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EARLY PREGNANCY LOSS AS AN INDICATION FOR PREIMPLANTATION GENETIC TESTING

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▪ ***Hypothesis/aims of study.*** Approximately 10–15% of clinical pregnancies end in spontaneous abortions. The main cause of early miscarriages is chromosomal aberrations of the embryos. Chromosomal abnormalities are detected in 70% of sporadic miscarriages and in 30–50 % of recurrent miscarriage. Modern assisted reproductive technologies allow not only to treat infertility, but also to provide access to embryos, which makes it possible to test them for hereditary diseases and chromosomal abnormalities before implantation. This study aimed to assess the efficacy of preimplantation genetic testing (PGT) in patients with infertility and early pregnancy loss.

Study design, materials and methods. IVF outcomes were studied retrospectively in 84 patients under the age of 39 years. The first group consisted of 22 women with a normal karyotype, who underwent 34 IVF cycles with PGT for aneuploidies and 22 transfers of euploid embryos. The second group comprised 48 women with a normal karyotype, who underwent IVF treatment without PGT. In this group, we preformed 45 frozen and 18 fresh embryo transfers. The third group included 14 couples with chromosomal structural rearrangements, who underwent 22 IVF cycles with PGT for chromosomal structural rearrangements.

Results. The cumulative pregnancy rate and the birth rate did not significantly differ between the study groups. The early miscarriage rate and the multiple pregnancy rate were significantly lower in groups with PGT compared to the group without PGT. The aneuploidy rate was significantly higher in women with two or more pregnancy losses in history compared to patients with only one pregnancy loss.

Conclusion. The data obtained allow recommending IVF with PGT to women with recurrent pregnancy loss in order to avoid subsequent miscarriage.

▪ **Keywords:** spontaneous abortion; infertility; preimplantation genetic testing; in vitro fertilization; chromosomal structural rearrangements; aneuploidy.

НЕВЫНАШИВАНИЕ БЕРЕМЕННОСТИ КАК ПОКАЗАНИЕ К ПРЕИМПЛАНТАЦИОННОМУ ГЕНЕТИЧЕСКОМУ ТЕСТИРОВАНИЮ

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▪ ***Актуальность.*** Примерно 10–15 % клинических беременностей заканчиваются самопроизвольными выкидышами, главная причина которых, особенно в I триместре беременности, — хромосомные аберрации эмбриона. При спорадических выкидышах хромосомные аномалии выявляют в 70 % случаев, а при привычном невынашивании — в 30–50 %. Современные вспомогательные репродуктивные технологии не только позволяют эффективно лечить бесплодие, но и обеспечивают доступ к эмбрионам, что дает возможность проводить их тестирование с целью диагностики наследственных заболеваний и хромосомных дефектов еще до имплантации.

Цель — оценить эффективность преимплантационного генетического тестирования у пациенток с бесплодием и невынашиванием беременности.

Материалы и методы исследования. Ретроспективно изучены исходы экстракорпорального оплодотворения (ЭКО) у 84 пациенток в возрасте до 39 лет. Первую группу составили 22 женщины с нормальным кариотипом, которым в общей сложности было выполнено 34 цикла ЭКО с преимплантационным генетическим тестированием на анеуплоидии и 22 переноса эуплоидных эмбрионов. Во вторую группу вошли 48 женщин с нормальным кариотипом, получивших лечение методом ЭКО без преимплантационного генетического тестирования. В общей сложности в этой группе проведено 45 переносов размороженных и 18 переносов свежих эмбрионов. В третью группу включили 14 супружеских пар с хромосомными аномалиями, которым проведено 22 цикла ЭКО с преимплантационным генетическим тестированием на хромосомные перестройки.

Результаты исследования. Кумулятивная частота наступления беременности и частота родов достоверно не отличались между группами. Частота прерывания беременности в сроке до 12 нед. и частота многоплодия были достоверно ниже в группах с преимплантационным генетическим тестированием по сравнению с группой без преимплантационного генетического тестирования. Частота анеуплоидии оказалась достоверно выше у пациенток с двумя и более потерями беременности в анамнезе по сравнению с пациентками после одной потери беременности.

Выводы. Полученные данные позволяют рекомендовать ЭКО с преимплантационным генетическим тестированием женщинам с несколькими потерями беременности для снижения риска невынашивания последующей беременности.

