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RESEARCH

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Predictive factors of aneuploidy in infertile patients undergoing IVF: a retrospective analysis in a private IVF practice

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Abstract

Background PGT-A has become an important part of IVF treatments. Despite its increased use, there are contradicting results on its role in improving reproductive outcomes of ART cycles. Given that aneuploidy is a main limiting factor for IVF success, we aimed to study the predictive factors of aneuploidy in infertile patients undergoing IVF and hence highlight the patients who would benefit the most from genetic testing.

Results A retrospective analysis of 1242 blastocysts biopsied in the setting of PGT-A cycles was performed. The euploid group included 703 embryos, while the aneuploid group had 539 embryos. The factors included in the analyses were the couple's history as well as the embryo characteristics. The primary outcome was the rate of aneuploid embryos per patient's history as well as per embryo characteristics. The aneuploidy rate (AR) in our cohort was 43.4%. The woman's age was found to be a significant predictor (OR 1.045, 95% CI 1.008–1.084, $p=0.016$). Biopsy on day 5 as well as degree of expansion 3 was also found to affect significantly (OR 0.724, 95% CI .541–.970, $p=0.03$ and OR 2.645, 95% CI 1.252–5.585, $p=0.011$). Lack of consanguinity decreased the AR by an OR 0.274 with 95% CI .137–.547, $p<0.001$. The number of blastocysts available, trophectoderm quality, embryo grade, gonadotropins as well as trigger used were not found to be significant predictors ($p=0.495$, 0.649, 0.264, 0.717 and 0.659 respectively).

Conclusion Advanced female age, consanguinity, the day of embryo biopsy, and the degree of blastocyst expansion were all found to affect the incidence of AR. The age of the male partner, cause of infertility, and grade of embryo at biopsy were not found to correlate with aneuploidy.

Keywords Preimplantation genetic testing for aneuploidy, Aneuploidy, Blastocyst, In vitro fertilization, Maternal age, Consanguineous marriage

Introduction

Embryo aneuploidies are a leading cause of implantation failure, miscarriages, and congenital defects. It is believed to be due to an error in meiotic divisions of the oocyte, the incidence of which increases with advanced woman's age. This is of major concern as many women are delaying conception at a risk of increased embryo aneuploidies. Add to that, due to multiple factors like social, economic, religious, and personal, couples in some parts of the world are becoming more interested in family balancing and consequently the need for preimplantation

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genetic testing for aneuploidy (PGT-A) [1]. Hence, the increased use of PGT-A in recent years [2, 3]. It is well known that when PGT-A is performed, only 50–60% of the tested embryos will be euploid and are candidates for transfer into the uterus. Multiple reports have highlighted the deterioration of oocyte quality with ovarian aging and hence an increased rate of aneuploidy [4, 5]. Increased rates of meiotic errors during oogenesis, decreased oocyte concentrations of mitochondrial DNA, mitotic spindle instability, and shortened telomeres in the granulosa cells of the oocytes have all been suggested as possible markers of oocyte aging [5, 6]. Yet aneuploidies are encountered in all age groups accounting for 23% of tested embryos in the age group younger than 38 years [7, 8]. As per La Marca et al., female age was found to be the strongest predictor of embryo aneuploidy, while other factors such as the male's age, body mass index, fertilization rate, and ovarian reserve were only marginal contributing factors [9]. As per Cimadomo et al., other than the patient's age, the patient's reproductive history did not affect the euploidy status of the tested embryos [10]. Melado et al. concluded that a couple's consanguinity predisposed to increased rates of segmental aneuploidies [11]. In a recent study by Zhang et al., the authors reported that maternal age, previous history of pregnancy loss due to aneuploidy, blastulation rate, and estrogen level on trigger day were significant predictors of aneuploidy in good responders [12]. Another recent study showed that in addition to maternal age, low ovarian reserve defined as less than 5 antral follicular counts on ultrasound, teratospermia, morphologic low-grade embryos, and grade C inner cell mass or trophectoderm, to be significantly associated with aneuploidies [13]. Other studies showed no correlation between male parameters and aneuploidy [14]. Low ovarian reserve remains a controversial topic as there is no consensus on whether low AMH increases the incidence of aneuploidies or not [15, 16].

