The GnRH Antagonist Protocol is Associated with a Higher Embryo Aneuploidy Rate Than the GnRH Agonist Long Protocol in Chinese Women

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Research

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Abstract

Background: Studies in oocytes have suggested increased aneuploidy rates after ovulation induction in mammals and humans. Conversely, some studies have shown that ovarian stimulation does not significantly increase the embryo aneuploidy rate in humans compared with an unstimulated cycle. In addition, the potential effect of the gonadotropin-releasing hormone (GnRH) antagonist (GnRH-ant) protocol and GnRH agonist (GnRH-a) long protocol on embryo aneuploidy remains unknown.

Methods: This is the retrospective cohort study from university-affiliated fertility center. In total, 578 early miscarriage patients who conceived through IVF/intracytoplasmic sperm injection (ICSI) after receiving the gonadotropin-releasing hormone (GnRH) antagonist (GnRH-ant) protocol or the GnRH agonist (GnRH-a) long protocol were analyzed to compare the aneuploidy rates in early aborted tissues. In addition, a total of 466 preimplantation genetic testing for aneuploidy (PGT-A) cycles undergoing GnRH-ant protocol or GnRH-a long protocol were also analyzed to compare the aneuploidy rates in embryo.

Results: For early miscarriage patients who conceived through IVF/ICSI, compared to the GnRH-a long protocol group, the GnRH-ant protocol group had a significantly higher rate of aneuploidy in early aborted tissues (48.70% vs. 64.52%), and increased aneuploidy was associated with a significantly higher incidence of trisomy 13, 18, and 21 ($p<0.01$). Regarding PGT-A cycles, compared to the GnRH-a long protocol group, the rate of embryo aneuploidy was also significantly higher in the GnRH-ant protocol group (48.01% vs. 58%). After stratification and multiple linear regression, the GnRH-ant regimen remained significantly associated with an increased risk of aneuploidy in early aborted tissues and embryos [OR (95% CI) 1.767 (1.174, 2.661), OR (95% CI) 1.465 (1.020, 2.102)]. Furthermore, the embryo aneuploidy rate in the GnRH-ant protocol group was significantly higher than that in the GnRH-a long protocol group but only in young and normal ovarian responders [OR (95% CI) 3.54 (1.48, 8.46)].

Conclusions: The GnRH-ant protocol is associated with a higher aneuploidy rate in early aborted tissues and embryos than the GnRH-a long protocol in Chinese women. A multicenter, randomized controlled trial would be the optimal strategy to confirm these results.

Background

Aneuploidy is one of the most detrimental factors causing failed implantation, miscarriage, and disordered embryo development [1]. The vast majority of human aneuploidies arise from errors in the first meiotic division of the oocyte, which is initiated prenatally and is not complete until ovulation [2]. Recent studies have confirmed that both young and older women have high rates of aneuploidy [1, 3]. However, the proportions of human preimplantation embryos with aneuploidy are increased in patients of advanced maternal age [4, 5]. In addition to maternal age, chromosome abnormalities in embryos may be induced by factors involved in assisted reproductive technology (ART), including embryo culture conditions [6]. Ovarian stimulation plays a crucial role in ART. The goal of ovarian stimulation is to induce ongoing development of multiple dominant follicles and obtain many mature oocytes to improve the

chances of conception [7]. However, the potential deleterious effect of ovarian stimulation on oocyte and embryo euploidy is still the subject of lively debate. In 2020, Gang Li et al. confirmed that the long-acting gonadotropin-releasing hormone (GnRH)-a protocol after follicular phase (FP) stimulation resulted in a lower aneuploidy blastocyst rate than the short-acting GnRH agonist (GnRH-a) protocol after luteal phase (LP) stimulation [8]. It was also found the duration of the ovarian stimulation treatment was correlated with the aneuploidy rate: patients requiring more days of stimulation presented a lower rate of aneuploid embryos[9]. However, Filippo Maria Ubaldi et al. [10] demonstrated no differences in the aneuploidy blastocyst rate after FP and LP stimulations using follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in combination with the GnRH-ant in patients with reduced ovarian reserve.

