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Comparative analysis of the effectiveness of assisted reproductive technology in oncology patients

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ANNOTATION

BACKGROUND: The annual increase in the number of oncology patients and improvements in their quality of life are among the top priorities of modern medicine. Gonadotoxic treatments lead to premature ovarian insufficiency and infertility. To address this issue, various assisted reproductive technologies (ART) have been developed. First-line approaches for fertility preservation involve oocyte and embryo vitrification. Ovarian stimulation and *in vitro* maturation (IVM) of oocytes are used to achieve this goal.

AIM: To assess the effectiveness of different ART methods for preserving reproductive material in oncology patients.

MATERIALS AND METHODS: A prospective study was conducted with 48 women of reproductive age diagnosed with oncological diseases. In the first stage, fertility preservation potential was assessed. After stratifying the patients into groups, ovarian stimulation followed by transvaginal oocyte retrieval was performed, or immature oocytes were obtained for *in vitro* maturation. The collected material was then assessed by an embryologist, who carried out fertilization and vitrification.

RESULTS: The average age of the patients was 33.9±1.7 years, anti-Müllerian hormone levels ranged from 1.26 to 3.02 ng/mL, and the number of antral follicles was approximately 10. In the first group, 256 oocyte-cumulus complexes were retrieved, with 73.0% of them being mature. In the second group, 149 complexes were obtained, with 38.9% suitable for vitrification. Structural abnormalities were more commonly observed in the oocytes matured *in vitro*. The number of embryos obtained was 161 and 78 in the first and second groups, respectively.

CONCLUSION: Fertility preservation in oncology patients remains a critical challenge in modern healthcare. Ovarian stimulation shows high efficiency in obtaining reproductive material. The *in vitro* maturation method should be used only as an alternative to ovarian stimulation or in cases of high ovarian reserve.

Keywords: oncofertility; ovarian stimulation; IVM; assisted reproductive technology; oncology disease.

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Сравнительный анализ эффективности методов вспомогательных репродуктивных технологий у пациенток онкологического профиля

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АННОТАЦИЯ

Обоснование. Ежегодное увеличение количества онкологических больных и улучшение качества их жизни являются одним из приоритетных направлений современной медицины. Гонадотоксичное лечение приводит к развитию преждевременной недостаточности яичников, а также бесплодия. Для преодоления данной проблемы разрабатываются различные методы вспомогательных репродуктивных технологий. Первой линией для сохранения фертильности рекомендовано использовать витрификацию ооцитов и эмбрионов. Для достижения данной цели используются овариальная стимуляция и метод дозревания ооцитов *in vitro*.

Цель. Оценить эффективность различных методик вспомогательных репродуктивных технологий для сохранения репродуктивного материала у пациенток с онкологическими заболеваниями.

Материалы и методы. Проведено проспективное исследование, в которое вошли 48 женщин репродуктивного возраста с онкологическими заболеваниями. На первом этапе оценивали возможность сохранения фертильности, после распределения пациенток на группы проводили овариальную стимуляцию с последующей трансвагинальной пункцией или получали незрелые ооциты с последующим их дозреванием. Затем эмбриолог оценивал весь полученный материал, проводил оплодотворение и витрификацию.

Результаты. Средний возраст пациенток, вошедших в исследование, составил 33,9±1,7 года, уровень антимюллерова гормона колебался от 1,26 до 3,02 нг/мл, количество антральных фолликулов — около 10. В первой группе было получено 256 ооцит-кумулюсных комплексов, из них 73,0% зрелых яйцеклеток; во второй группе — 149, из них 38,9% пригодных для витрификации. Также при дозревании *in vitro* наиболее часто наблюдали аномалии строения полученных клеток. Эмбрионов получено 161 и 78 соответственно.

Заключение. Сохранение фертильности у пациенток с онкологической патологией является актуальной проблемой современного здравоохранения. Овариальная стимуляция демонстрирует высокие показатели эффективности получения репродуктивного материала. Метод дозревания ооцитов *in vitro* стоит применять только как альтернативу овариальной стимуляции или при высоких значениях овариального резерва.

Ключевые слова: онкофертильность; овариальная стимуляция; IVM; вспомогательные репродуктивные технологии; онкологические заболевания.