The use of PGT-A for all patients undergoing fertility treatments is not considered the standard of care for infertile patients so far. PGT-A is directed mainly toward the selection of euploid embryos carrying the highest rate of pregnancy and subsequently live birth contrary to non PGT-A stimulation cycles where embryo selection is based on the morphologic assessment of the developing embryo. Given the lack of consensus in the literature so far on the predisposing factors to aneuploidy other than age related, the primary aim of our retrospective analysis was to underline the predictive factors for aneuploidy in patients undergoing IVF treatment. The findings of our retrospective study can lay the foundation for creating a formula to quantify the couple's risk of aneuploidy in the setting of IVF and hence recommend PGT-A testing when needed.

Data and methods

In a retrospective analysis, all patients undergoing ovarian stimulation cycles with subsequent PGT-A in the Fakih Fertility Center, Al Ain branch in the United Arab Emirates during the period expanding between December 1st, 2018, and December 31st, 2021, were included. The reviewed charts accounted for 1242 blastocysts biopsied in the setting of PGT-A cycles resulting from 177 ovarian stimulation cycles. The studied embryos resulting from the stimulated patients were divided into 2 groups: euploid and aneuploid based on the PGT-A results. The inclusion criteria involved patients who presented for IVF treatment with PGT-A testing. PGT-A cycles involving cleavage stage biopsies were excluded from the chart review. The factors included in the analyses for the predisposing factors for aneuploidy were the age of the couple, consanguinity, cause of infertility, semen analysis abnormalities (azoospermia (AZO), oligozoospermia (Oligo), asthenozoospermia (AST), teratozoospermia (Terato), Oligoasthenoteratozoospermia (OAT)), gonadotropins used for stimulation, trigger taken, day of the biopsy, grade of the embryo, degree of the expansion of the blastocyst, and the grade of the trophectoderm at the biopsy day. Patients were considered as low ovarian reserve patients based on Bologna criteria [17]. The aneuploidy rate (AR) was defined as the number of resulting aneuploid embryos out of the total number of tested embryos in a specific period of time.

The study was approved by the internal research ethical committee (REC) of the fertility center.

Description of treatment cycles and genetic testing

The patients underwent PGT-A for the following indications: advanced reproductive age, severe male factor, consanguinity, recurrent pregnancy loss, recurrent implantation failure, gender selection, and personal choice. The controlled ovarian stimulation protocol used was the antagonist protocol. The type and dose of the gonadotropins used were decided based on the baseline hormonal profile, antral follicular count, and ovarian response to stimulation. The type and dose of the ovulation trigger depended on the number of follicles (more than 15 follicles above 12 mm) on trigger day and estrogen level (more than 3000 pg/ml) as well as on the fact that the studied cycles involved PGT-A testing and hence no subsequent fresh embryo transfer.

Microfluidic sperm sorting chips were used for sperm preparation in the laboratory. An untreated semen sample would be pipetted into the inlet chamber and then sorted sperms would be collected from the outlet.

Embryo biopsy procedure was performed based on the facility's internally validated standard of procedure. Using the Laser (LYKOS®—Hamilton Thorne) of 200

pulses, a hole of 7 μm was created in the zona pellucida on cleavage stage embryos as a part of assisted hatching. On day 5, the well-expanded blastocyst with herniating trophectoderm from the zona breach was biopsied to get an ambient number of 6–7 cells using the pulling technique with additional low pulse Laser (LYKOS®—Hamilton Thorne). The non-expanded blastocysts were further cultured to day 6 for adequate expansion. After the aspiration, the embryologist made sure that the cells were not lysed with an intact nucleus seen, washed them twice in wash buffer (ORIGIO® Handling™ IVF Medium), placed them in a 10 μ HEPES buffered drop of media, and then transferred the cells into a post biopsy four-well dish (ORIGIO® Handling™ IVF Medium) labeled with the patient's information. In case the biopsied cells were not intact, then they were discarded and additional blastomeres would be sampled. The samples were then transferred to the genetic lab that is present in-house where the received samples were then loaded into PCR tubes (tubing) to start the DNA amplification process. The number of the blastomeres received ranged between 5 and 9 cells. The DNA was amplified by Multiple displacement amplification (MDA). The MDA product would then be processed for library preparation and sequencing by high-resolution next-generation sequencing (NGS-HR). The sequencing platform used is MiSeq from Illumina. Once the sequencing data was generated, it was transferred to the computer to view the results using Bluefuse software. The biopsied blastocysts were washed twice in culture media and then placed back in the corresponding drop of culture medium in a new culture dish followed by cryopreservation using Cryotech ® Vitrification. The grading of the embryos on the day of the biopsy was based on the grading system adapted by Capalbo et al. and Gardner's grading method [18]. Excellent embryos include AA blastocysts; good embryos include AB and BA blastocysts; average embryos include BB, AC, BC, and CA blastocysts, while poor embryos include CC and below embryos. To note that in our lab, when embryos were graded as poor embryos, only CC embryos were biopsied.