The GnRH agonist and GnRH-ant protocols are well-established methods for controlled ovarian hyperstimulation among patients who are receiving assisted reproductive technology (ART) [11]. The GnRH-a protocol was developed in the 1980s and is used to prevent premature LH outpouring, thereby increasing the number of retrieved oocytes and pregnancy rates. The GnRH-a protocol has become the gold standard for IVF. The GnRH-ant protocol was introduced in the 1990s; it can competitively block GnRH receptors and cause a rapid suppression of Gn release. The GnRH-ant protocol results in a lower risk of severe ovarian hyperstimulation syndrome (OHSS) and is more convenient for patients because of the shorter duration of Gn administration and lower Gn dose [11, 12].

Current reports are not consistent in comparing the clinical outcomes of the GnRH-a and GnRH-ant protocols. Wang et al. [12] and Xiao et al. [13] showed that compared with the standard long GnRH-a protocol, the GnRH-ant protocol resulted in similar ongoing pregnancy and live birth rates. Yang et al. [11] revealed that the long-acting GnRH-a protocol was beneficial in improving the clinical pregnancy rate and live birth rate compared with the GnRH Antagonist Protocol. Moreover, all studies reported that the incidence of OHSS was significantly lower in women receiving the GnRH-ant protocol. Accounting for various patient populations, C.B. Lambalk et al. [14] systematically reviewed the GnRH-ant and GnRH-a long protocols in IVF. This meta-analysis confirmed that compared to the GnRH-a long protocol, the GnRH-ant protocol was associated with lower ongoing pregnancy rates in a general IVF population but did not seem to compromise ongoing pregnancy rates in individuals with polycystic ovarian syndrome (PCOS) and poor responders. Studies also suggest that embryos conceived after GnRH antagonists may have higher early pregnancy loss rates [15–17]. Furthermore, the Chinese birth cohort study on ART and birth defects published in *The Lancet Regional Health, 2021*, showed that the GnRH-ant regimen for ovulation induction in women was associated with an increased risk of birth defects in their offspring [18]. It is evident that aneuploidy is a major factor that results in pregnancy loss and birth defects [19–21]. However, to date, no study has compared the effects of the GnRH-ant protocol and GnRH-a long protocol on embryo aneuploidy.

In the present study, we aimed to analyze whether women who received the GnRH-ant protocol for ovarian stimulation generate different aneuploid embryos than those who received the GnRH-a long protocol. We also elucidated the aneuploidy rate in early aborted tissues after receiving the GnRH-ant or GnRH-a long

protocol. These findings help to reveal the different effects of the GnRH-ant and GnRH-a long protocols on embryo aneuploidy and provide guidance for clinical treatment.

Methods

Inclusion and Exclusion Criteria

This was a retrospective study to investigate the effect of ovarian stimulation on embryo aneuploidy. The data were collected at a single tertiary reproductive medical center between January 2017 and December 2020. Patients with multiple pregnancies, second trimester abortions, unexplained infertility, and basic data deficiency were excluded. In total, 578 patients with a singleton pregnancy and early miscarriage (gestational age 6-12 wk) undergoing IVF/ICSI were included. Patients with the chromosomal balanced structural abnormity who underwent preimplantation genetic testing for chromosomal structural rearrangement (PGT-SR) were also excluded. In total, 1912 embryos from 466 cycles undergoing preimplantation genetic testing for aneuploidy (PGT-A) were included. The GnRH-a long protocol or the GnRH-ant protocol was used for ovarian stimulation. A flow chart of the data processing procedure is provided in *Supplemental Figure 1.*

This study was approved by the Ethics Committee of the Tang Du Hospital of the Air Force Military Medical University, China (K202108-09).

Clinical Characteristics

Baseline demographic parameters, including age (in years, y) of the female and male subjects, body mass index (BMI) (kg/m^2), infertility factors, and infertility diagnosis, were collected. Baseline IVF-specific data, including the basal plasma FSH level (mIU/mL), basal LH level (mIU/mL) and basal estradiol (E2) level (pg/mL), all measured on menstrual cycle Day 2 or Day 3, were also collected. Anti-Mullerian hormone (AMH) levels (ng/mL) measured on any day of the menstrual cycle were also documented. COS parameters documented for all patients included the ovulation-inducing scheme, duration of ovarian stimulation (days), and total dose of gonadotropins (IU). The chromosome aneuploidy of embryo or early aborted tissues after the next-generation sequencing (NGS) diagnosis.