▪ **Ключевые слова:** невынашивание беременности; бесплодие; преимплантационное генетическое тестирование; экстракорпоральное оплодотворение; хромосомные перестройки; анеуплодия.

In recent decades, assisted reproductive technologies (ARTs) have become widespread in clinical practice. Modern ARTs are an effective method of not only treating infertility but also obtaining embryos to study them for possible hereditary diseases and chromosomal defects. To date, according to the ART registry of the Russian Association of Human Reproduction (RAHR), more than 100,000 ART cycles per year are performed annually in the Russian Federation. In 2016, a total of 113,976 ART cycles were performed, and 5,222 of them were *in vitro* fertilization (IVF) cycles with preimplantation genetic testing (PGT) [1].

PGT includes a whole range of methods and approaches for obtaining genetic material and highly accurate diagnostics of aneuploidy (PGT-A), structural chromosomal rearrangements (PGT-SR), and monogenic mutations (PGT-M), as well as for human leukocyte antigen typing. From 2011 to 2016, the number of IVF cycles with PGT performed annually in the Russian Federation increased seven times (from 762 to 5,222) [1]. According to the ART register, every fifth IVF cycle is performed together with PGT in the USA [2].

Clinical recommendations for ART, released with the participation of RAHR in 2019, contain the indications for PGT-A, including the woman's age of 35 years and older (with normal somatic karyotype), two or more spontaneous abortions at early terms in history, repeated unsuccessful attempts of transfer of fresh or thawed embryos (three or more in women under 35 years old and two or more for over 35 years of age), and severe disorders of spermatogenesis (oligoasthenoteratozoospermia, severe oligozoospermia, and azoospermia) [3].

Chromosomal aberrations are the main cause of miscarriages, especially under 10 weeks of pregnancy. The frequency of chromosomal aberrations in amblomas in trimester I in the case of natural conception increased from 50 % to 85 % [4, 5].

Cytogenetic studies of amblomas tissue after miscarriages have shown that chromosomal abnormalities occur *de novo* during gametogenesis, fertilization, or embryo development. Most of them are quantitative abnormalities, mainly autosomal trisomies, triploidy or tetraploidy, and monosomy X, while the minor group includes structural chromosomal abnormalities, mosaicism, and other disorders. Autosomal trisomies are most often found on chromosomes 16, 22, 21, 15, 18, and 2 [4–6].

Chromosomal abnormalities are detected in 70 % of cases with sporadic miscarriages, whereas they are revealed in 30%–50% of cases with recurrent miscarriage [4]. In approximately 2 %–4 % of cases, one of the partners in a couple with recurrent miscarriage has structural chromosomal rearrangements. The most common of them are balanced translocations (52 %) and inversions (26 %), which are more common among men than women. Mosaicism is registered in approximately 21 % of cases and more often in women. In carriers of balanced chromosomal rearrangements, 50%–70 % of gametes and, accordingly, embryos are aneuploid because of discrepancy errors during meiosis [4].

It was established that the risk of miscarriage after IVF, including using the method of intracytoplasmic sperm injection (ICSI), does not differ from that during natural conception [5].

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Wu et al. [5] performed a chromosome microarray analysis of 560 samples of chorionic villi after a spontaneous miscarriage and did not find significant differences in the frequency of aneuploidy for individual chromosomes in the natural conception, IVF, and ICSI groups. The frequency of chromosomal abnormalities and aneuploidy on one chromosome increased with the age of the mother [5].

The effect of embryo quality and maternal age on the risk of pregnancy loss was reported by Hourvitz et al. [7]. In their study, from 2000 to 2004, 2,902 embryo transfers at the cleavage stage resulted in 816 pregnancies. The average pregnancy loss rate was quite high (32%), while for women under 35 years old, it was 28%, and in those over 35 years old, it was 38%. The risk of miscarriage was higher with the transfer of embryos having five blastomeres and less on the third day of development, compared with embryos having more than five blastomeres.

Currently, most IVF clinics practice embryo transfer at the blastocyst stage (days 5 to 6 of development) [1, 2]. Wang et al. [8] analyzed 509,938 IVF cycles with the transfer of fresh and thawed embryos conducted from 2004 to 2013 and revealed that the risk of losing pregnancy in trimester I is higher when transferring embryos at the cleavage stage compared with at the blastocyst stage, as well as in transfer of thawed embryos compared with that of fresh ones.