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肿瘤患者辅助生殖技术方法的比较分析

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摘要

背景。肿瘤患者数量的逐年增加及其生活质量的改善是现代医学的重点方向之一。生殖毒性治疗可导致卵巢早衰及不孕。为解决这一问题，正在开发多种辅助生殖技术方法。卵母细胞和胚胎玻璃化冷冻被推荐作为首选的生育力保存方法。为了实现这一目标，通常使用卵巢刺激和体外卵母细胞成熟技术。

目的。评估不同辅助生殖技术在肿瘤患者生殖材料保存中的有效性。

材料与方法。本研究为一项前瞻性研究，共纳入48名育龄期肿瘤女性。首先评估生育力保存的可能性，随后将患者分组，并分别实施卵巢刺激后经阴道穿刺取卵，或获取未成熟卵母细胞并进行体外成熟处理。随后，胚胎学家评估所有获得的生殖材料，并进行受精和玻璃化冷冻处理。

结果。研究人群的平均年龄为33.9±1.7岁，抗苗勒管激素水平在1.26 – 3.02 ng/mL之间，窦卵泡数约为10个。第一组共获得256个卵母细胞-卵丘复合体，其中73.0%为成熟卵母细胞；第二组获得149个，其中38.9%适用于玻璃化冷冻。此外，在体外成熟过程中，最常观察到所得卵母细胞的结构异常。最终获得的胚胎数量分别为161和78个。

结论。肿瘤患者的生育力保护是当代医疗保健的重要问题。卵巢刺激在获取生殖材料方面具有较高的效率。而体外成熟应作为卵巢刺激的替代方法，仅适用于卵巢储备功能较高的患者。

关键词：肿瘤生育力；卵巢刺激；IVM；辅助生殖技术；肿瘤病。

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BACKGROUND

Improving the quality of life in patients with cancer is one of the most pressing challenges which the society faces today. The number of cancer cases is increasing every year, with approximately 5% of all cases reported in women of childbearing age. According to the Global Cancer Observatory: Cancer Today (GLOBOCAN) statistics [1], 940,667 women under the age of 40 years have cancer. Modern diagnostic and treatment options are improving survival in this patient population. However, treatment is often associated with adverse effects that affect the quality of life, including the development of premature ovarian failure and infertility [2].

Fertility preservation in patients with cancer is an important medical and social need. In recent years, research has focused on developing management strategies for this population in order to benefit from the reproductive potential of these women in the future. A new area of research, oncofertility, has been developed to address this problem by sharing information about the potential of assisted reproductive technology (ART) in women with cancer [3, 4]. Various options for reproductive function preservation are now available, including suppression of ovarian function with gonadotropin-releasing hormone (GnRH) agonists, oocyte and embryo vitrification, ovarian tissue transplantation, and ovarian transposition. Each of these options has its own characteristics and effectiveness, and their use requires a personalized approach [5].

Global clinical protocols recommend oocyte and embryo vitrification as the first-line option for fertility preservation. However, it is important to remember that the effectiveness of ART decreases with age. For example, the pregnancy rate after embryo transfer is 44% at the age of 31–35 years, 23% at the age of 41–42 years, and only 4% at the age of over 43 years [6–8]. Ovarian stimulation and retrieval of immature oocytes for subsequent *in vitro* maturation (IVM) may be used. Ovarian stimulation is not currently contraindicated in patients with cancer. The clinical protocol for the treatment of female infertility (2024) recommends the use of letrozole at 5 mg/day from menstrual days 2–3 for the entire period until the final trigger of oocyte maturation, with possible prolongation in case of high estradiol levels [9]. Any of the ovarian stimulation protocols can be used for this technique. However, Rodgers et al. demonstrated higher efficacy with GnRH antagonists and recombinant human chorionic gonadotropin as the final trigger. The 2020 European Society of Human Reproduction and Embryology (ESHRE) guidelines for fertility preservation in patients with cancer [10, 11] also provide these data.

IVM collects oocyte-cumulus complexes by transvaginal puncture of all visible follicles. However, this technique does not require hormonal stimulation or ovulation trigger [12]. The procedure takes only 48 hours and does not cause ovarian hyperstimulation syndrome, which may affect the course of cancer [13, 14]. Immature oocytes can be retrieved from ovarian tissue samples. However, insufficient data on the use of IVM in women with cancer and the lack of consistent management algorithms for these women require further research into the efficacy and safety of IVM.

Aim

The study aimed to evaluate the efficacy of different ARTs in preserving reproductive material in patients with cancer.

METHODS

This prospective, randomized trial enrolled 48 women of childbearing age with newly diagnosed cancer. The study also included three patients who underwent oophorectomy with immature oocytes retrieved from ovarian tissue.

The study was approved by the Local Ethics Committee (Protocol No. 213, dated 13 December 2021).

In the first phase, after diagnosis, a tumor conference was held to determine further treatment strategies and the need to preserve the patient's reproductive function. Certain techniques were chosen based on the conclusion of a fertility specialist. A key criterion for group assignment was the possibility to perform ovarian stimulation. In case of contraindications or insufficient time for ovarian stimulation, patients were immediately assigned to the second group. The other women were randomized. Figure 1 shows the study design.