COS Protocols and Oocyte Retrieval

For the GnRH-a long protocol, subcutaneous injection of 0.05 mg-0.1 mg triptorelin acetate for injection (IPSEN) was started between the 18th and 21st day of the menstrual cycle before the IVF/ICSI cycle and was continued for 12-14 days. Down-regulation was confirmed by a linear endometrium, as observed by ultrasonography, and suppressed ovaries, as indicated by a concentration of serum estradiol < 50 pg/ml. Recombinant Human Follitropin Alfa for Injection (rFSH, Merck Serono) stimulation was commenced with a dose of 50 IU-300 IU from the 2nd to 5th days in the menstrual period in the IVF/ICSI treatment cycle and was continued until oocyte retrieval.

For the GnRH-ant protocol, the initial dose of rFSH ranged from 112.5 to 200 IU per day on day 2 or day 3 of the menstrual cycle. Gonadotropin-releasing-hormone antagonist (Cetrorelix acetate, Baxter Oncology GmbH), at a daily dose of 250 μg, was started after 4-5 days of rFSH initiation until the day of hCG administration.

For both protocols, the initial dose of rFSH was based on age, antral follicle count, and basal FSH levels, and the ovarian response was monitored through serum sex steroids and serial transvaginal ultrasound examinations during stimulation. Gonadotrophin doses were adjusted when needed. Human menopausal gonadotropin or recombinant Human Lutropin Alfa for Injection (Merck Serono) could be added. Urinary human chorionic gonadotropin was administered at a dose of 5000 to 10000 IU to induce oocyte maturation when two or more follicles measured 17 mm or more. Oocyte retrieval was performed 34 to 36 hours later.

IVF and Embryo Culture

Fertilization was performed by IVF, intracytoplasmic sperm injection (ICSI) or a combination of IVF and ICSI, either by an active choice of half IVF and half ICSI or passive use of rescue ICSI; the latter was applied when conventional IVF failure was confirmed by the fact that two polar bodies could not be observed 6 hours after insemination. All normally fertilized embryos were cultured in culture media, and the culture fluid was replaced on the third day of embryo culture. Embryo vitrification and warming were performed in accordance with the method reported by Ozgur and colleagues [22].

Biopsy and PGT

In PGT patients, embryo biopsy was performed by removing five to eight trophectoderm cells from day 5 or day 6 fully expanded blastocysts through a small opening in the zona pellucida, which was created by laser, with a pipette. The biopsied trophoblasts were lysed, and DNA was extracted and amplified by the SurePlex DNA amplification system (SurePlex; Illumina, USA). Chromosomal aneuploidy screening was performed using NGS-based technology with Preimplantation Chromosomal Aneuploidy Detection Kit-Semiconductor Sequencing (Suzhou Basecare Medical Co., Ltd., China). Each sample met the 0.1-fold average sequencing depth.

Acquisition of and Testing Early Aborted Tissues

The early aborted tissues of early miscarriage patients were rinsed with 0.9% saline solution, and then DNA was extracted from 0.5 cm^3 sections with a QIAamp DNA Mini Kit (Qiagen, Germany). Chromosomal aneuploidy screening was performed using a MiSeq® Dx Reagent Kit V3 (Illumina, USA), and each sample met the 0.01-fold average sequencing depth.

Statistical Analysis

Data processing and statistical analysis were performed using EmpowerStats software (www.empowerstats.com) and the statistical software package R.

Categorical variables are expressed as the number (n) and percentage (%), and continuous variables are expressed as the mean ± standard deviation (SD). Normality was assessed through the Shapiro-Wilk normality test in addition to visual inspection of the distribution. Subject characteristics in the GnRH-a long protocol group and the GnRH-ant protocol group are described separately. Student's t-test was applied for the primary comparison between the two groups. To assess the odds ratio (OR) of aneuploidy in the two groups, a multivariable regression model was established with potential confounding factors as the variables and adjusted for female age, female BMI, male age, infertility diagnosis, infertility factors, basal plasma levels of FSH, LH and E2, menstrual cycle Day 2 or Day 3, AMH, duration of ovarian stimulation, and total dose of gonadotropins. Stratified analysis by AMH (≤1.3 ng/mL, >1.3 ng/mL≤3.36 ng/mL, >3.36 ng/mL) and female age (<35 y, ≥35 y) was also conducted to assess the primary outcome of embryo aneuploidy in PGT patients between the two COS groups. Differential types of chromosome aneuploidy in early aborted tissues from the two COS groups were examined using chi-square analysis.

