European Journal of Medical Genetics 62 (2019) 103707

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Controlled ovarian hyperstimulation (COH) parameters associated with euploidy rates in donor oocytes

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ARTICLE INFO

Keywords:
Preimplantation genetic testing for aneuploidy (PGT-A)
Controlled Ovarian Hyperstimulation (COH)
Donor Oocytes
Euploidy rate
Human menopausal gonadotropin (hMG)
Array comparative genomic hybridization (aCGH)

ABSTRACT

Although oocyte donors are young and are expected to provide a high rate of euploid oocytes, significant differences of euploidy rates for donor embryos exist between different IVF centers (1). Laboratory conditions can lead to differences of euploidy (2,3,4,5,6,7); but, the role of COH has not been investigated. In this study, we investigated whether euploidy rates in the embryos created from donor oocytes are influenced by controlled ovarian hyperstimulation parameters used during assisted reproduction. Euploidy rates in egg donor cycles undergoing PGT-A (N = 423) were examined retrospectively for associations with donor age, gonadotropin doses (dose per day), the fraction of gonadotropin provided by hMG (F(hMG)), days of stimulation, estradiol per mature oocyte on day of trigger, number of mature oocytes retrieved, number of embryos biopsied, incidence of euploidy and physician of record. Differences in euploidy rates between physicians were examined using analysis of variance. The proportion of euploid embryos per donor cycle was examined for associations with COH parameters using pairwise post-hoc comparisons, adjusting for multiple testing. The set of variables from this analysis was then submitted to a principal component analysis. Linear regression analysis was used to assess the relationships between stimulation parameters and the incidence of euploidy (the dependent variable). Euploidy rates and cycle parameters varied significantly among treating physicians. Euploidy rates (expressed as a fraction of biopsied embryos) were associated ($p = 0.01$) only with the F(hMG) but not with the number of MII retrieved or other variables. On the other hand, the number of euploid embryos (in contrast to the euploidy rate) was associated with the number of MII produced. Donor euploidy rates are significantly associated with the fraction of total gonadotropin comprising human menopausal gonadotropin (or F(hMG)) during controlled ovarian hyperstimulation but are not associated with other cycle parameters. The study provides the first suggestion that patient stimulation parameters can affect the incidence of euploidy in embryos generated through the use of standard assisted reproductive techniques. The study is limited by its retrospective approach and because the aCGH analysis used is less sensitive than more recent NGS technology. Further, it provides a suggestion that the use of hMG is beneficial for obtaining euploid embryos.

1. Introduction

The success of human IVF since its earliest days has been and continues to be hampered by low efficacy. Live birth rates per transferred embryo have been generally low, encouraging practitioners to transfer more than one embryo and leading to adverse outcomes including multiple pregnancy, pre-term deliveries and increased infant mortality (Reynolds et al., 2003; Norwitz et al., 2005; Black and Bhattacharya, 2010).

Recent advances in embryo selection include trophectoderm biopsy

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https://doi.org/10.1016/j.ejmg.2019.103707
Received 1 May 2019; Accepted 19 June 2019
Available online 25 June 2019
1769-7212/

in combination with preimplantation genetic testing of all 24 chromosomes (PGT-A). This approach has been quite successful, showing that euploid embryos implant equally well at any maternal age, provided they are replaced in a thaw cycle (Harton et al., 2013; SART, 2015). Without this selection, implantation potential decreases with age, indicating that chromosome abnormalities are a major factor in failure of embryos to result in live birth with advancing age.

The low incidence of euploid embryos, especially in advanced maternal age, provides us with an impetus to search for methods that may reduce the occurrence of aneuploidy, caused by malsegregation of chromosomes during meiosis. In addition to maternal age (Hassold et al., 1980; Munné et al., 1995), chromosome abnormalities may be induced by assisted reproductive technologies (Munné et al., 2017). Aneuploidy has been attributed to numerous treatments including embryo culture conditions (Munné et al., 1997; Hunt et al., 2003; Susiarjo et al., 2007; Munné and Alikani, 2011; Sachdev et al., 2016; Hickman et al., 2016). 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 (Munné et al., 2017). This led us to question whether treatment of the donor *per se* can affect the incidence of euploidy.