Inclusion criteria: age 18–42 years, adequate ovarian reserve (anti-Müllerian hormone >1.2 ng/mL, total antral follicle count >5), and patient desire to preserve reproductive material.

Exclusion criteria: a serious somatic disorder, stage IV cancer, or ovarian cancer.

In the second phase, after further screening and randomization, group 1 patients received stimulation protocols. In most cases, a GnRH antagonist protocol was used. In addition, letrozole was used. A transvaginal puncture was then performed to obtain mature oocytes. Group 2 patients did not receive any additional medication, but immediately underwent transvaginal puncture of all visible follicles. All obtained oocyte-cumulus complexes were transferred to an embryologist to mature.

The third phase was an embryological one. An embryologist evaluated the retrieved oocytes. All immature oocytes were placed in an IVM maturation medium. If a patient had a sexual partner, IVF/ICSI was offered to fertilize the retrieved material. The retrieved material (oocytes and embryos) was then vitrified.

StatSoft STATISTICA 10 was used for statistical analysis of the obtained data.

RESULTS

The study included 48 women of childbearing age with cancer of different sites. Table 1 demonstrates that patients in both groups are comparable in age and ovarian reserve.

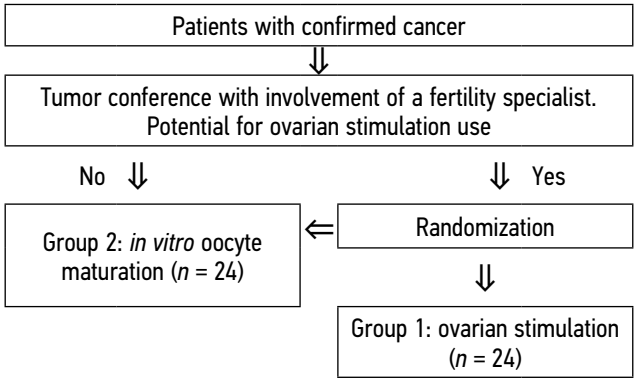

Fig. 1. Study design.

No statistically significant differences were found ($p > 0.05$). Previous pregnancies were observed in <12% of cases, and only 4% of pregnancies resulted in delivery. This confirms the relevance of this area of research.

Breast cancer was the most common cancer (31 patients); 5 women had cervical cancer, 2 had endometrial cancer, and 10 had hematologic cancer (Fig. 2). Gynecological cancers were diagnosed at an early stage and organ-sparing treatment was given to preserve reproductive function for the future.

Ovarian reserve plays an important role in assessing the potential for fertility preservation. In our study, most patients had anti-Müllerian hormone levels <2.0 ng/mL and antral follicle counts <10 in both ovaries. This may be related to the age of the patients; the mean age was 33.2 ± 2.4 years in group 1 and 34.1 ± 1.3 years in group 2.

Ovarian stimulation was performed to retrieve oocytes in group 1. Initially, the procedure did not need to be linked to menstrual function because of the use of a random start protocol, i.e., ovarian stimulation was performed on any day of the cycle. A GnRH antagonist, recombinant follicle stimulating hormone, and human menopausal gonadotropin were used. The average duration of stimulation ranged from 10 to 12 days. GnRH or recombinant human chorionic gonadotropin was used to induce final oocyte maturation. Group 2 received no medication, but underwent an immediate transvaginal puncture (Fig. 3), so the average duration of this oncofertility program was 1–2 days.

The primary aim of our study was to evaluate the efficacy of these techniques for the collection of reproductive material. In group 1, 256 oocyte-cumulus complexes were obtained, with 73.0% of mature oocytes suitable for fertilization. Significantly less material was obtained in group 2: 149 oocyte-cumulus complexes, of which 38.9% were mature. *In vitro* matured oocytes were 5 times more likely to degenerate than oocytes after ovarian stimulation (Table 2). In addition, grading of the collected material showed that the oocytes derived by IVM had more structural abnormalities (Fig. 4).

The oocytes were then fertilized using IVF/ICSI techniques to obtain embryos. Group 1 had the significantly higher percentage of fertilized oocytes (62.9% vs. 52.3%). However, both groups showed comparable grade of blastocysts. The obtained data may indicate a lower fertilization potential of *in vitro* matured oocytes.

DISCUSSION

Current options for cancer diagnosis and treatment provide at least a 75% survival rate. However, in the future, the treatment may negatively affect the patients' quality of life [15]. The phenomenon of postponed maternity, which often leads to the development of cancer before reproduction, should also be considered [16].

