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# Ovarian stimulation methods at a single-assisted reproductive technology institution: a retrospective study of successful outcomes

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**Abstract**

**Aim:** to examine an efficacy of various stimulation methods in a group of successful pregnancies.

**Materials and Methods.** In a single center retrospective study 47 pregnancy cases were examined after performing embryo transfers at our institution from the years 2017 to 2021. Patients were divided into four groups: i) clomiphene hyperstimulation (CH), ii) stimulation with gonadotropin-releasing hormone (GnRH) agonist, iii) with GnRH antagonist, and iv) progestin-primed ovarian stimulation (PPOS). Age, anti-Mullerian hormone, presence of chronic disease, number of in-vitro fertilizations prior to conception, dosage of follicle stimulating hormone (FSH), presence of premature luteinizing hormone surge, number of egg retrievals, fertilization rate, and live birth rate were assessed.

**Results.** The number of pregnancies obtained by CH, agonist, antagonist, and PPOS methods comprised 25, 12, 2, and 8 cases, respectively. No significant difference in parameters between CH group and non-CH groups, excepting FSH was observed. The FSH was used in CH group and non-CH group at dose of 1108 ± 468 IU and 1756 ± 394 IU, respectively ($p < 0.0001$).

**Conclusion.** CH is not commonly used due to potential luteal phase defects, but it is thought to be cost-effective, requiring lower FSH doses, requiring no ovulation suppression antagonists, and exerting no effect on frozen embryos or fetuses. Hence, CH could be a suitable protocol for egg retrieval in Japan.

**Keywords:** ovarian stimulation, clomiphene, pregnancy, assisted reproductive technology, in-vitro fertilization

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# Методы стимуляции яичников в клинике репродуктивной медицины: ретроспективное исследование успешных результатов

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**Резюме**

**Цель:** изучить эффективность различных методов стимуляции яичников в группе успешных беременностей.

**Материалы и методы.** В рамках одноцентрового ретроспективного исследования с 2017 по 2021 гг. нами было изучено 47 случаев беременности после переноса эмбрионов. Пациентки были разделены на 4 группы: 1) кломифеновая гиперстимуляция (КГ) яичников, 2) стимуляция агонистом гонадотропин-рилизинг-гормона (ГнРГ), 3) стимуляция антагонистом ГнРГ и 4) прогестин-праймированная стимуляция яичников (ППСЯ). Оценивали возраст пациенток, уровень антимюллерова гормона, наличие хронических заболеваний, количество попыток экстракорпорального оплодотворения до зачатия, дозу фолликулостимулирующего гормона (ФСГ), наличие преждевременного всплеска лютеинизирующего гормона, количество извлечений яйцеклеток, частоту оплодотворения и частоту живорождения.

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**Результаты.** Количество беременностей, наступивших при использовании метода КГ, стимуляции яичников агонистами ГнРГ, антагонистами ГнРГ и метода ППСЯ, составили 25, 12, 2 и 8 случаев соответственно. Не установлено существенной разницы в изученных параметрах между группами с КГ и без таковой, за исключением ФСГ, который применяли в группах с КГ и без таковой в дозе 1108 ± 468 МЕ и 1756 ± 394 МЕ соответственно (p < 0,0001).

**Заключение.** КГ обычно не используется из-за возможного нарушения лютеиновой фазы, но считается, что она экономически эффективна, требует более низких доз ФСГ, не требует применения антагонистов ГнРГ, подавляющих овуляцию, и не оказывает влияния на замороженные эмбрионы или плоды. Следовательно, в Японии метод КГ может быть признан подходящим протоколом для извлечения яйцеклеток.

**Ключевые слова:** стимуляция яичников, кломифен, беременность, вспомогательные репродуктивные технологии, экстракорпоральное оплодотворение

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**Highlights**

**What is already known about this subject?**

- There is no golden-standard ovarian stimulation protocol in assisted reproductive technology, and protocol is various at each hospital.

**What are the new findings?**

- The efficacy of various stimulation methods in a group of successful pregnancies is presented.