Statistical analysis

Statistical analysis was done using IBM SPSS© Statistics version 29.0 (IBM, Chicago, IL, USA). Model assumptions for the continuous variables were checked by the D'Agostino and Pearson test and the Shapiro–Wilk test. When model assumptions were violated, the difference between study groups (euploid and aneuploid embryos) was determined by the non-parametric Mann–Whitney *U* test. Chi-square or Fisher's exact test was used for categorical variables. Continuous variables are presented as mean ± SD, and categorical variables are presented as percentages and counts. All variables were entered using a forced entry method, and all the predictor variables were tested in one block to assess their predictive ability while controlling for other predictors in the model for the regression analysis. Results are presented as an adjusted odds ratio with 95% CI. A two-sided *p*-value of 0.05 was considered statistically significant.

Results

A total of 1242 blastocysts were included in the analysis, from 153 patients. The age range of the women was 17–43, and the men's age ranged between 23 and 63. The euploid result group (ERG) included 703 embryos, while the aneuploid result group (ARG) had 539 embryos. The women and men were younger in the euploid group (30.45 vs 32.47 years, $p = < 0.001$ and 34 vs 36 years, $p = < 0.001$, respectively). The other demographic data is presented in Table 1. The stimulation cycle characteristics between the euploid and the aneuploid embryos were comparable. The values are presented in Table 2. As for embryo characteristics, excellent-grade embryos were most commonly biopsied in the ERG when compared to the ARG (52.3% vs 34.5%, $p = 0.003$). In the ERG, when compared to the ARG, biopsies were performed more on day 5 (56.5% vs 45.3%, $p = 0.0001$). The remainder of the embryo characteristics are presented in Table 3. When comparing the AR based on the patient's history, the rate was higher in the non-consanguineous couples (24.8% vs 18.4%, $p = 0.0001$). There was no difference in the AR when taking the cause of infertility into consideration ($p = 0.15$). Abnormal semen analysis was not found to increase the rate of aneuploidy significantly (normal 12%, AST 18.4%, AZO 0.4%, OAT 9.5%, Oligo 1.6%, and Tera 1.4%, $p = 0.77$). Also, low ovarian reserve in comparison to the other female infertility causes was not found to increase the AR (6.4%, no cause 22.5%, PCOS 7.4%, $p = 0.289$). The AR per patient's history is presented in Fig. 1. The impact of the gonadotropins as well as the trigger used was also studied. The AR showed no difference based on the type of gonadotropin used for stimulation (hpHMG 20%, rFSH 13.2%, and rFSH + rLH 9.7%, $p = 0.708$). The trigger mostly used is the GnRH agonist trigger due to the fact that the studies' cycles involved PGT-A testing with the aim of shortening the luteal phase. The AR per medications used during the stimulation cycles are presented in Fig. 2. In Fig. 3, the AR was compared based on the different embryo characteristics. The AR was found to be the highest in excellent grade embryos (14.9%, good grade 9.5%, average grade 12%, and poor grade 6.5%, $p < 0.0001$). Biopsies on day 6 were found to have higher AR (day 5 19.6%, day 6 22.9%, and day 7 0.8%,