Results

Studies have suggested that embryos conceived after GnRH antagonists may have higher early pregnancy loss rates. In the precent study, we first compared the abortion rates of GnRH-a long protocol or GnRH-ant protocol. In the multivariable model, when maternal age, paternal age and potential influencing factors, including the basal FSH level, infertility diagnosis, basal E2 level, infertility factors, total dose of Gn and duration of Gn, were considered, the GnRH-ant regimen remained significantly associated with an increased risk of non-pregnant and miscarriage [(OR 1.16, 95%CI 1.04-1.29, P=0.0094) and (OR 1.40, 95%CI 1.11-1.77, P=0.0044)] (*Supplemental Table 1*).

Effect of the Ovarian Stimulation Protocol on the Rate of Aneuploidy in Early Aborted Tissues in Patients Receiving IVF/ICSI

Baseline Characteristics of Early Miscarriage Patients

In total, 578 early miscarriage patients conceived through IVF/ICSI, and the demographics and clinical characteristics of those who received the GnRH-ant and GnRH-a long protocols were compared. A total of 423 patients in the GnRH-a long protocol group and 155 patients in the GnRH-ant protocol were analyzed. The baseline characteristics of the patients are presented in *Supplemental Table 2*. As shown in Supplemental Table 2, compared to the GnRH-a long protocol group, the maternal and paternal ages of those in the GnRH-ant protocol group were significantly higher (31.51 ± 4.31 vs. 32.95 ± 4.40) (32.97 ± 5.13 vs. 34.30 ± 5.71). The rate of aneuploidy in early aborted tissues in patients in the GnRH-ant protocol group was significantly higher than that in patients in the GnRH-a long protocol group [206 (48.70%) vs. 100 (64.52%)]. In addition, women in the GnRH-ant protocol group had a higher basal plasma FSH level and received a higher total gonadotropin dose during the cycle but received a shorter duration of ovarian

stimulation. In addition, there was a statistically significant distribution of infertility factors between the two groups.

GnRH Antagonists Increase the Aneuploidy Rate in Early Aborted Tissues in Patients Receiving IVF/ICSI

We assessed whether any specific infertility diagnosis or clinical characteristic was associated with the risk of aneuploidy in aborted tissues. We observed significant associations of aneuploidy with maternal and paternal ages, the basal FSH level, the basal E2 level and infertility diagnosis (*Supplemental Tables 3*). When assessing specific ovarian stimulation procedures, the GnRH-ant protocol was associated with an increased risk of aneuploidy compared with the GnRH-a long protocol (OR 1.92, 95%CI 1.31-2.80, *P*=0.0008) (*Supplemental Tables 3*). We observed null associations of aneuploidy with BMI and 5 infertility diagnoses: tubal factor, male factor, PCOS, POR and female and male factors. We did not observe significant associations between aneuploidy and the fertilization method (conventional IVF, ICSI, and IVF/ICSI) or maternal AMH, Gn dose or duration.

Due to the significant relationship between aneuploidy in early aborted tissues and the GnRH-ant protocol observed, multivariable regression analysis was performed to corroborate the impact of the GnRH-ant protocol on aneuploidy. In the multivariable model, when maternal age, paternal age and potential influencing factors, including the basal FSH level, infertility diagnosis, basal E2 level, infertility factors, total dose of Gn and duration of Gn, were considered, the GnRH-ant regimen remained significantly associated with an increased risk of aneuploidy in early aborted tissues (P=0.0064) with an adjusted odds ratio of 1.767 (95% confidence interval, 1.174-2.661) (Table 1).

Significant Difference in Aneuploidy Types in Early Aborted Tissues After the GnRH-ant Protocol and GnRH-a Long Protocol

Aneuploidy in early aborted tissues was divided into two types: the type with the highest incidence involved trisomy 15, 16, 21, and 22, and the other type (aneuploidy causing birth defects) involved trisomy 13, 18, and 21. Two Pearson's chi-squared tests were run separately. As shown in Table 4, compared with the GnRH-a long protocol, the GnRH-ant protocol was associated with a significantly higher incidence of trisomy 13, 18, and 21 ($p<0.01$) (Table 2), but the two protocols had the same incidence of trisomy 15, 16, 21, and 22 (Table 2).