In this second phase of the study, we have identified a large dataset of egg donor cycles and hormonal stimulation protocols from a single fertility center. The laboratory aspects of the procedure were common to all egg donors. Therefore, this new analysis focused on hormonal stimulation, follicle aspiration practices, and patient management by the different physicians. These variables were assessed to determine if any of the individual physician practices were associated with euploidy rate.

2. Material and methods

2.1. Patients

A total of 423 PGT-A oocyte donation cycles managed by 6 different physicians from January 2013 to April 2016 at a single fertility center were studied. PGT-A was performed by a single laboratory (CooperGenomics, Los Angeles laboratory, CA) between January 2013 and April 2016. Signed informed PGT-A consents were obtained from oocyte recipients by the referring fertility center, which also provided demographic, hormonal stimulation and follow up information on every cycle to the principal investigator in a de-identified manner. This study was approved by Aspire IRB as a retrospective study of archived clinical data with de-identification (Protocol number PGSP-2015).

2.2. Obtaining and testing samples

Trophectoderm biopsy was performed at the blastocyst stage on Day 5 or 6 of development using a laser; biopsy samples were sent to CooperGenomics for analysis by array CGH as described previously (Colls et al., 2012; Gutiérrez-Mateo et al., 2011) with minor modifications. These modifications comprised improving software versions with 24SureV3 (Illumina, San Diego, CA) between January to August 2013, and version 4.1 starting in August 2014. “Testing technology” was considered as a variable in the current analysis, as it was in the previous analysis (Munné et al., 2017).

Samples were classified as euploid (46,XX or 46,XY), aneuploid if they had an extra or missing full chromosome, or segmental abnormal if they were missing or had an extra chromosome piece above the resolution limit of the technique (6 MB in size). In a minority of cases (about 4%; [17]), mosaicism (40–60% abnormal cells) could be identified by aCGH but at the time, such embryos were diagnosed as abnormal/aneuploid. For purposes of this analysis, all chromosome abnormalities (mosaicism and aneuploidy) were grouped together as aneuploidy.

In order to avoid any bias that might be imposed by individual personnel in the genetic testing laboratory, samples are randomly analysed by different analysts. Each sample is tested and independently analysed by two different technologists. If their results do not agree, a third person, the Lab director, makes the final decision concerning the result. Furthermore, the reference laboratory performs proficiency testing. The satisfactory completion of proficiency testing is a requirement for the laboratory's certification by the New York State Department of Health.

2.3. Statistical analysis

Descriptive variables were tested for normality using Shapiro-Wilk tests. Differences between groups (physicians) were determined using ANOVA for the normally distributed variables, or Kruskal-Wallis tests for non-normal distribution. Pairwise post-hoc comparisons adjusting for multiple testing were also performed using the methods of Dunn for normally-distributed variables or Benjamini & Hochberg (Walters, 2016) for variables that were not normally distributed. Next, the set of patient treatment variables from the analysis was submitted to a principal component analysis. The strength of the association among variables was calculated as Pearson's correlation coefficients. Likewise, the p-values for trends were computed from the Pearson test.

Linear regression analysis was used to assess the relationships between stimulation parameters with the incidence of euploidy as the dependent variable. Residuals analysis was performed, and diagnostic plots were made to verify model assumptions. Data were analyzed using R version 3.1.3 (http://www.r-project.org) and the appropriate packages. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Physician-specific stimulation parameters and bivariate analyses

The incidence of euploidy (F(euploid)) was significantly different

Table 1
Summary of Cycle Parameters for each Physician.[a].