Table 1 Demographic data of the Euploid group versus the aneuploid group

	Euploid (*N* = 703)	Aneuploid (*N* = 539)	Total (1242)	*p*-value[a]
Age (female)	30.45 ± 5.83	32.47 ± 6.34		< 0.001
Age (male)	34.02 ± 7.75	36.02 ± 8.31		< 0.001
Duration of the infertility	2.46 ± 2.55	2.82 ± 2.33		< 0.001
Consanguinity				< 0.0001
None	477 (68.4%)	309 (57.5%)	786 (63.7%)	
Present	220 (31.6%)	228 (42.5%)	448(36.3%)	
Cause of infertility				0.0591
Male factor	278 (39.5%)	204 (37.8%)	482(38.8%)	
Mixed	188 (26.7%)	170 (31.5%)	358(28.8%)	
Female factor	138 (19.6%)	85 (15.8%)	223(18%)	
Unexplained	99 (14.1%)	80 (14.8%)	179(14.4%)	
Semen analysis				0.7626
Asthenospermia	299 (42.5%)	229 (42.5%)	528(42.5%)	
Normal	214 (30.4%)	150 (27.8%)	364(29.3%)	
Oligoasthenoteratospermia	131 (18.6%)	118 (21.9%)	249(20%)	
Oligospermia	27 (3.8%)	20 (3.7%)	47(3.8%)	
Tertatospermia	24 (3.4%)	17 (3.2%)	41(3.3%)	
Azoospermia	8 (1.1%)	5 (0.9%)	13(1%)	
Subdivision of female infertility causes				0.1498
No female cause	373 (53.1%)	280 (51.9%)	653(52.6%)	
PCOS	125 (17.8%)	93 (17.3%)	218(17.6%)	
Uterine factor	87 (12.4%)	54 (10.0%)	141(11.4%)	
Low ovarian reserve	77 (11.0%)	80 (14.8%)	157 (12.6%)	
Tubal factor	34 (4.8%)	29 (5.4%)	63(5.1%)	
Endometriosis	7 (1.0%)	3 (0.6%)	10(0.8%)	

[a] *p*-value less than 0.05 is considered significant

$p = 0.0003$). The degree of expansion of the blastocyst as well as the trophectoderm quality were to significantly affect the AR ($p < 0.0001$ for both). The results are documented in Fig. 3. Pearson's correlation between the age of the female and the embryo characteristics showed that age is positively correlated with blastulation as well as the degree of expansion of the embryo is correlated negatively. The results are presented in Table 4. Binary logistic regression analysis was performed to verify the significant predictors of aneuploidy. The woman's age was found to be a significant predictor (OR 1.045, 95% CI 1.008–1.084, $p = 0.016$). Biopsy on day 5 as well as degree of expansion 3 was also found to affect significantly (OR 0.724, 95% CI 0.541–0.970, $p = 0.03$ and OR 2.645, 95% CI 1.252–5.585, $p = 0.011$). The absence of consanguinity significantly decreased the AR with an OR 0.247, 95% CI 0.137–0.547, $p < 0.001$). The number of blastocysts available, trophectoderm quality, embryo grade, gonadotropins as well as trigger used were not found to be significant predictors ($p = 0.495$, 0.649, 0.264, 0.717 and 0.659 respectively). The regression analysis is presented in Table 5.

Discussion

Our retrospective analysis has revealed a 43.4% AR in blastocysts biopsied. The biopsies were mainly performed on excellent grade day 5 blastocysts (44.6%, $p = 0.003$ and 51.6%, $p = 0.0001$, respectively). Most of the embryos had a degree of expansion 5 and involved trophectoderm quality A (60.6%, $p < 0.0001$ and 54%, $p < 0.0001$, respectively). This might be explained by the good ovarian reserve of our cohort as well as the young age of our patients (< 35 years old) especially when taking into account the results of Pearson's correlation in Table 4. Even the patients that were initially diagnosed as low ovarian reserve patients had an unexpectedly good response which could be explained partially by the young age of the patients in addition to individualized approaches in choosing the stimulation medication doses. Nevertheless, the percentage of low

Table 2 Stimulation cycle characteristics of the Euploid embryos group versus the aneuploid embryos group