**How might it impact on clinical practice in the foreseeable future?**

- Reductio of visiting hospital by using home injection during COVID-19 pandemic.
- From successful pregnancy group, any ovarian stimulation protocol might be effective.

**Основные моменты**

**Что уже известно об этой теме?**

- В настоящее время при проведении вспомогательных репродуктивных технологий не существует «золотого стандарта» протокола стимуляции яичников, поэтому в каждом медицинском центре применяется свой протокол.

**Что нового дает статья?**

- Изучена эффективность использования различных методов стимуляции яичников в группе успешных беременностей.

**Как это может повлиять на клиническую практику в обозримом будущем?**

- Сокращение частоты посещения больниц за счет использования инъекций на дому во время пандемии COVID-19.
- В группе успешных беременностей любой протокол стимуляции яичников может быть эффективным.

## Introduction / Введение

Japan is one of the countries in the world where assisted reproductive technology (ART) is most commonly practiced resulting in 1 per 16.7 births being conceived [1]. The average age of patients receiving ART treatment is 38 years old, which is higher than the average age of 35 years [2, 3]. Patients over 40 years old account for 38 % of those currently undergoing fertility treatment. Patient age at initial treatment is also high [4], with 5.0 % of patients being over 40 at first ART-treatment visit [5]. Japanese ART treatment is characterized by a broad use of clomiphene peaking among developed countries, along with China [6]. In Japan, the use of oral stimulation drugs is higher than that of agonist and antagonist methods [7].

Although there have been studies on ovarian stimulation methods based on ovarian reserve capacity and poor responders, studies on the number of eggs obtained as well as fertilization and pregnancy rates are limited. The use of clomiphene for hyperstimulation currently applied in our institution has been reported only at academic conferences in Japan. Despite several different protocols being described for ovarian stimulation in ART, there is no consensus on a standard method [8].

**Aim:** to examine the efficacy of various stimulation methods in a group of successful pregnancies.

## Materials and Methods / Материалы и методы

### Study design / Дизайн исследования

In a single center retrospective study 47 pregnancy cases were examined after performing embryo transfers at our institution from the years 2017 to 2021. Based on the stimulation methods, patients were stratified as follows: clomiphene hyperstimulation (CH) group (n = 25), gonadotropin-releasing hormone (GnRH) agonist group (n = 12), gonadotropin-releasing hormone (GnRH) antagonist group (n = 2), and progestin-primed ovarian stimulation (PPOS) group (n = 8). There was no uniform protocol to choose from stimulation methods; it was individually determined by the patient's medical history and unique circumstances.

The data collected were as follows: age, anti-Mullerian hormone (AMH), chronic comorbidities, number of in-vitro fertilization (IVF) procedures prior to conception, dose of follicle stimulating hormone (FSH), presence of premature luteinizing hormone (LH) surge, number of egg retrievals, fertilization rate, and live birth rate.

### Ethical aspects / Этические аспекты

All procedures were conducted in accordance with the ethical standards of the responsible committee on human

experimentation (institutional and national) and with the 1964 Declaration of Helsinki and updated amendments. The study protocol was approved by a suitably constituted Ethics Committee of Tokyo Women's Medical University (01.01.1999 to 31.12.2030, Protocol No 5704). The study design was approved by the institutional review board of Tokyo Women's Medical University. Informed consent was obtained from all patients to be enrolled in the study.

### Statistical analysis method / Статистический анализ

Statistical data processing was carried out using specialized software R 4.2.1. For statistical analysis, the Student's t-test and $\chi$-square test were used. Statistical significance was set at $p \leq 0.05$.