Cycle Parameter	Physician 1 N = 100	Physician 2 N = 155	Physician 3 N = 20	Physician 4 N = 6	Physician 5 N = 50	Physician 6 N = 92	P[b]
#MII	17.0 (7.83)	16.2 (6.55)	22.9 (10.3)	18.3 (5.05)	20.1 (9.61)	17.2 (8.00)	0.001
E2/MII (pg/ml/MII)	187 (130)	248 (166)	216 (93.6)	161 (77.7)	156 (84.1)	182 (99.1)	< 0.001
Dose/Day (IU/day)	259 (66.6)	264 (75.0)	238 (66.0)	304 (51.9)	246 (88.6)	380 (79.0)	< 0.001
F(hMG)	0.36 (0.11)	0.30 (0.10)	0.34 (0.08)	0.28 (0.05)	0.18 (0.15)	0.35 (0.14)	< 0.001
Duration of stimulation (days)	9.93 (1.51)	10.8 (1.55)	10.2 (1.29)	10.3 (1.03)	10.3 (1.54)	9.04 (1.38)	< 0.001
Age (years)	25.5 (2.73)	25.4 (3.17)	25.1 (2.39)	24.0 (2.45)	25.5 (2.54)	24.0 (3.18)	0.005
#embryos biopsied	9.91 (4.71)	6.44 (3.33)	9.10 (4.22)	10.2 (4.17)	11.1 (5.32)	7.15 (3.40)	< 0.001
F(euploid)	0.62 (0.17)	0.74 (0.21)	0.72 (0.16)	0.78 (0.16)	0.58 (0.19)	0.72 (0.18)	< 0.001

[a] Displaying mean (standard deviation).
[b] The probability that physician's values in the same row were not significantly different (one-way ANOVA).

Table 2
P-values resulting from the post-hoc differences among physicians for each stimulation parameter studied.

	P1 vs P2	P1 vs P3	P1 vs P4	P1 vs P5	P1 vs P6	P2 vs P3	P2 vs. P4	P2 vs P5	P2 vs P6	P3 vs P4	P3 vs. P5	P3 vs P6	P4 vs P5	P4 vs P5	P5 vs P6
MII[a]	0.646	0.066	0.646	0.167	0.894	0.036	0.616	0.066	0.649	0.628	0.628	0.066	0.894	0.646	0.167
E2/MII[b]	< 0.001	0.103	0.415	0.177	0.429	0.463	0.130	< 0.001	< 0.001	0.186	0.026	0.132	0.422	0.471	0.142
Dose/Day[a]	0.941	0.129	0.143	0.117	< 0.001	0.117	0.129	0.097	< 0.001	0.056	0.941	< 0.001	0.075	0.012	< 0.001
F(hMG).[a]	< 0.001	0.488	0.044	< 0.001	0.787	0.057	0.538	< 0.001	0.004	0.237	< 0.001	0.277	0.003	0.237	< 0.001
Duration of stimulation[a]	< 0.001	0.504	0.504	0.326	< 0.001	0.189	0.540	0.062	< 0.001	0.900	0.900	0.001	0.900	0.041	< 0.001
Age[a]	0.825	0.825	0.436	0.912	0.001	0.912	0.626	0.825	0.005	0.634	0.825	0.223	0.456	0.912	0.007
Total embryos biopsied[a]	< 0.001	0.503	0.740	0.207	< 0.001	0.003	0.062	< 0.001	0.197	0.531	0.113	0.065	0.740	0.113	< 0.001
F(euploid)[a]	< 0.001	0.044	0.091	0.438	0.001	0.692	0.795	< 0.001	0.600	0.600	0.017	0.942	0.066	0.600	< 0.001

[a] Values listed for each cycle parameter were determined using Dunn's test for normally-distributed variables using a correction for multiple testing according to Benjamini & Hochberg (Walters, 2016).
[b] Values listed for E2/MII were determined using the methods of Kruskal and Wallace (for variates that were not normally distributed).

when donors were compared among physicians managing the donor cycle (Table 1). There were significant differences among physicians with respect to donor's age, total gonadotropin administered, the fraction of gonadotropin comprising hMG (or F(hMG)), days of gonadotropin administration, estradiol per mature follicle, number of oocytes retrieved, as well as number of embryos biopsied ($P < 0.05$, ANOVA, Table 1).