	Euploid (*N* = 703)	Aneuploid (*N* = 539)	*p*-value[a]
Estrogen (pg/ml)	42.94 ± 16.31	42.90 ± 15.90	0.839
FSH (mIU/ml)	6.33 ± 1.67	6.23 ± 1.78	0.499
LH (mIU/ml)	6.19 ± 2.75	5.83 ± 2.51	0.062
Progesterone (ng/ml)	0.25 ± 0.14	0.25 ± 0.15	0.251
No of oocytes	23.85 ± 10.73	24.40 ± 11.59	0.738
No of mature oocytes	19.45 ± 8.85	19.63 ± 9.38	0.963
No of fertilized oocytes	16.40 ± 7.41	16.69 ± 7.78	0.739
No of blasts	9.42 ± 5.10	9.43 ± 4.99	0.783
Gonadotropin used			0.252
hpHMG[1]	316 (44.9%)	253 (47%)	
rFSH[2] alone	230 (32.7%)	165 (30.6%)	
rFSH + rLH[3]	157 (22.4%)	121 (22.5%)	
Trigger taken			0.221
GnRH agonist	577 (82.1%)	421 (78.3%)	
Dual trigger	70 (10%)	72 (13.4%)	
Rec hCG[4]	56 (8%)	45 (8.4%)	

[1] Highly purified human menopausal gonadotropin

[2] Recombinant FSH

[3] Recombinant LH

[4] Recombinant human chorionic gonadotropin

[a] *p*-value less than 0.05 is considered significant

ovarian reserve patients in our cohort was extremely low as shown in Table 1. Day 6 biopsies were associated with higher AR in comparison to day 5 biopsies (22.9% vs 19.6%, $p = 0.0003$). Regression analysis confirmed the association of day 5 biopsies with a decreased rate of aneuploidy (OR 0.724, 95% CI 0.541–0.970, $p = 0.03$). In a study by Tong et al. in 2022, the authors found that day 5 embryos were associated with lower AR in comparison to day 6 (31% vs 41%, $p < 0.001$) which is consistent with our findings [19]. The authors of two other studies also concluded that day 5 blastocysts are significant predictors of higher euploidy rate when compared to day 6 embryos [20, 21]. This finding supports the prioritizing of day 5 blastocysts for embryo transfer in the setting of non PGT-A cycles. Excellent grade embryos as well as degree of expansion 5 were found to have significantly higher AR as presented in Fig. 3. This can be explained by the higher incidence of biopsies involving excellent embryos with a degree of expansion 5. However, the regression analysis revealed that a lower degree of expansion 3 is a significant predictor of aneuploidy (OR 3.284, 95% CI 1.46–7.37, $p = 0.004$). One would hypothesize here that waiting until the embryo reaches a higher degree of expansion might involve genetic self-correction and thus reduce the incidence of aneuploidy, but definitely more research are needed before drawing firm conclusions on this subject. Interestingly, the grade of the whole embryos based on Gardner's criteria adapted by Capalbo et al. at biopsy was not found to affect the AR ($p = 0.064$) [18]. Bilibio et al. in 2022 showed that trophectoderm grade

Table 3 Blastocyst characteristics between the euploid blastocysts and the aneuploid blastocysts

	Euploid (*N* = 703)	Aneuploid (*N* = 539)	Total (1242)	*p*-value[a]
Grade at biopsy				0.0033
Excellent	368 (52.3%)	186 (34.5%)	554(44.6%)	
Good	128 (18.2%)	118 (21.9%)	249(19.8%)	
Average	162 (23%)	154 (28.6%)	316(25.4%)	
Poor	45 (6.4%)	81(15%)	126(10.1%)	
Day of the biopsy				0.0001
Day 5	397 (56.5%)	244 (45.3%)	641(51.6%)	
Day 6	299 (42.5%)	285 (52.9%)	584(47%)	
Day 7	7 (1%)	10 (1.9%)	17(1.4%)	
Degree of expansion				< 0.0001
3	147(20.9%)	192(35.6%)	339(27.3%)	
4	52(7.4%)	48(8.9%)	100(8.1%)	
5	474(67.4%)	279(51.8%)	753(606%)	
6	30(4.3%)	20(3.7%)	50(4%)	
Trophectoderm quality				< 0.0001
A	432(61.5%)	239(44.3%)	671(54%)	
B	265(37.7%)	291(54%)	556(44.8%)	
C	6(0.9%)	9(1.7%)	15(1.2%)	