## Results / Результаты

The number of pregnancies from embryos obtained by CH, agonist, antagonist, and PPOS methods were 25, 12, 2, and 8, respectively (**Fig. 1**). Statistical analyses were performed for the CH and agonist methods, which accounted for 25 and 22 cases. There were no significant differences between the CH and agonist protocols in the age, number of eggs retrieved, fertilization rate, AMH, number of IVF before conception, and live birth rate among pregnancies. The comparison between the CH and non-agonist groups also showed no significant differences (**Table 1**). Moreover, pregnancy rates were not significantly different between the protocols. Clomiphene was used more frequently in patients with chronic diseases (6 out of 7 cases). In CH and non-CH group, FSH was used at a dose of 1,108 ± 468 IU and 1,756 ± 394 IU, respectively that differed significantly ($p < 0.0001$). Two cases of ovulation suppression injection were recorded in CH group due to mildly elevated LH.

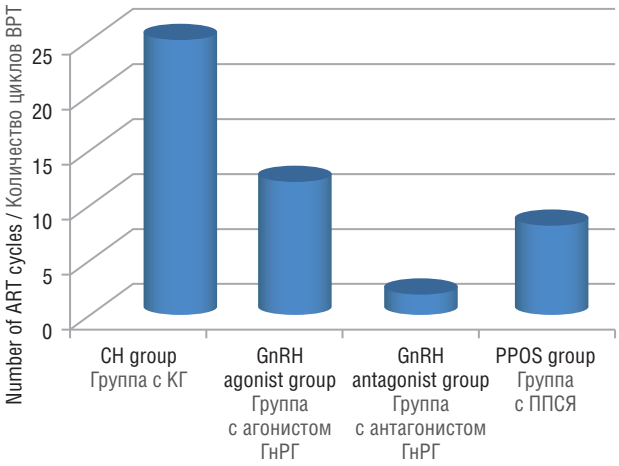


**Figure 1.** Stimulation protocols used in pregnancy cases from 2017 to 2021.

***Note:*** *CH – clomiphene hyperstimulation; GnRH – gonadotropin-releasing hormone; PPOS – progestin-primed ovarian stimulation; ART – assisted reproductive technology.*

**Рисунок 1.** Протоколы стимуляции при успешных беременностях, выполненные с 2017 по 2021 гг.

***Примечание:*** *КГ – кломифеновая гиперстимуляция; ГнРГ – гонадотропин-рилизинг-гормон; ППСЯ – прогестин-праймированная стимуляция яичников; ВРТ – вспомогательные репродуктивные технологии.*

**Table 1.** Comparison of clomiphene hyperstimulation (CH) method and other protocols.

**Таблица 1.** Сравнение метода кломифеновой гиперстимуляции (КГ) и других протоколов.

| Parameters<br>Показатель | CH group<br>Группа с КГ | Other protocols<br>Прочие протоколы | p |
|---|---|---|---|
| Age, years, M ± SD<br>Возраст, лет, M ± SD | 36.0 ± 5.0 | 36.9 ± 3.7 | 0.48 |
| AMH, ng/ml<br>АМГ, нг/мл | 2.98 ± 2.35 | 4.01 ± 2.81 | 0.15 |
| Chronic disease, n<br>Хронические заболевания, n | 6 | 1 | N/A |
| Number of previous ART, M ± SD<br>Количество предшествовавших ВРТ, M ± SD | 1.40 ± 0.58 | 2.00 ± 1.74 | 0.08 |
| Use of FSH, IU<br>Применение ФСГ, МЕ | 1108 ± 468 | 1756 ± 394 | 0.0001 |
| Premature LH surge<br>Преждевременный подъем ЛГ | 0 | 0 | N/A |
| Number of egg retrieval, M ± SD<br>Количество извлечений яйцеклеток, M ± SD | 6.2 ± 2.9 | 8.5 ± 5.7 | 0.07 |
| Rate of fertilization, %<br>Степень фертилизации, % | 73.1 | 71.7 | 0.88 |
| Number of deliveries out of clinical pregnancy, n<br>Количество родов при клинической беременности, n | 16 | 11 | 0.72 |

***Note:*** *AMH – anti-Mullerian hormone; ART – assisted reproductive technology; FSH – follicle stimulating hormone; LH – luteinizing hormone.*

***Примечание:*** *АМГ – антимюллеров гормон; ВРТ – вспомогательные репродуктивные технологии; ФСГ – фолликулостимулирующий гормон; ЛГ – лютеинизирующий гормон.*