We investigated whether differences in the incidence of euploidy were associated with differences in cycle parameters. The post-hoc analysis showed parameters with great variability among physicians, including F(hMG) and the number of days of gonadotropin administration (Table 2).

Principal Component Analysis (PCA) is a very useful method for reducing a complex data set. We used PCA to reveal hidden patterns within the analyzed variables among physicians. For this, all the variables included in the bivariate analysis were converted into a set of linearly uncorrelated (orthogonal) principal components (PCs). Fig. 1 is a bi-plot where each point represents a composite of the stimulation parameters and euploidy outcomes of a single cycle projected on the first 2 principal components. The first principal component (PC1) accounts for 24.4% of the variability in the data. Visual inspection showed that all six physicians grouped similarly along the first principal component (the horizontal axis). PC1 captured treatment parameters, number of MII and number of embryos biopsied.

The second principal component (PC2) accounts for 16.6% of the variability in the data. In contrast to PC1, five physicians clustered more or less together, but physician 6 was moderately separated along the second principal component (the vertical axis). PC2 captured the duration of stimulation and the gonadotropin dose per day indicating that differences in those two variables separated physician 6 from the rest of the physicians. Interestingly, the same variables appeared significantly different in the post-hoc analyses when physician 6 was compared to the other physicians.

3.2. Multiple regression analysis

Multiple regression determined which parameters were associated with the incidence of euploidy per PGT-A cycle. First, we determined the existence of multicollinearity (strong correlations) between stimulation parameters, as it may lead to an inability to deduce the relative contribution of the independent (stimulation) parameters to the final outcome (euploidy) during multivariate analysis. We therefore calculated the pairwise Pearson correlation coefficients between the stimulation parameters (Table 3). Although some significant correlations were indeed observed, MII with both E2/MII and Gnd/Day, and duration of stimulation with Gnd/day and F(hMG), only the former appeared to provide a significant multicollinearity problem. We retained E2/MII as an independent parameter and removed MII as an independent parameter in the analysis in order to avoid problems with multicollinearity.

Following the above, a model was built with stimulation parameters as predictors of euploidy. Regression analysis revealed that only one parameter was associated with the incidence of euploidy: the fraction of gonadotropin comprising hMG (F(hMG)) (Table 4). Further examination of the data (after removal of 9 cycles with F(hMG)s > 0.5 considered outliers) for association between euploidy and F(hMG) revealed that euploidy rate reached a peak at F(hMG)s of $0.45 + 0.022$ ($p = 0.018$, Davies' test) with euploidy rates declining as F(hMG) diverged from 0.45.

3.2.1. Segmented analysis

We repeated the bivariate analysis, segmenting the patients according to MII number based on the premise that more MII eggs may be obtained with higher dosages of gonadotropins. Four categories were created (4–10, 11–15, 16–20, and > 20 MIIs) and Table 5 shows their association with the stimulation parameters. Overall, the number of