In a study of risk factors for pregnancy loss after IVF, performed by Chinese authors based on a retrospective analysis of 5,485 pregnancies resulted after the IVF program until 2015, it was shown that the total frequency of miscarriage after IVF amounted to 12.5 %, with 67 % of losses occurred in trimester I of pregnancy. The risk of miscarriage increased significantly with age (1.6 and 4.1 times in the groups of women aged 36–40 years and older than 40 years, respectively, compared with women under 35 years old) and was higher in overweight women, with ovarian stimulation using antagonists of gonadotropin-releasing hormone (GnRH), minimal stimulation, and in transfer of thawed embryos [9].

In a retrospective study, Murugappan et al. [10] found that the cumulative frequency of pregnancy and the birth rate after 6 months of treatment or monitoring do not differ in groups of women with recurrent miscarriage who underwent IVF with PGT (n = 112) and who had recurrent miscarriage after conception in a natural way (n = 188).

Shahine et al. [11] examined the frequency of aneuploidy in blastocysts in 239 patients with recurrent miscarriage and found that, regardless of age, in women with reduced ovarian reserve, their rate was significantly higher than in patients with normal ovarian reserve.

Kort et al. [12] studied the incidence of aneuploidy among blastocysts obtained from fertile and infertile wedded couples. A trophectoderm biopsy was performed on 18,387 embryos in 3,378 IVF/PGT cycles. Among women of the same age, the risk of aneuploidy was higher in patients with miscarriage (risk coefficient 1,330, $p < 0.001$), with aneuploid pregnancy in history (risk coefficient 1,439, $p < 0,001$), and with an unsuccessful attempt of IVF in history (risk coefficient 1,356, $p = 0.0012$) compared with fertile women. The authors concluded that patients with miscarriage, regardless of age, have an increased risk of aneuploid embryos.

Thus, studies by various authors confirm the leading role of chromosomal abnormalities in miscarriage, especially in early terms. Using this mechanism (abortion), *in vivo* embryos with an abnormal chromosome complement are eliminated. A similar mechanism works for pregnancies achieved with IVF. There is a reason to expect that PGT-A of embryos obtained in the IVF program can significantly reduce the likelihood of an undeveloped pregnancy and the risk of spontaneous miscarriage up to 12 weeks since embryos with known chromosomal abnormalities are not transferred to the uterine cavity.

This study aimed to evaluate the efficiency of PGT in patients with infertility and miscarriage.

Material and methods

From January 2012 to December 2018, the FertiMed Center for Reproduction and Genetics conducted 4,140 IVF/ICSI programs in patients with infertility of various origins. A total of 4,418 transfers were performed (2,600 fresh embryos and 1,995 thawed ones). In 450 cycles, PGT-A, PGT-M, and PGT-SR were performed. A total of 1,875 pregnancies occurred, 236 pregnancies of which were terminated in the period up to 12 weeks (12.6%), and miscarriage occurred in 41 cases in the period of up to 20 weeks.

A retrospective study of IVF outcomes was performed in 84 patients under the age of 39 years with infertility and miscarriage (at least one pregnancy loss up to 12 weeks in history).

Before going to the clinic, 36 patients previously had between one and four spontaneous pregnancy losses, and 48 patients were included in the study after a miscarriage or an undeveloped pregnancy

that occurred as a result of IVF/ICSI treatment in our clinic. An age limit was established to exclude the effect of late reproductive age on IVF program results.

Before the start of the IVF program, all patients and their partners underwent an examination under Order of the Ministry of Health No. 107n "On the Procedure for Using Assisted Reproductive Technologies, Contraindications and Restrictions on Their Use." There were no contraindications to participation in ART.

To diagnose the cause of miscarriage, karyotyping was additionally prescribed to both spouses, and women underwent a study for thrombophilia, hormonal examination, and genital tract infection screening.

Carriage of chromosomal structural rearrangements was detected in 14 couples. According to the results of other studies, no significant deviations from the norm were revealed.

Hysteroscopy with endometrial biopsy was performed to 12 patients, one of them had an endometrial polyp, and three had chronic endometritis.