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## Discussion / Обсуждение

Although there are reports indicating the non-inferiority of clomiphene-based stimulation methods to other methods, the importance of this difference remains controversial [9–12]. However, CH is not generally used due to potential luteal phase defects [13], but it is thought to be cost-effective, requiring lower FSH doses or ovulation suppression antagonists, nor affecting frozen embryos or fetuses [14–16]. There were no cases of premature LH surge with CH, and we concluded that clomiphene, with its mild ovulation suppression effect, was sufficient for multiple egg retrievals and protocols; similar results have been reported elsewhere [14]. Recent reports suggest that the agonist method is coupled to the highest risk of ovarian hyperstimulation syndrome (OHSS) and should be avoided; therefore, clomiphene use is reasonable in Japan, where many ART procedures are performed in non-bedded clinics [5]. The use of clomiphene for the treatment of poor responders has the lowest clinical pregnancy rate. As our results showed no significant differences in pregnancy rate or number of eggs retrieved, care should be taken when selecting cases [17].

The advantage of clomiphene-based stimulation is that it can reduce the FSH dose required in the egg retrieval cycle, which is desirable in patients with chronic diseases, such as diabetes, who receive other medications. We have observed many births with clomiphene use in our institution. It may be effective in cases of multiple failed transplants and infertility complications; however, prospective studies with larger sample sizes are required. The CH method has the disadvantage that fresh embryos cannot be transferred due to endometrial thinning. This is not a problem in Japan, where majority of the embryos are frozen using the vitrification method and once thawed are transferred in the next cycle or later [4].

Ovarian stimulation methods are actively being switched to home injections of recombinant FSH products, which in Japan recently become covered by insurance. This partly minimizes a risk of exposure by reducing the number of hospital visits during the COVID-19 epidemic. The hope is that the reduced risk of exposure could potentially lower the number and frequency of egg retrieval appointment cancellations and treatment delays due to COVID-19. A retrospective study at our institution showed no significant differences in the pregnancy rate or number of egg retrievals based on home injections compared to in-hospital methods, such as human menopausal gonadotrophin (hMG), and is expected to become a mainstream method for ovarian stimulation in the future [18].

### Limitations / Ограничения

This study had some limitations. The number of cases was limited because it was designed and conducted as a single-center retrospective study.

## Conclusion / Заключение

In conclusion, since fertility treatment is strongly correlated with the economic, social, and cultural conditions in any country, CH is a suitable protocol for egg retrieval in our country. We will continue to accumulate more cases and investigate an efficacy of clomiphene-mediated hyperstimulation.


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## References / Литература:


