proportion of dominant follicles and oocyte developmental competence. *J Assist Reprod Genet* 2014,
 263 31(12):1599-1604.

264 9. Ectors FJ, Vanderzwalmen P, Van Hoeck J, Nijs M, Verhaegen G, Delvigne A, Schoysman R,
 265 Leroy F: Relationship of human follicular diameter with oocyte fertilization and development after
 266 in-vitro fertilization or intracytoplasmic sperm injection. *Hum Reprod* 1997, 12(9):2002-2005.

267 10. Haines CJ, Emes AL: The relationship between follicle diameter, fertilization rate, and
 268 microscopic embryo quality. *Fertil Steril* 1991, 55(1):205-207.

269 11. Bergh C, Broden H, Lundin K, Hamberger L: Comparison of fertilization, cleavage and
 270 pregnancy rates of oocytes from large and small follicles. *Hum Reprod* 1998, 13(7):1912-1915.

271 12. Mehri S, Levi Setti PE, Greco K, Sakkas D, Martinez G, Patrizio P: Correlation between
 272 follicular diameters and flushing versus no flushing on oocyte maturity, fertilization rate and embryo
 273 quality. *J Assist Reprod Genet* 2014, 31(1):73-77.

274 13. Braga DP, Bonetti TC, da Silva ID, Setti AS, Iaconelli AJ, Borges EJ: The developmental
 275 competence of oocytes retrieved from the leading follicle in controlled ovarian stimulated cycles. *Int*
 276 *J Fertil Steril* 2013, 6(4):272-277.

277 14. Morley L, Tang T, Yasmin E, Hamzeh R, Rutherford AJ, Balen AH: Timing of human chorionic
 278 gonadotrophin (hCG) hormone administration in IVF protocols using GnRH antagonists: a
 279 randomized controlled trial. *Hum Fertil (Camb)* 2012, 15(3):134-139.

15. Nogueira D, Friedler S, Schachter M, Raziel A, Ron-El R, Smitz J: Oocyte maturity and preimplantation development in relation to follicle diameter in gonadotropin-releasing hormone agonist or antagonist treatments. *Fertil Steril* 2006, 85(3):578-583.
16. Akbariasbagh F, Lorzadeh N, Azmoodeh A, Ghaseminejad A, Mohamadpoor J, Kazemirad S: Association among diameter and volume of follicles, oocyte maturity, and competence in intracytoplasmic sperm injection cycles. *Minerva Ginecol* 2015, 67(5):397-403.
17. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC: Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update* 2013, 19(5):433-457.
18. Wirleitner B, Okhowat J, Vistejnova L, Kralickova M, Karlikova M, Vanderzwalmen P, Ectors F, Hradecky L, Schuff M, Murtinger M: Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval. *Ultrasound Obstet Gynecol* 2018, 51(1):118-125.
19. Ou J, Xing W, Li Y, Xu Y, Zhou C: Short versus Long Gonadotropin-Releasing Hormone Analogue Suppression Protocols in IVF/ICSI Cycles in Patients of Various Age Ranges. *PLoS One* 2015, 10(7):e0133887.
20. Hugues JN, Masse-Laroche E, Reboul-Marty J, Boiko O, Meynant C, Cedrin-Durnerin I: Impact of endogenous luteinizing hormone serum levels on progesterone elevation on the day of human chorionic gonadotropin administration. *Fertil Steril* 2011, 96(3):600-604.
21. Sun L, Ye J, Wang Y, Chen Q, Cai R, Fu Y, Tian H, Lyu Q, Lu X, Kuang Y: Elevated basal luteinizing hormone does not impair the outcome of human menopausal gonadotropin and medroxyprogesterone acetate treatment cycles. *Sci Rep* 2018, 8(1):13835.

302 22. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H: The relationship between pregnancy outcome
303 and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod 2004, 19(7):1591-
304 1597.

305 23. Setti AS, Figueira RC, de Almeida Ferreira Braga DP, Azevedo MC, Iaconelli A, Jr., Borges E,
306 Jr.: Oocytes with smooth endoplasmic reticulum clusters originate blastocysts with impaired
307 implantation potential. Fertil Steril 2016, 106(7):1718-1724.

308

309 **Table 1.** Baseline Characteristics and Clinical Outcomes.

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

ET 1 embryo

Clinical pregnancy rate(%)	58.20(188/323)	65.30(510/781)	62.82(98/156)	0.084
----------------------------	----------------	----------------	---------------	-------

ET 2 embryos

Clinical pregnancy rate(%)	68.00(51/75)	73.94(332/449)	67.38(95/141)	0.232
----------------------------	--------------	----------------	---------------	-------

Implantation rate (%)	48.67(73/150)	57.57(517/898)*	50.71(143/282)	0.031
-----------------------	---------------	-----------------	----------------	-------

310 BMI = body mass index; AFC = antral follicle counting; FSH = follicle stimulating hormone; IVF =

311 in vitro fertilization; ICSI = intracytoplasmic sperm injection.

312 * Group B versus Group A, $P < 0.05$; ** Group C versus Group A, $P < 0.05$.

313

314 **Table 2.** Logistics Regression Analysis of Clinical Pregnancy.

	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

315 REF = reference value. P-value of < 0.05 indicated statistical significance.

316

317 **Table 3.** Multiple linear regression analysis of number of blastocysts frozen.

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

318 REF = reference value. P-value of < 0.05 indicated statistical significance.

319

320 **Table 4.** Multiple linear regression analysis of blastocyst formation rate.

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

321 REF = reference value. P-value of < 0.05 indicated statistical significance.

322

323 **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

324 Low Blastocysts Frozen = number of blastocysts frozen ≤ 1 ; REF = reference value. P-value of <
325 0.05 indicated statistical significance.