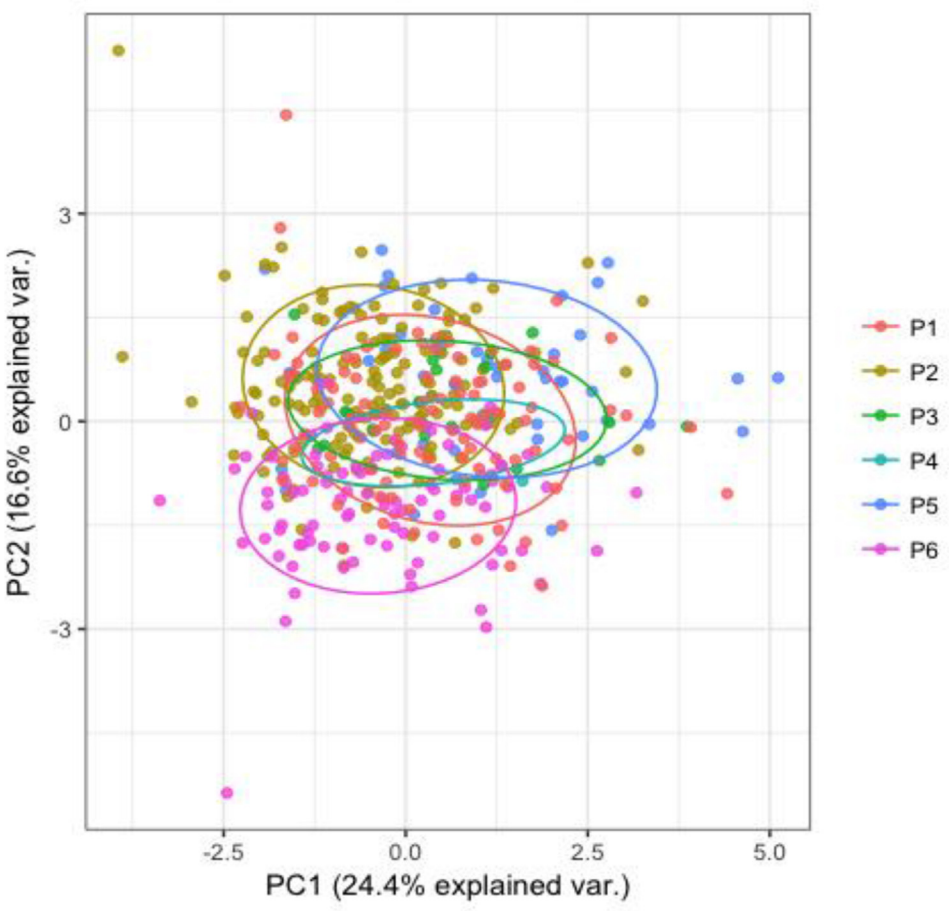

Fig. 1. Principal component analysis (PCA) of the cycle parameters and incidence of euploidy. The PCA biplot shows the PC1 and PC2 values for each PGS cycle. Each donor cycle is represented by a closed circle with a color corresponding to the physician (P1, P2, P3, P4, P5, P6) that treated the donor. Each colored ellipse encircles 68% of the closed circles of the same color for that physician. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Correlations between the independent variables[a].

	MII	E2/MII	Gnd/Day	F(hMG)	duration of stimulation	Age
MII	1.000	**−0.334**	**−0.143**	−0.043	0.012	−0.029
E2/MII		1.000	−0.071	0.087	0.073	0.087
Gnd/Day			1.000	0.074	**−0.191**	−0.022
F(hMG)				1.000	**−0.120**	−0.011
Duration of stimulation					1.000	0.095

[a] Values for Pearson correlation coefficients. Bold characters represent significant correlations at the ($p < 0.05$) level. The significance level for each of these was ($p \leq 0.01$).

Table 4
Multivariate association between incidence of euploidy and stimulation parameters.

Cycle Parameter	β[a]	Std. Error[b]	P[c]
E2/MII	0.00005	0.00007	0.496
Dose/ Day	0.00005	0.0001	0.666
F(hMG)	0.195	0.076	**0.010**
Duration of stimulation	−0.001	0.006	0.931
Age	−0.005	0.003	0.162

[a] The parameter estimating the cycle parameter's contribution to variation in the incidence of euploidy, comparable to a slope in regression analysis.
[b] The standard error of the estimate of β
[c] The probability that β was not different than zero.

MIIs was not associated with F(hMG) or the proportion of euploid embryos. The number of embryos biopsied and the absolute number of euploid embryos increased with the number of MII's. By contrast, as expected, both E2/MII and Gnd/Day decreased as the number of MIIs increased (Table 5). These trends remained relatively consistent among the 6 physicians (**Supplemental data** Table 1).

4. Discussion

4.1. Differences in euploidy rates among physicians

In a previous study, we demonstrated that euploidy rates in embryos generated from donor eggs were center-dependent (Munné et al., 2017). Taking the analysis a step further in search of reasons for these differences, we analyzed data from a single center with six practicing physicians using the same IVF and genetic laboratories. The analysis shows that euploidy rates vary among physicians, and that this variation is due to physician-specific ovarian stimulation protocols used for the donor. Specifically, the dosage of hMG appears to influence euploidy rate.