A variety of techniques are available for use in fertility preservation programs, and the first-line technique should be oocyte and embryo vitrification. Ovarian stimulation and *in vitro* oocyte maturation can be used for this purpose [17].

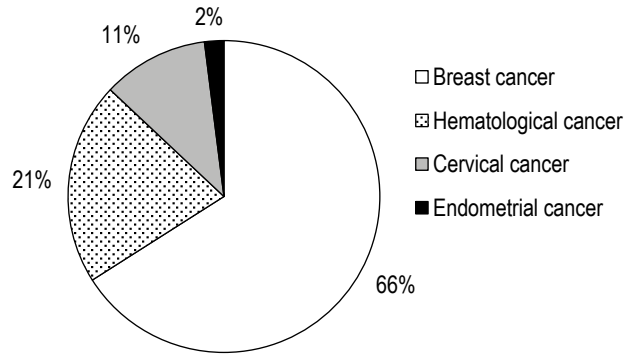

Fig. 2. Structure of oncological pathology.

Table 1. Clinical and medical history characteristics of the studied groups

Criterion	Group 1	Group 2
Age, years	33.2±2.4	34.1±1.3
Anti-Müllerian hormone ng/mL	1.92 [1.32; 3.02]	1.64 [1.26; 2.89]
Total number of antral follicles in both ovaries, *n*	7.8±2.7	8.2±2.9
Menstrual cycle duration, days	28.3±2.1	29.4±1.9
Pregnancies in medical history	3 (12.5%)	2 (8.3%)
Deliveries in medical history	1 (4.2%)	0 (0.0%)

Note. No statistically significant differences were found ($p > 0.05$).

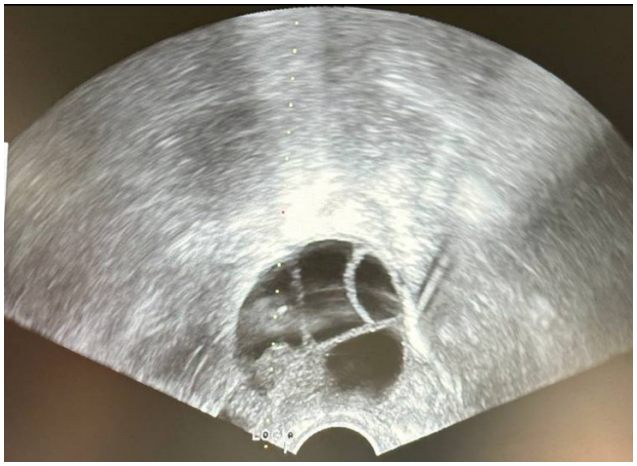

Fig. 3. Ultrasound during transvaginal puncture (dashed line indicates ultrasound guidance).

Initial ovarian reserve is a key factor in choosing a fertility preservation technique. Our study showed low efficacy of IVM due to low anti-Müllerian hormone levels and antral follicle counts. Mostinckx et al., Seok et al. suggested that anti-Müllerian hormone levels >8.5 ng/mL is required for successful reproduction [18, 19]. Ovarian stimulation shows better results even with low levels of anti-Müllerian hormone, in our study it was <1.92 ng/mL.

The key point is to evaluate the amount of retrieved material. IVM is an innovative technique for patients with cancer. For example, Cohen et al. [20] obtained from 2 to 6 mature oocytes in this group of patients, and Krasnopolskaya et al. [6] obtained an average of 5.8 mature oocytes. In our study, the average number of oocytes in metaphase II was 4.00 ± 2.37. These differences may be due to the use of different maturation media, as well as the use of an ovulation trigger or minimal doses of hormonal stimulation before a transvaginal puncture. Ovarian stimulation is more effective. For example, we obtained an average of 6.71 ± 1.92 fertilizable oocytes. Virant-Klun et al. reported this parameter to be 11.0 ± 9.0 [21]. This may be due to our use of letrozole, which, according to some authors, may negatively affect oogenesis. However, there is insufficient data to support this finding.

Creux et al. compared ICSI outcomes by evaluating mature oocytes obtained *in vitro* and *in vivo*. On average, 5 stimulation embryos and 3 IVM embryos were cryopreserved [22]. We also observed a lower fertilization potential in oocytes obtained by maturation (52.3% vs. 62.9%). Therefore, the IVM technique showed lower efficacy compared with ovarian stimulation for fertility preservation in patients with cancer.

CONCLUSION

Fertility preservation in patients with cancer is an extremely urgent medical issue that requires a multidisciplinary approach involving oncologists and fertility specialists. When cancer treatment is needed, a timely and informed decision about preserving reproductive function is important. It can significantly expand a patient's future options, including the potential for motherhood after treatment.