Effect of the Ovarian Stimulation Protocol on Embryo Aneuploidy in PGT Cycles

Baseline Characteristics of Embryos from PGT Cycles

In the present study, a total of 1912 embryos from 466 cycles and were obtained, of which 1612 embryos were obtained from 383 cycles receiving the GnRH-a long protocol and 300 embryos were obtained from 83 cycles receiving GnRH-ant protocol. The baseline characteristics of the patients are presented in *Supplemental Table 4*. As shown in *Supplemental Table 4*, compared to the GnRH-a long protocol group, the maternal and paternal ages of individuals in the GnRH-ant protocol group were significantly higher (31.47 ± 4.11 vs. 32.32 ± 4.61 y; 32.95 ± 4.35 vs. 33.65 ± 4.91 y). Significant differences were observed in the rate of aneuploidy between the GnRH-a long protocol and GnRH-ant protocol groups, and the rate of aneuploidy was significantly higher in the GnRH-ant protocol group (48.01% vs. 58%). In addition, patients who received the GnRH-ant protocol had a higher basal FSH level and AMH level and a lower basal E2 level. These patients also had a higher rate of primary infertility and received a larger total dose of gonadotropins. The two groups differed significantly in PGT indications.

GnRH Antagonists Increase the Embryo Aneuploidy Rate in Patients Receiving IVF/ICSI

We assessed whether any specific infertility diagnosis or clinical characteristic was associated with the risk of blastocyst aneuploidy. We observed significant associations of embryo aneuploidy with maternal and paternal ages, as well as the basal E2 level and total dose of Gn (*Supplemental Table 5*). When assessing specific ovarian stimulation procedures, the GnRH-ant protocol was associated with an increased risk of aneuploidy compared with the GnRH-a long protocol (OR 1.5, 95%CI 1.07-2.10, P=0.0201) (*Supplemental Table 5*). We observed null associations of aneuploidy with other clinical factors.

Multivariable regression analysis was performed to corroborate the impact of the GnRH-ant protocol on the aneuploidy of embryos. In the multivariable model, when maternal age, paternal age and potential influencing factors, including infertility diagnosis, PGT indications, basal FSH level, basal E2 level, total dose of Gn and duration of Gn, were considered, the GnRH-ant regimen remained significantly associated with an increased risk of aneuploidy in embryos from PGT cycles (P=0.0386) with an adjusted odds ratio of 1.465 (95% confidence interval, 1.020-2.102) (Table 3).

Comparison of Embryo Aneuploidy in PGT Patients Receiving the Two Stimulation Protocols Stratified by AMH and Age Using Multivariable Regression Analysis

The ovarian response to gonadotropins may be related to oocyte aneuploidy, and advanced maternal age is the determining factor of embryo aneuploidy. AMH is a key predictive biomarker for the ovarian response. The aneuploidy rate between the two groups was stratified by AMH and maternal age. We divided the patients into expected low (AMH ≤1.30 ng/mL), medium (AMH >1.30 ng/mL, and AMH ≤3.36 ng/mL), and high (AMH >3.36 ng/mL) response groups. Females were divided into two groups according to (<35 y and ≥35 y).

As shown in Table 4, the multivariable regression stratified analysis showed that women younger than 35 y and with an AMH concentration between 1.30 ng/mL and 3.36 ng/mL had a significantly higher rate of embryo aneuploidy after receiving the GnRH-ant protocol (P=0.0045) with an adjusted odds ratio of 3.54 (95% confidence interval, 1.48-8.46). This tendency was not observed in other groups.

Interactions between the AMH subgroups and the stimulation protocols were tested in two subgroups according to female age, and the results showed that all the AMH subgroups failed to have an interaction effect with the stimulation protocol on embryo aneuploidy.