[a] *p*-value less than 0.05 is considered significant

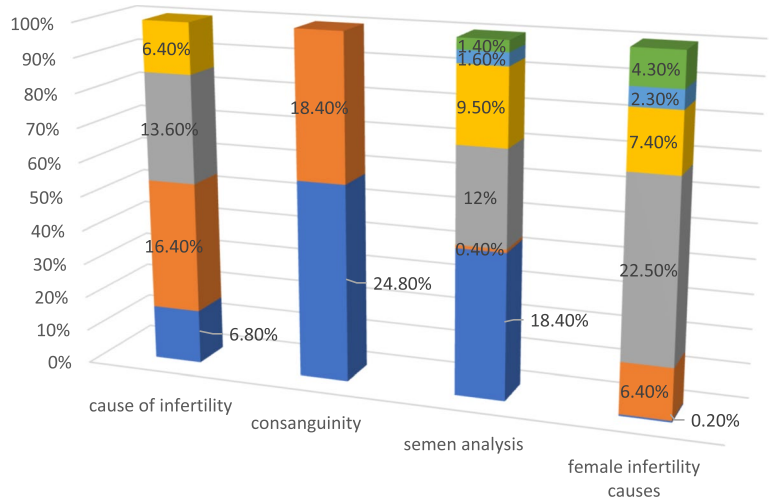

Fig. 1 Aneuploidy rate based on patients' history (color coded). Cause of infertility: blue, female cause; orange, male cause; gray, mixed cause; yellow, unexplained; $p=0.15$. Consanguinity: blue, none; orange, present; $p=0.0001$. Semen analysis: blue, asthenospermia; orange, azoospermia; gray, normal; yellow, oligoasthenospermia; light blue, oligospermia; green, teratospermia; $p=0.77$. Female infertility causes: blue, endometriosis; orange, low ovarian reserve; gray, no cause; yellow, PCOS; light blue, tubal factor; green, uterine factor; $p=0.289$

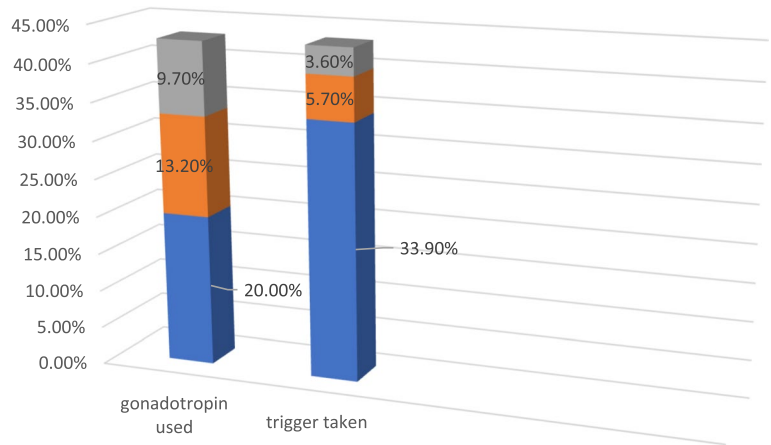

Fig. 2 Aneuploidy rate based on the medications given (color coded). Gonadotropin used: blue, hpHMG; orange, rFSH alone; gray, rFSH + rLH; $p=0.708$. Trigger taken: blue, GnRH agonist; orange, dual trigger; gray, rec hCG; $p=0.154$

C was significantly associated with aneuploidy [13]. In our series, the incidence of trophectoderm grade C was very low, i.e., 1.2% of the whole population studied. This extremely low frequency hindered the proper assessment of grade C's influence on the AR.

As expected, advanced female age was the most important risk factor from the couples' history that was found to predispose to embryo aneuploidy (OR 1.045, 95% CI 1.008–0.084, $p=0.016$). This finding is consistent with the previously published data [9, 22]. Minasi et al. in 2016 reported that the mean euploid blastocyst rates decreased from 48.1% in younger than 32 years old women to 10.3% in women older than 42 years respectively highlighting the impact of the female's age on AR [23]. However, the chances of finding euploid embryos significantly increased in advanced-age women with the increase in the number of embryos available for biopsies [23, 24]. Additionally, low ovarian reserve diagnosed by AMH < 1.1 ng/ml was not associated with an increased AR in comparison to the other causes of female infertility (7.4%, $p=0.289$). It is important to note that generalization of this result is questionable given that only 12.6% of the population included in our study was diagnosed with low ovarian reserve. Moreover, this finding was inconsistent in the published literature, and larger cohort studies are needed to elucidate this matter.