PGT with frozen embryos remaining after the IVF program or a subsequent IVF attempt with PGT of fresh embryos was offered to all the patients. Depending on the chosen treatment approach, all patients were divided into three groups.

Group 1 consisted of 22 women with a normal karyotype, who had a total of 34 cycles of IVF with PGT-A and 22 transfers of euploid embryos in history.

Group 2 included 48 women with a normal karyotype who continued treatment with IVF without PGT. In total, 45 transfers of thawed embryos and 18 transfers of fresh embryos were performed to them.

Group 3 included 14 couples with karyotype abnormalities, who underwent 22 cycles of IVF with PGT-SR. The ovaries were stimulated with preparations of recombinant follicle-stimulating hormone (Gonal F and Puregon), urinary gonadotropins (Menopur, Merional, and Meriofert), or a combination of recombinant follicle-stimulating hormone and recombinant luteinizing hormone (Pergoveris) according to the long protocol with the GnRH agonist (decapeptyl) or the protocol with the GnRH antagonist (Cetrotide).

Ovarian puncture was performed under intravenous anesthesia according to the standard technique. All mature oocytes obtained after aspiration were fertilized using ICSI. Embryos were cultured in plate incubators using LIFEGLOBAL LGGG-020 culture medium according to the manufacturer's recommendations.

Material for PGT was obtained on day 5 or 6 of embryo development by laser biopsy of the embryos trophectoderm at the blastocyst stage. The blastocysts of only good and excellent quality were tested (grades AA, AB, and BB). After a biopsy, freezing of each blastocyst was performed on a separate carrier.

DNA was extracted from the obtained biopsy samples using the method of whole genome amplification, which was then analyzed using array comparative genomic hybridization or next-generation sequencing.

Thawed embryos were transferred into the uterine cavity in the next menstrual cycle after IVF/PGT on day 5 or 6 after ovulation diagnosed by ultrasound. After ovulation, all patients received gestagens (Utrogestan or Krinon) to support pregnancy.

The main primary outcome of the study was the rate of progressing pregnancy. We also analyzed the incidence of embryo aneuploidy in groups 1 and 3 and estimated the frequency of pregnancy loss, the frequency of multifetal pregnancy, and the period from the start of treatment to the onset of progressing (more than 10 weeks) pregnancy in all groups.

Statistical processing of the results was performed using standard software packages for applied statistical analysis (Statistica for Windows v. 10.0, Microsoft Excel). The parameters of the sign distribution in the sample were evaluated using the Shapiro–Wilk test. Normal distribution data were described using the mean and error of the mean and nonparametric data using the median and quantiles. The Student *t*-test was used to assess the intergroup differences of values with a normal distribution and the Mann–Whitney criterion in the case of distribution other than normal. The critical level of significance of the null statistical hypothesis was equal to 0.05.

Results

The average age of patients, parity, and the average number of pregnancy terminations in history did not significantly differ between groups. Patients who refused from PGT and continued treatment with the IVF method made, on average, one attempt more than patients who underwent PGT.

In group 1, 34 cycles of IVF with PGT (from 1 to 5) were performed to 22 patients in the "fresh" protocol (30) or with frozen and thawed embryos

(4). A total of 97 embryos were tested, 53 of which had a normal chromosome complement (55%), and 44 (45%) were aneuploid.

Among 44 embryos with aneuploidy, monosomy on one or more chromosomes (16/44, 36%) prevailed, as well as trisomy (10/44, 23%), combined karyotype abnormalities (8/44, 18%), and segmental deletions or duplication of chromosomes (10/44, 23%). Two patients became pregnant on their own before embryo transfer, and both pregnancies progressed; in two patients, according to the results of three IVF attempts, euploid embryos were not detected.

The remaining 18 patients underwent a total of 22 embryo transfers; 14 pregnancies occurred, and the development of one of them stopped at 9 weeks. Six of the remaining pregnancies progressed, and seven resulted in timely delivery (38–40 weeks). Seven full-term babies were born (four boys and three girls) without visible malformations. In group 2, 48 patients underwent IVF without PGT. Thawed embryos were transferred in the natural cycle to 40 women, and 27 pregnancies occurred: 4 of them did not progress, 18 resulted in delivery, and 5 are progressing. Another 14 women underwent one to three repeated IVF attempts and 18 transfers of fresh (12) and thawed (6) embryos, including one IVF with donor oocytes. There were 12 pregnancies: two of them stopped developing at 6 and 8 weeks, while the remaining 10 ended in delivery at term.