Discussion

The present study suggested that embryos conceived after GnRH antagonists may have higher early pregnancy loss rates. This study also demonstrated that the GnRH-ant protocol was associated with a higher embryo aneuploidy rate than the GnRH-a long protocol in Chinese women receiving PGS cycles. Additionally, the rate of aneuploidy in early aborted tissues in patients who conceived after receiving the GnRH-ant protocol was significantly increased compared with that in patients who conceived after receiving the GnRH-a long protocol. Furthermore, this study showed that the embryo aneuploidy rate in the GnRH-ant protocol was significantly higher than that in the GnRH-a long protocol but only in young and normal ovarian responders. These results suggest that an increased embryo aneuploidy rate may account for the higher early pregnancy loss rate associated with GnRH-ant protocol. This is the first study that evaluated whether a difference in aneuploidy rates exists between the GnRH-ant protocol and GnRH-a long protocol.

Aneuploidy is one of the most detrimental factors causing failed implantation, miscarriage, and disordered embryo development [23]. Many researchers have explored the influencing factors of embryonic aneuploidy. Several studies in animal oocytes have suggested increased aneuploidy rates after ovulation induction [24, 25]. In humans, studies have also reported that the proportions of human preimplantation embryos with aneuploidy are increased by ovarian stimulation [26, 27]. Studies have also suggested that a higher daily dose of gonadotropin during IVF is associated with greater aneuploidy rates than a lower daily dose of gonadotropin [28, 29]. It was also found patients requiring more days of stimulation presented a lower rate of aneuploid embryos[9]. Conversely, Labarta et al. showed that ovarian stimulation did not significantly increase the embryo aneuploidy rate in IVF-derived human embryos compared with an unstimulated cycle (24). Three retrospective studies of PGS outcome data showed that the total dose of exogenous gonadotropins was not significantly associated with blastocyst aneuploidy and that a high dosage of gonadotropin did not affect euploidy or pregnancy rates [30–32]. A retrospective cohort study that included 2230 embryos conceived from IVF that underwent PGT-A also demonstrated that the gonadotropin dosage, duration of ovarian stimulation, estradiol level, follicle size at ovulation trigger and number of oocytes retrieved, within certain ranges, do not appear to significantly influence euploidy rates, regardless of the woman's age [33]. Therefore, the potential deleterious effect of ovarian stimulation on oocyte and embryo euploidy is still the subject of lively debate. The conflicting results may be attributed to the operation of different physicians in different IVF centers. It has been

found that euploidy rates for embryos created using donor oocytes can vary significantly between different IVF centers and even between donors treated by different physicians at the same IVF center [26, 34]. Studies have also demonstrated different mosaicism rates between IVF centers, implicating differences in stimulation protocols as a potential reason. Therefore, in the present study, we compared the effects of the GnRH-ant protocol and GnRH-a long protocol on embryo aneuploidy.

It has been reported that in a general IVF population, GnRH-ant protocol is associated with lower ongoing pregnancy rates than the GnRH-a long protocol [14, 35]. Studies have also suggested that embryos conceived after receiving GnRH antagonists may be associated with higher early pregnancy loss rates [15–17]. It is evident that aneuploidy is a major factor that results in early pregnancy loss [19]. However, the rate of aneuploidy in early aborted tissues had not been evaluated in patients who conceived after receiving the GnRH-ant or GnRH-a long protocol. In the present study, we noted a difference in aneuploidy rates between our two cohorts and found that the GnRH-ant protocol was associated with a significantly higher aneuploidy rate. Univariable analysis revealed that female age, male age, the basal FSH level, the basal E2 level, infertility diagnosis, and the stimulation protocol were associated with the aneuploidy rate in aborted tissues. Furthermore, after regression analysis controlling for age, the basal FSH level, the basal E2 level, infertility diagnosis, the total dose of Gn and the duration of Gn, this difference in aneuploidy rates between the GnRH-ant and GnRH-a long protocol groups was still statistically significant. The present study also showed that the GnRH-ant stimulation protocol mainly caused an increased incidence of trisomy 13, 18, and 21 compared with the GnRH agonist stimulation protocol. An increase in the rate of aneuploidy in early aborted tissues may account for the higher early pregnancy loss rates of women who conceived after receiving GnRH antagonists. Thus, it is believed that blastocysts treated with GnRH-ant protocol may exhibit more aneuploidy than blastocysts treated with GnRH-a long protocol.