4.2. Differences in cycle parameters among physicians

There were significant differences in cycle parameters among physicians responsible for treatment of the donors. These parameters included donor age, total gonadotropin administered, the fraction of gonadotropin comprising hMG (F(hMG)), duration of gonadotropin administration, estradiol level per mature oocyte, number of oocytes retrieved and number of embryos biopsied.

It is known that a number of laboratory and environmental factors, such as culture media (Hickman et al., 2016), plastics, and volatile

Table 5
Trends in stimulation parameters and euploidy outcomes according to MII categories the physician performing the PGS cycle.[a].

Cycle Parameter	4–10 N = 86	11–15 N = 109	16–20 N = 99	> 20 N = 129	P[b]
#MII	8.16 (1.78)	13.0 (1.43)	17.8 (1.45)	27.0 (5.85)	< 0.001
E2/MII (pg/ml/MII)	294 (141)	219 (173)	182 (99.3)	152 (79.7)	< 0.001
Dose/Day (IU/day)	317 (117)	288 (71.3)	271 (81.2)	273 (86.1)	< 0.001
F(hMG)	0.35 (0.13)	0.30 (0.14)	0.31 (0.10)	0.30 (0.13)	0.038
Duration of stimulation (days)	9.93 (1.69)	10.2 (1.76)	10.4 (1.46)	10.0 (1.55)	0.667
Age (years)	25.4 (3.39)	25.0 (2.97)	25.4 (2.65)	24.7 (3.01)	0.161
# embryos biopsied	4.95 (2.07)	6.30 (2.29)	8.24 (3.27)	11.8 (4.99)	< 0.001
F(euploid)	0.68 (0.22)	0.72 (0.21)	0.68 (0.20)	0.66 (0.18)	0.128
# euploid embryos	3.29 (1.49)	4.45 (1.89)	5.52 (2.48)	7.66 (3.78)	< 0.001

[a] Values are mean (standard deviation).
[b] Probability that values in the same row were not different, indicating a trend with the number of MIIs retrieved (as described in methods).

organic compounds (Hunt et al., 2003; Susiarjo et al., 2007) can influence chromosome integrity in gametes and embryos. However, we eliminated laboratory practices as potential confounders in this study since all embryology was performed in the same laboratory and gamete and embryo treatment parameters remained unchanged throughout the study.

4.3. Associations between %euploidy and cycle parameters

Since there were significant physician-specific differences for both euploidy rates and cycle parameters, we investigated whether the cycle parameters were associated with euploidy rates. After considering all cycle parameters, multiple regression revealed that the incidence of euploidy was not associated with any of the cycle parameters except the fraction of gonadotropin that comprised hMG (F(hMG)). The main distinction between gonadotropin compounds consisting of FSH and those consisting of hMG is the presence of LH-like activity in hMG that is predominantly provided by LH (with a short half-life) and hCG (with a longer half-life). The higher incidence of euploidy occurring with greater F(hMG) suggests that LH-like activity has a positive influence on follicular/oocyte development and maturation. The observation that euploidy rate attained a significant maximum value at F(hMG) = 0.45 suggests that an optimal F(hMG) may exist for recruitment and maintenance of euploid oocytes.

The study confirmed a previous finding that euploidy rate per cycle was not associated with number of blastocysts biopsied (Ata et al., 2012). However, the study of Ata et al. (19. 2012) did not take into consideration the number of eggs retrieved. Since the number of embryos biopsied is correlated with the number of MIIs retrieved, the observed association between number of euploid embryos and number biopsied (Ata et al., 2012) would also be expected between number of euploid embryos and number of MII oocytes retrieved (consistent euploidy rate for each age group). However, we must note that there is a distinct difference between the two scenarios that may affect euploidy rates. In one scenario, patient-to-patient differences in response to consistent stimulation practices lead to retrieval of different numbers of oocytes (reported by Ata et al., [19]). In the other scenario, differences in stimulation (e.g., low vs. high doses, short vs. long duration) lead to retrieval of different numbers of oocytes. In the latter case, it can be expected that differences in treatment of the same patient may result in more or fewer oocytes. We believe that the data in this study represents the former rather than the latter scenario because a higher number of MII oocytes was associated with a lower daily dose of gonadotropin and was not associated with the duration of stimulation (see Table 3). Therefore, this study does not refute observations that milder stimulation reduces aneuploidy (Baart et al., 2007).