In total, as a result of 63 transfers of fresh and thawed embryos, 39 pregnancies occurred: 6 were terminated in periods of 6–9 weeks, 28 ended in childbirth in the term of 38–40 weeks, and 5 are progressing.

Thus, the cumulative pregnancy rate and the birth rate did not significantly differ between the groups.

Statistical analysis of the data revealed that the groups studied did not differ in such parameters as the frequency of clinical and progressive pregnancy. In this case, the frequency of abortion up to 12 weeks and the incidence of multifetal pregnancy were significantly lower in groups with PGT compared with the group without PGT (Table 1).

An analysis of the outcomes of IVF treatment with PGT in the subgroups of women who had a history of only one episode of pregnancy loss and women with recurrent miscarriage revealed significant differences in the incidence of embryo aneuploidy (Table 2). The results of IVF with PGT in 14 married couples with chromosomal rearrangements, making up group 3, are presented in Table 3. In total, 22 IVF programs with PGT were completed in this group. A total of 61 blastocysts were tested, 37 of which were euploid (61%). In five programs, no euploid embryos were detected, and therefore, embryo transfer was not performed in two couples. In total, 14 transfers were performed in 12 women (no more than one euploid embryo was transferred). Ten singleton pregnancies occurred: eight of them ended in delivery at term, and two are progressing.

Table 1 / Таблица 1

Treatment outcomes in the study groups
Исходы лечения в исследуемых группах

Patient characteristics	Group 1 of IVF with PGT-A (*n* = 22)	Group 2 of IVF without PGT (*n* = 48)	Group 3 of IVF with PGT-SR (*n* = 14)
Average age, years	33,4	31,7	32,1
Average number of miscarriages in history	2	1	1
Number of IVF attempts (median)	1	2*	1
Incidence of aneuploidy in embryos	45 % (44/97)	Нет данных	39 % (24/61)
Total number of embryo transfers	22	63	13
Frequency of pregnancy per transfer	64 % (14/22)	62 % (39/63)	71 % (10/14)
Multifetal pregnancy rate	0	13 % (5/39)*	0
Pregnancy termination rate	7 % (1/14)	15 % (6/39)*	0
Frequency of progressing pregnancy per transfer	59 %	52 %	71 %
Frequency of progressing pregnancy per patient	72 % (13/18)	69 % (33/48)	83 % (10/12)

IVF with PGT-A, in vitro fertilization with preimplantation genetic testing for aneuploidy; IVF without PGT, in vitro fertilization without preimplantation genetic testing; IVF with PGT-SR, in vitro fertilization with preimplantation genetic testing for chromosomal rearrangements.
* Differences are significant.

Discussion

The problem of determining indications for PGT in a group of women with miscarriage is currently widely discussed in the scientific literature and at international conferences. It was revealed that up to 70% of pregnancy losses at a period of up to 12 weeks occur because of chromosomal abnormalities in embryos. The mechanisms of the formation of aneuploidy in oocytes of women of older reproductive age have been described in detail. At that, disorders in the disjunction of sister chromatids in some women can be genetically determined and cause recurrent miscarriage. It is known that in married couples with chromosomal rearrangements, the probability of losing pregnancy at early terms reaches 50%. Each case of spontaneous abortion leads to the psychological trauma of the spouses and can be accompanied by medical complications, the most common of which is the inflammatory process with the formation of intrauterine synechias, endometrial hypoplasia, and chronic uterine inflammation. Surgical removal of the remains of the fetal egg, like any other intrauterine intervention, can lead to the inflammatory process of the pelvic organs and provoke secondary infertility. PGT avoids psychological trauma associated with the loss of pregnancy, as well as complications after abortion.

The disadvantages of PGT include significant financial costs. In some patients, despite several IVF programs with PGT, all embryos received carry chromosomal abnormalities. In such a situation, the only way out for the couple is the oocyte donation program or the use of donor sperm. The problem of embryonic cell mosaicism, which, according to various authors, occurs in 10 %–20 % of cases, has still not been resolved. When testing for aneuploidy in a mosaic embryo, there is a chance of getting a false-positive or false-negative result. In doubtful cases, the embryo may be subjected to repeated biopsy to analyze an additional cohort of trophectoderm cells. The results of the study revealed that IVF with PGT does not increase the likelihood of pregnancy in married couples with a single history of pregnancy loss but reduces significantly the risk of spontaneous abortion. In addition, the high pregnancy rate after transferring a single euploid embryo enables to avoid the approach of transferring two embryos within IVF programs, which reduces significantly the risk of multifetal pregnancy in such patients.