Based on this hypothesis, we compared the rates of aneuploidy in blastocysts treated with GnRH antagonists and long agonists in PGS-A cycles. Given the potential variation in different PGT-A testing platforms, we evaluated blastocysts using NGS only [34]. The rate of blastocyst aneuploidy was significantly higher in the GnRH-ant protocol group. In the multivariable model, when maternal age, paternal age and potential influencing factors, including infertility diagnosis, PGT indications, the basal FSH level, the basal E2 level, the total dose of Gn and the duration of Gn, were considered, the GnRH-ant regimen remained significantly associated with an increased risk of blastocyst aneuploidy in women receiving PGT cycles. Previous studies reported that embryonic aneuploidy rates were not influenced by the dose of gonadotropins used in ovarian stimulation [30, 31]. Our study results reinforce the idea of their findings, showing that blastocyst aneuploidy is independent of the dose of gonadotropins in both the GnRH-ant and GnRH-a long protocols. Previous data have indicated that the aneuploidy rates in embryos produced from eggs collected from ovarian stimulation are between 39.1 and 53.2%, which are higher than the proportion of eggs with abnormal chromosomes in young women during the natural cycle (17%) [4, 36]. Our present data indicate that the aneuploidy rate in embryos produced from the GnRH-ant protocol is 58.00%.

The effect of the hormones administered during stimulation at the cellular level is still unknown. It has been reported that mitochondrial dysfunction that causes a decrease in ATP and/or an increase in reactive oxygen species (ROS) is sufficient to disrupt meiotic spindles [37, 38]. The percentage of mitochondria that were vacuolated in oocytes was significantly increased after ovarian stimulation in mice [39]. Moreover, the ratio of activated mitochondria to inactivated mitochondria and ATP synthesis in mouse oocytes decreased after ovarian stimulation [39]. Evidence has also demonstrated that repeated superovulation has adverse effects on the mitochondrial function of cumulus cells in rhesus monkeys or mice [40, 41]. Evidence has also indicated that repeated superovulation induces oxidative stress by elevating ROS levels in oocytes [42]. A significant decrease in ATP generation and increase in ROS caused by ovarian stimulation may adversely affect chromosomal segregation and meiotic spindle adjunction, which result in embryo aneuploidy [43, 44]. It has been reported that the use of antagonists may present an endocrinologically unfavorable scenario in which the suppression of endogenous pituitary gonadotrophin secretion may be insufficient [14]. The nonphysiological microenvironment caused by the GnRH-ant may result not only in abnormal follicular fluid biochemistry but also aberrant oocyte cytoplasmic development, which compromises mitochondrial function. However, no report has compared the effects of the GnRH-ant and GnRH-a long protocols on mitochondria and metabolism in human oocytes. In the future, it will be necessary to analyze the effects of GnRH antagonists on oocyte mitochondria and ATP synthesis.

Our study has several strengths and a few limitations. Strengths included its relatively large sample size from a single state over several years, which included 578 early miscarriage patients and a total of 466 cycles after receiving the GnRH-ant or GnRH-a long protocol. We controlled for maternal age, paternal age, infertility diagnosis, PGT indications, basal FSH level, basal E2 level, and total dose of Gn and duration of Gn were confounders in the study.

This study has some limitations. First, it was retrospective in nature, and some key statistical parameters may not have been calculated. We are aware that differences in baseline characteristics exist between patients who received the GnRH-ant and GnRH-a long protocols and that a greater proportion of low responders would be expected to receive the GnRH-ant protocol. Therefore, stratification and multiple linear regression were used to control for these parameters, most notably age. To control for age, it would be best to compare patients of similar age and ovarian reserve who receive the long agonist or GnRH-ant protocol. Second, the analyses performed in the study showed associations between an increased rate of embryonic aneuploidy and the GnRH-ant protocol, but they did not establish causality. In addition, this study was performed at a single IVF center, which may limit its generalizability. As euploidy rates for embryos created using donor oocytes can vary significantly between different IVF centers and even between donors treated by different physicians at the same IVF center [26, 34], a multicenter, randomized controlled trial would be the optimal strategy to confirm these results.