Medication type and dosage in ovarian stimulation have been linked to chromosome abnormalities (Baart et al., 2007; Weghofer et al., 2008; Rubio et al., 2010; Munné, 2006). However, these studies were limited by the use of FISH (low resolution), single blastomere biopsies, and non-homogeneous populations of patients. In contrast, the current study relied upon comprehensive chromosome analysis of all 24 chromosomes using trophectoderm biopsy of blastocysts from donor oocytes.

The number of euploid embryos per donor cycle was associated only with the number of MII oocytes. Two previous studies found a negative correlation between dosage of gonadotropins and pregnancy rates, with Baker et al. (2015) reporting that increasing dosages of gonadotropins were correlated with diminished live birth rates; Sunkara et al. (2011) reported that maximum pregnancy rates were achieved with an optimal number of eggs. However, both studies involved IVF cycles with transfer of fresh embryos. Therefore, it remains unclear whether the negative effects of higher egg numbers are a result of high gonadotropin dosage on the endometrium or the oocytes. The increasing practice of deferring transfer to a frozen cycle (Maheshwari et al., 2018) is predicated on the notion that the endometrial environment in fresh IVF cycles is not optimal.

In this study we have shown that the proportion of euploid blastocysts is associated with F(hMG) dosage but independent of the number of MII eggs in oocyte donors. In contrast, the absolute number of euploid blastocysts is associated with the number of MII eggs retrieved and is independent of F(hMG). These observations may help fine-tune strategies for egg donor ovarian stimulation during ART that promote both safety and efficacy.

4.4. Limitations

The study has several limitations. One, it is a retrospective observational study and other uncontrolled variables may have affected the outcomes; therefore we can detect associations but not causality. For example, in this study we could not eliminate the effect of F(hMG) from the size of the follicles being aspirated or the technique used for aspiration. Follicular size was not recorded per egg retrieved since many eggs are aspirated into the same tube and henceforth are indistinguishable.

Another limitation of the study is the technology used. All the embryos were analyzed by array CGH, while a more complete analysis, such as NGS, would have been able to determine more subtle differences in chromosome abnormalities by differentiating some aneuploidies from mosaicism. However, even the use of next generation sequencing may be incapable of distinguishing aneuploidy from mosaicism in some blastocysts since the small biopsy sample may be taken from a region of the trophectoderm that is euploid or aneuploid and not mosaic even if other regions of the blastocyst actually would prove it to be mosaic. While aneuploidy is known to increase with maternal age, mosaicism is associated with embryo morphology and since mosaicism occurs during mitosis (in the lab), it may be influenced by laboratory conditions. Here we controlled for both age and culture conditions, but we still do not know if F(hMG) dosage directly influences aneuploidy or mosaicism. A previous study (Sachdev et al., 2016) showed differences

between centers in egg donor cycles not only in aneuploidy but also in mosaicism. Because DNA samples stored for over 6 months at −20C tend to degrade, reanalysis of the DNA samples with NGS for this study is not feasible.

5. Summary

This is the third study beginning with Munné et al., 2007, 2017 to show an association between center-specific ART treatment practices and the incidence of chromosome abnormality in human embryos. Prospective randomized and blinded clinical trials are needed to corroborate these findings and obviate the problems of bias and confounding by inter-dependent variables.

Authors' roles

David H. McCulloh and Jose Maria Arbones performed data and statistical analyses and participated in writing of the manuscript. Mina Alikani participated in writing of the manuscript and discussion of results. John Norian and Bradford Kolb participated in assembly of data and writing of the manuscript. Santiago Munné participated in assembly of data, data analysis and writing of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmg.2019.103707.

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