Ovarian stimulation is a well-established method of oocyte retrieval with high success rates. However, it should be noted that this procedure is time-consuming (at least 7 days) and may have contraindications that limit its use in some clinical situations. Therefore, *in vitro* oocyte maturation is a promising alternative for the preservation of reproductive material. However, the efficacy of IVM depends on adequate ovarian reserve, which should be considered when choosing a technique. For patients awaiting oophorectomy for any reason, IVM is the only way to preserve reproductive material.

Table 2. Amount of material obtained in oncofertility programs

Criterion	Group 1	Group 2
Oocyte-cumulus complexes (total number)	256	149*
Blastocele stage	26 (10.1%)	30 (20.1%)
Metaphase I stage	35 (13.6%)	38 (25.5%)
Metaphase II stage	187 (73.0%)	58 (38.9%)
Degenerated	8 (3.1%)	23 (15.4%)
Embryos	161 (62.9%)	78 (52.3%)*

* $p < 0.05$ compared to group 1.

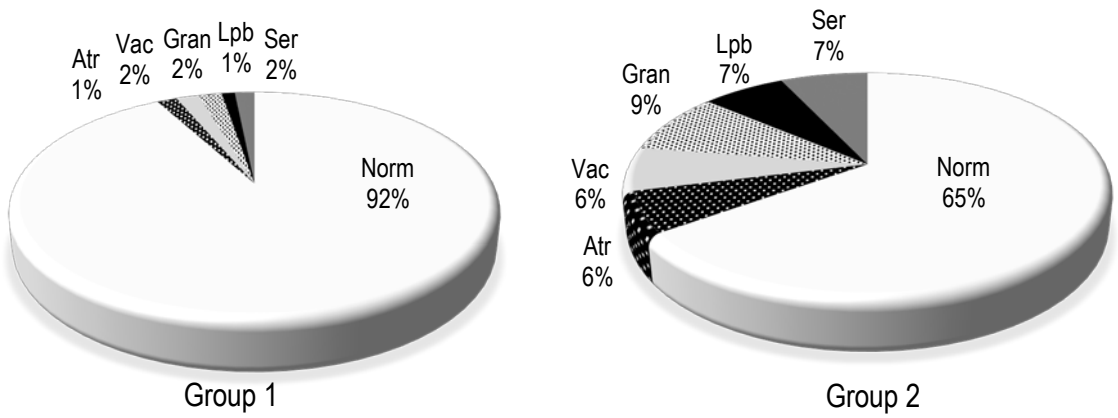

Fig. 4. Oocyte dysmorphisms in the study groups.

Fig. 5. Evaluation of follicular fluid and presence of oocyte-cumulus complexes.

It is possible to perform several cycles of ovarian stimulation or to combine techniques (e.g., IVM combined with ovarian stimulation) to increase the effectiveness of AST. This multidisciplinary approach not only optimizes outcomes, but also significantly increases the chances of successful fertility restoration after cancer treatment.

Therefore, a comprehensive approach to fertility preservation, including active collaboration between oncologists and fertility specialists, is essential for patients to realize their future reproductive potential, which is critical to their quality of life and psychological well-being.

ADDITIONAL INFORMATION

Authors' contribution. Yu.E. Dobrokhotova: approval of the final version of the article; I.A. Lapina: design development of the study, approval of the final version; A.A. Malakhova: design development, preparation and editing of the text; T.G. Chirvon: text editing, literature search; V.M. Gomzikova: collection and analysis of patients with oncological pathology; Yu.A. Sorokin: collection and the analysis of patients with oncological pathology. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work).

Ethics approval. The study was performed within the framework of the dissertation of A.A. Malakhova and was approved by the local ethical committee of N.I. Pirogov Russian National Research Medical University (extract from protocol No. 213 dated 13 December 2021).

Consent for publication. The patients who participated in the study signed an informed consent to participate in the study and publish medical data.

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Disclosure of interest. The authors declares that there are no obvious and potential conflicts of interest associated with the publication of this article.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Ю.Э. Доброхотова — утверждение окончательного варианта статьи; И.А. Лапина — разработка дизайна исследования, утверждение окончательного варианта; А.А. Малахова — разработка дизайна, подготовка и редактирование текса; Т.Г. Чирвон — редактирование текста, поиск литературы; В.М. Гомзикова — сбор и анализ пациенток с онкологической патологией; Ю.А. Сорокин — сбор и анализ пациенток с онкологической патологией. Все авторы подтверждают соответствие своего авторства международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией).

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