PGT enables to achieve high frequency of pregnancy progression in couples in which one of the spouses carries chromosomal rearrangement, which increases the risk of miscarriage. The frequency of a progressive pregnancy per female patient in this group amounted to 83 %; all pregnancies that have occurred are progressing or terminated with delivery at term.

When analyzing a cohort of young women with a history of two or more pregnancy losses, we revealed a higher incidence of embryo aneuploidy compared with female patients who had no more than one episode of pregnancy loss (52 % vs. 2 8 %, respectively). This phenomenon

Table 2 / Таблица 2

Treatment outcomes in subgroups with a different history of miscarriages
Исходы лечения в подгруппах с разным количеством выкидышей в анамнезе

Patient characteristics	Subgroup 1A IVF with PGT-A in women with a single loss in history (*n* = 10)	Subgroup 1B IVF with PGT-A in women with two or more loss in history (*n* = 12)
Average age	34,4	34,2
Average number of miscarriages in history	1	2,5
Number of IVF attempts	18	16
Incidence of aneuploidy in embryos	28 % (9/36)	52 % (26/50)
Total number of embryo transfers	13	9
Frequency of pregnancy per transfer	54 % (7/13)	78 % (7/9)
Pregnancy termination rate	0	1/7
Frequency of progressing pregnancy per transfer	54 % (7/13)	67 % (6/9)
Frequency of progressing pregnancy per patient	70 % (7/10)	74 % (6/8)

Note. IVF with PGT-A, in vitro fertilization with preimplantation genetic testing for aneuploidy.

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Table 3 / Таблица 3

Treatment outcomes in the group with karyotype abnormalities
Исходы лечения в группе с аномалиями кариотипа

Patient number	Type of chromosomal abnormality	Number of losses in history	Share of euploid embryos	Treatment outcome
1	46,XX,der(13;14)(q10;q10)	2	58 % (7/12)	3 IVF, 2 transfers, childbirth
2	46,XY,der(13;14)(q10;q10)	1	50 % (3/6)	2 IVF, 2 transfers, 1 childbirth
3	46,XY,t(2;15)(g21;g22)	2	33 % (1/3)	2 IVF, 1 transfer, pregnancy did not occur
4	46,XY,t(1;2)(p32;q37)	1	44 % (4/9)	2 IVF, 1 transfer, childbirth
5	45,X/46,XX	2	100 % (4/4)	1 IVF, 1 transfer, childbirth
6	45,XY(13;14)(q10;q10)P	1	100 % (4/4)	1 IVF, 1 transfer, childbirth
7	47,XY,+mar	1	50 % (1/2)	1 IVF, 1 transfer, childbirth
8	46,XX,t(4;9)(p15.2;p13)	1	33 % (2/6)	2 IVF, 1 transfer, childbirth
9	46,XY,inv(10)(q21;q24)	2	66 % (4/6)	1 IVF, 1 transfer, childbirth
10	46,XX,t(2;13)(q34;q34)	1	0 % (0/2)	1 IVF, no euploid embryos
11	46,XY,t(4;13)(q24;q14)	1	25 % (1/4)	2 IVF, 1 transfer, pregnancy progresses
12	46,XX,der(13;14)(q10;q10)	1	17 % (1/6)	2 IVF, 1 transfer, pregnancy did not occur
13	46,XY,inv(9)(p13;p22)	1	75 % (3/4)	1 IVF, 1 transfer, pregnancy progresses
14	46,XX,t(11;22)(q23.3;q11.2)	4	0 % (0/3)	1 IVF, transfer was cancelled

can be explained by a genetic predisposition to disorders of a meiotic division of oocytes, which manifests itself regardless of the woman's age and leads to a high frequency of oocyte aneuploidy.

The data obtained enable to recommend IVF with PGT to women with several pregnancy losses to reduce the risk of loss of future pregnancy.

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