1. Ishihara O., Jwa S.C., Kuwahara A. et al. Assisted reproductive technology in Japan: a summary report for 2017 by the Ethics Committee of the Japan Society of Obstetrics and Gynecology. *Reprod Med Biol*. 2019;19(1):3–12. https://doi.org/10.1002/rmb2.12307.
2. Pierce N., Mocanu E. Female age and assisted reproductive technology. *Glob Reprod Health*. 2018;3(2):e9. https://doi.org/10.1097/GRH.0000000000000009.
3. Newman J.E., Paul R.C., Chambers G.M. Assisted reproductive technology in Australia and New Zealand 2018. *Sydney: National Perinatal Epidemiology and Statistics Unit, the University of New South Wales*, 2020. 93 p. Available at: https://npesu.unsw.edu.au/sites/default/files/npesu/surveillances/Assisted%20Reproductive%20Technology%20in%20Australia%20and%20New%20Zealand%202018_0.pdf. [Accessed: 25.02.2023].
4. Kushnir V.A., Barad D.H., Albertini D.F. et al. Systematic review of worldwide trends in assisted reproductive technology 2004-2013. *Reprod Biol Endocrinol*. 2017;15(1):6. https://doi.org/10.1186/s12958-016-0225-2.
5. 2020 Survey and Research Project for the Promotion of Support for Children and Child Rearing: Survey and Research on the Actual Condition of Infertility Treatment. *Japanese Ministry of Health Labour and Welfare report*, 2020. Available at: https://www.mhlw.go.jp/content/000766912.pdf. [Accessed: 25.02.2023].
6. Audibert C., Glass D. A global perspective on assisted reproductive technology fertility treatment: an 8-country fertility specialist survey. *Reprod Biol Endocrinol*. 2015;13:133. https://doi.org/10.1186/s12958-015-0131-z.
7. Kuroda K., Nagai S., Ikemoto Y. et al. Incidences and risk factors of moderate-tosevere ovarian hyperstimulation syndrome and severe hemoperitoneum in 1,435,108 oocyte retrievals. *Reprod Biomed Online*. 2021;42(1):125–32. https://doi.org/10.1016/j.rbmo.2020.09.001.
8. Kuroda K., Katagiri Y., Ishihara O. Optimal individualization of patient-oriented ovarian stimulation in Japanese assisted reproductive technology clinics, a review for unique setting with advanced-age patients. *J Obstet Gynaecol Res*. 2022;48(3):521–32. https://doi.org/10.1111/jog.15150.
9. Gibreel A., Maheshwari A., Bhattacharya S. Clomiphene citrate in combination with gonadotropins for controlled ovarian stimulation in women undergoing in vitro fertilization. *Cochrane Database Syst Rev*. 2012;11:CD008528. https://doi.org/10.1002/14651858.CD008528.pub2.
10. Ochin H., Ma X., Wang L. et al. Low dose clomiphene citrate as a mild stimulation protocol in women with unsuspected poor in vitro fertilization result can generate more oocytes with optimal cumulative pregnancy rate. *J Ovarian Res*. 2018;11(1):37. https://doi.org/10.1186/s13048-018-0408-x.
11. Songa D., Shi Y., Zhong Y. et al. Efficiency of mild ovarian stimulation with clomiphene on poor ovarian responders during IVF/ICSI procedures: a meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2016;204:36–43. https://doi.org/10.1016/j.ejogrb.2016.07.498.
12. Teramoto .S, Kato O. Minimal ovarian stimulation with clomiphene citrate: a large-scale retrospective study. *Reprod Biomed Online*. 2007;15(2):134–48. https://doi.org/10.1016/s1472-6483(10)60701-8.
13. Derksen L., Tournaye H., Stoop D. et al. Impact of clomiphene citrate during ovarian stimulation on the luteal phase after GnRH agonist trigger. *Reprod Biomed Online*. 2014;28(3):359–68. https://doi.org/10.1016/j.rbmo.2013.10.019.
14. Triantafyllidou O., Sigalos G., Gkoles L. et al. The addition of clomiphene citrate to ovarian stimulation protocols for poor responders. *Eur J Obstet Gynecoland Reprod Biol*. 2020;251:136–40. https://doi.org/10.1016/j.ejogrb.2020.05.026.
15. Singh A., Bhandari S., Agrawal P. et al. Use of clomiphene-based stimulation protocol in oocyte donors: a comparative study. *J Hum Reprod Sci*. 2016;9(3):159–63. https://doi.org/10.4103/0974-1208.192054.
16. Muasher S.J., Abdallah R.T., Hubayter Z.R. Optimal stimulation protocols for in vitro fertilization. *Fertil Steril*. 2006;86(2):267–73. https://doi.org/10.1016/j.fertnstert.2005.09.067.
17. Zhang Y., Zhang C., Shu J. et al. Adjuvant treatment strategies in ovarian stimulation for poor responders undergoing IVF: a systematic review and network meta-analysis. *Hum Reprod Update*. 2020;26(2):247–63. https://doi.org/10.1093/humupd/dmz046.
18. Horibe Y., Nakabayashi A., Murata S. et al. COVID-19 infection control protocol and acceptance in an ART in vitro fertilization hospital. *Middle East Fertil Soc J*. 2022:27(1):19. https://doi.org/10.1186/s43043-022-00112-8.


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