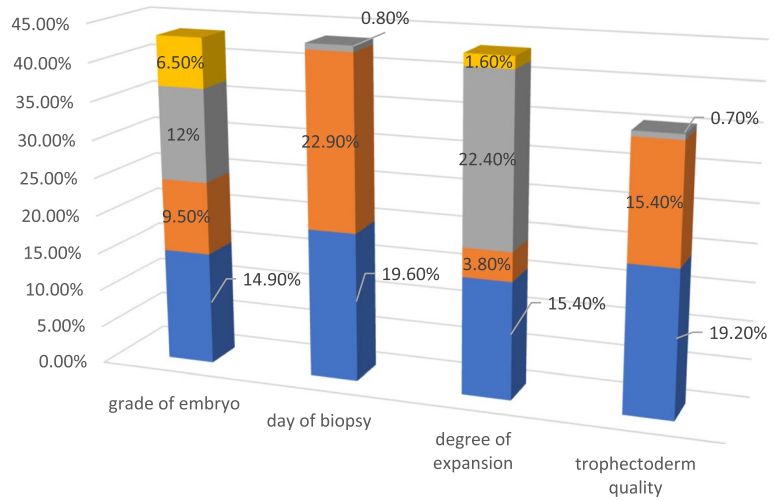

Fig. 3 Aneuploidy rate as per embryo characteristics (color coded). Grade of embryo: blue, excellent; orange, good; gray, average; yellow, poor; $p < 0.0001$. Day of biopsy: blue, day 5; orange, day 6; gray, day 7; $p = 0.0003$. Degree of expansion: blue, 3; orange, 4; gray, 5; yellow, 6; $p < 0.0001$. Trophectoderm quality: blue, A; orange, B; gray, C; $p < 0.0001$

Table 4 Pearson correlation between the age of the female and embryo characteristics

	Pearson Correlation	95% CI	*p*-value[a]
Age of the female—day of biopsy	.085	.029, 0.14	.003
Age of the female—grade at the biopsy	.024	−.032, 0.08	.403
Age of the female—degree of expansion	−.088	−.143, -0.032	.002
Age of the female—trophectoderm quality	.006	−0.05, 0.062	.834

[a] *p*-value less than 0.05 is considered significant

For instance, Morin et al. in 2018 reported that decreased number of oocytes retrieved in patients younger than 38 years did not increase the AR (30% vs 29%, $p = 0.72$) [25]. La Marca et al. in 2017, on the other hand, showed that the woman's ovarian reserve as well as the number of oocytes retrieved were significant factors predicting the euploidy rate [26].

Despite that the AR was higher in consanguineous marriages, the regression analysis showed that the lack of consanguinity decreased the AR by an OR 0.274 with 95% CI 0.137–0.547, $p < 0.001$). These results support the ones recently published by Melado et al. in 2023. In the mentioned study, the authors concluded that consanguineous marriages resulted in significantly higher rates of segmental aneuploidies and not the aneuploidy rate in general when compared to non-consanguineous marriages even when corrected for the female's age (19% vs. 16.7%, $p = 0.029$) [11]. The suggested mechanism is the presence of runs of homozygosity in the embryos resulting from consanguineous marriages. These runs are thought to be at risk of having breakpoints due to areas of instability [11]. This is important in spreading awareness in societies where consanguineous marriages are still highly practiced similar to some countries in the Arab world. Consanguineous couples could be encouraged to undergo IVF with PGT-A especially in the setting of previous poor reproductive history. Male factor was also included in the analysis, and the different subtypes of semen analysis abnormalities were not found to be significant predictive factors ($p = 0.758$). Furthermore, Asthenozoospermia was not associated with higher AR when compared to the other semen analysis categories (18.4%, normal 12%, OAT 9.5%, $p = 0.77$). Paternal age was also found to be non-significant in predicting the AR. The last two findings are consistent with the previous publications by Capelouto et al. in 2018 and Kim et al. in 2019 [7, 8]. Our study showed that the medications used for stimulation as well as the type of oocyte trigger did not affect the AR. This was also supported by the findings of Hong et al. in 2019 concerning the effect of gonadotropins on aneuploidy incidence and in concordance with the previously published data by Thorne et al. in 2020 [27, 28]. The authors concluded that GnRH agonists as well