Conclusion

This study demonstrated that during IVF, the aneuploidy rate in early aborted tissues and embryos was significantly higher after receiving the GnRH-ant protocol than after receiving the GnRH-a long protocol. GnRH antagonists may be associated with detrimental effects on embryo quality, and the higher early pregnancy loss rates associated with GnRH antagonists may originate from the higher aneuploidy rate. Prospective, multicenter, and randomized controlled trials are needed to corroborate these findings and obviate the problems of bias and confounding by interdependent variables.

Abbreviations

IVF: in vitro fertilization, GnRH: gonadotropin-releasing hormone, GnRH-ant: GnRH antagonist, GnRH-a: GnRH agonist, ICSI: intracytoplasmic sperm injection, PGT-A: preimplantation genetic testing for aneuploidy

Declarations

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Authors' contributions

Jun Wang collected the data and prepared all the tables and wrote the main manuscript, Jing Zhang and Nan Zhao collected the data, Shuqiang Chen searched the literature and designed the study, Xiaohong Wang revised the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used are analyzed are during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of the Tang Du Hospital of the Air Force Military Medical University, China (K202108-09).All the patients provided the written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

TABLE 1 Outcomes of multivariable regression analysis of the impact of the stimulation protocol on aneuploidy in early miscarriage patients undergoing IVF/ICSI (n=578)

	OR[a]	95%CI	*P* Value[a]	OR adj[b]	95%CI	*P* Value adj[c]
GnRH agonist	1.0			1.0		
GnRH antagonist	1.915	1.309, 2.801	0.0008	1.767	1.174, 2.661	0.0064

Note: adj = adjusted, CI=confidence interval, OR=odds ratio.

[a] OR and P value were calculated using univariate logistic regression.

[b] OR adjusted based on female age, male age, basal FSH, E2, infertility diagnosis, infertility factors, total dose of Gn, duration of Gn.

[c] *P* values were calculated using multivariate logistic regression.

TABLE 2 The type of aneuploidy with the highest incidence or causing birth defect in two stimulation protocol (n=306)

	Aneuploidy with the highest incidence		Aneuploidy causing birth defect	
	15/16/21/22 trisomy	Other types	13/18/21 trisomy	Other types
GnRH agonist	102 (67.55%)	104 (67.10%)	10 (41.67%)	196 (69.50%)
GnRH antagonist	49 (32.45%)	51 (32.90%)	14 (58.33%)*	86 (30.50%)

Pearson's Chi-squared test. *p□0.01

TABLE 3 Outcomes of multivariable regression analysis of the impact of the stimulation protocol on aneuploidy in 466 PGT cycles (n=1912 blastocysts)

	OR[a]	95%CI	*P* Value[a]	OR adj[b]	95%CI	*P* Value adj[c]
GnRH agonist	1.0			1.0		
GnRH antagonist	1.495	1.065, 2.099	0.0201	1.465	1.020, 2.102	0.0386

Note: adj = adjusted, CI=confidence interval, OR=odds ratio.

[a] OR and P value were calculated using univariate logistic regression.

[b] OR adjusted based on female age, male age, basal FSH, E2, infertility diagnosis, PGT indications, total dose of Gn, duration of Gn.

[c] *P* values were calculated using multivariate logistic regression. Generalized estimate equation were used, the type is independence.

TABLE 4 Comparison of embryos aneuploidy of GnRH agonist long protocol vs. GnRH antagonist protocol using multivariable regression analysis in PGT patients stratified by AMH and age (n=1912 blastocysts)

	n=1912	OR adj[a]	95%CI	*P* Value adj[b]	*P* Interaction
Female age <35 y	1475				
AMH, ng/mL					0.1800
≤1.30	136	1.23	0.35, 4.36	0.7462	
>1.30, ≤3.36	629	3.54	1.48, 8.46	0.0045	
>3.36	710	1.07	0.64, 1.77	0.7943	
Female age ≥35 y	437				
AMH, ng/mL					0.1046
≤1.30	102	0.77	0.26, 2.26	0.6335	
>1.30, ≤3.36	187	0.63	0.19, 2.15	0.4632	
>3.36	148	2.75	0.67, 11.32	0.1601	

[a] OR adjusted based on female age, male age, basal FSH, E2, infertility diagnosis, PGT indications, total dose of Gn, duration of Gn.

[b] *P* values were calculated using multivariate logistic regression. Generalized estimate equation were used, the type is independence.

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