Table 5 Binary logistic regression analysis for risk factors for aneuploidy

Variables	OR	95% CI	*p*-value[a]
Age of the female	1.045	1.008–1.084	.016
Age of the male	1.020	.989–1.051	.208
Duration of the infertility	1.001	.941–1.064	.984
nb of blasts	1.017	.969–1.068	.495
Cause of infertility			.053
Female infertility causes			.997
Semen analysis			.662
Day of biopsy (day 5)	.724	.541–.970	.030
Grade at the biopsy			.064
Average	1.184	.658–2.130	.573
Excellent	.493	.229–1.064	.072
Good	.889	.481–1.642	.706
Degree of expansion			< .001
3	2.645	1.252–5.585	.011
4	1.523	.691–3.358	.297
5	1.081	.550–2.127	.821
Trophectoderm quality			.649
A	.540	.135–2.159	.384
B	.548	.154–1.953	.353
Consanguinity			< .001
None	.274	.137–.547	< .001
Gonadotropin used			.717
Trigger taken			.659

[a] *p*-value less than 0.05 is considered significant

as hCG trigger have similar euploidy rates (38.5% vs 36.8%, $p = 0.66$) which means similar AR [27].

The strength of our study is that it included consanguineous marriages as a variable in the predictive factors analysis given that consanguineous marriages are still happening. We also compared the gonadotropins used in their impact on AR. The results showed similar rates which is reassuring for the physicians around the globe especially where the choice of medications is limited due to economic reasons for example. Given that there is no uniform grading system for the embryos, and different studies used different systems, we included all the factors included in the grading of the embryo to better understand which parameter(s) is/are more associated with AR. This is of importance since as already mentioned earlier, the use of PGT-A is not recommended to be used in all patients undergoing IVF treatment. The findings of our study can help treating physicians identify patients who are at a higher risk of having aneuploidies. To avoid failed IVF cycles and aneuploidy-related miscarriages, the IVF specialist can thus counsel the at-risk infertile couples as well as consanguineous couples on when to use PGT-A as an add-on for IVF in the absence of the conventional indications such as the maternal age. In case, the couple elected to avoid the use of PGT-A, our data can aid the treating physician in choosing the embryo with potentially the highest implantation rate and the lowest AR based on the day of the blastulation and degree of expansion especially when a single embryo transfer is planned.

The limitation of our study was its retrospective nature, making it prone to more bias. Given the nature of the topic involved, analyzing a cohort retrospectively seems to be the best option available. Given the low incidence of low ovarian reserve, the results concerning the effect of the number of blastocysts should be utilized with caution.

Conclusion

Advanced female age, consanguinity irrespective of the female's age, the day of embryo biopsy, and the degree of blastocyst expansion were all found to affect the incidence of AR. The age of the male partner, cause of infertility, grade of embryo at biopsy, trophectoderm quality, and medications used were not found to correlate with AR.

Abbreviations

AR	Aneuploidy rate
ARG	Aneuploidy result group
ART	Assisted reproductive technologies
AST	Asthenoteratospermia
AZO	Azoospermia
ERG	Euploid result group
IVF	In vitro fertilization
OAT	Oligoasthenoteratospermia
Oligo	Oligospermia
Terato	Teratospermia
PGT-A	Preimplantation genetic testing for aneuploidy
REC	Research ethical committee

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Anastasia A. Salame, Elias M. Dahdouh, Mokhamad Zhaffal Rania Aljafari, Arya Muraleekrishnan, Aparna Bajpai, Shabin Kainoth, and Michael Fakih. The first draft of the manuscript was written by Anastasia Salame, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The study was approved by the internal research ethical committee (REC) of the fertility center. It is retrospective in nature; hence there is no need for a consent to participate.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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