

## Article

# Significant correlation between anti-müllerian hormone and embryo euploidy in a subpopulation of infertile patients

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## KEY MESSAGE

The current study demonstrates a significant inverse association between AMH and blastocyst euploidy among infertile couples with female age younger than 37 years old treated by IVF and pre-implantation genetic screening for all 24 chromosomes. Clinical outcome was similar after euploid blastocyst transfer regardless of maternal age.

## ABSTRACT

Anti-Müllerian hormone (AMH) is a standard marker of ovarian reserve. Correlation between AMH and egg euploidy is controversial. We evaluated the association between AMH and blastocyst euploidy rate examined by pre-implantation genetic screening (PGS). This retrospective study was conducted at the CReATe Fertility Centre. We included single IVF cycles of 216 infertile couples, which resulted in 911 blastocysts subjected to array comparative genomic hybridization and evaluated IVF outcome after embryo transfer. The average age and median AMH of female patients were 37.2 (SD = 3.8) and 20 pmol/l, respectively, and the average euploidy rate was 38.3%. Using multivariate regression controlling for age, antral follicle count, body mass index and parity, there was a significant association between serum AMH and proportion of euploid embryos ( $P = 0.02$ ), due to the dominant  $\leq 36$  age group in which significant correlation between AMH and euploidy rate ( $P = 0.02$ ) was demonstrated. Clinical outcome was similar, including biochemical, clinical and ongoing pregnancy rates as well as pregnancy loss. This study shows a correlation between AMH and aneuploidy rate, specifically among infertile patients younger than 37 years old. Study limitations are discussed.

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## Introduction

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor  $\beta$  (TNF- $\beta$ ) family. It was initially described in males as a regression factor for the Müllerian ducts, the precursors of female internal genitalia (Behringer, 1994; Lee et al., 1996). In females, AMH is produced by granulosa cells of early follicles, regardless of endogenous or exogenous gonadotrophin stimulation and therefore remains relatively consistent within and between menstrual cycles in both healthy ovulating women and among infertile patients. Because AMH is produced by resting follicles before cyclical recruitment, it represents the resting follicular pool and is a clinically useful tool to assess ovarian reserve (Kevenaar et al., 2006) in infertile populations prior to attempting treatment, as well as for menopause prediction (Broer et al., 2011) and to assess ovarian damage prior to chemotherapy exposure, radiation therapy or surgery (Dewailly et al., 2014). AMH levels have been correlated with oocyte yield, implantation rate, risk of ovarian hyperstimulation syndrome (OHSS), and probability of poor response in both autologous and egg donor cycles (Riggs et al., 2008, 2011). Numerous studies have demonstrated a linear correlation between AMH and number of retrieved oocytes after ovarian stimulation (Choi et al., 2011; La Marca et al., 2010; Nelson et al., 2007).

In addition to quantitative ovarian reserve correlation, it has been speculated that AMH levels may be associated with egg and embryo quality, with respect to egg (and consequently embryo) euploidy. Although higher AMH has been found to be associated with improved implantation, pregnancy and live birth rates (Lukaszuk et al., 2014; Tal et al., 2015), these findings can be explained by higher egg quantity and not necessarily by egg quality. The current data regarding that matter remains controversial and inconsistent. Some studies focused on AMH measured during pregnancy and its relation to prenatal tests for aneuploidy (Plante et al., 2010) and early pregnancy loss (Shim et al., 2015), and therefore could not be generalized to infertile populations. A recent publication demonstrated reduced AMH among patients with recurrent pregnancy loss compared with age-matched controls (Atasever et al., 2016). These findings suggest that AMH level may be correlated with the risk of pregnancy loss as a possible reflection of egg euploidy. Contrary to these findings, Lie Fong et al. (2008) found no correlation between AMH and day 3 embryo euploidy.

One of the major challenges of evaluating the association between AMH and egg quality is the need to quarantine the impact of the patient's age. There is a clear relationship between age and serum AMH levels (Kelsey et al., 2011). It is also well known that the incidence of early pregnancy loss increases for women over 35 years, and that most of these losses are secondary to chromosomally abnormal embryos (Hassold and Hunt, 2001), because older female age is associated with an increasing embryo aneuploidy rate (Hassold and Hunt, 2001). However, the available data regarding the predictive value of AMH for embryo aneuploidy is still unclear as there are very few studies assessing this relationship, resulting in conflicting results (Gleicher et al., 2012; Lie Fong et al., 2008; Shim et al., 2015). The primary aim of this study was to evaluate the association between AMH levels and proportion of aneuploid blastocysts among infertile women undergoing IVF and pre-implantation genetic screening (PGS) for 24 chromosomes. We postulated that serum AMH would be an independent predictor of proportion of euploid blastocysts. The secondary objective was to evaluate the possible impact of AMH and euploidy rate on clinical outcome after transfer of euploid blastocysts.

## Materials and methods

### Population

This retrospective study included patients who underwent IVF with PGS between 1 January 2009 and 30 June 2016 at the CReATe Fertility Centre, a university-affiliated infertility clinic. All couples underwent detailed evaluation before IVF, including medical history, physical examination, pelvic ultrasound, semen analysis and uterine cavity assessment. Additional testing such as sonohysterogram was performed as needed. The first IVF-PGS cycle was included for each couple. The patient population was divided into three age-related subgroups: up to 36 years old (Group 1), 37–40 years old (Group 2) and  $\geq 41$  years old (Group 3).

### AMH assay

Serum AMH quantification was performed in our clinic's biochemistry laboratory before IVF cycle initiation. The Beckman-Coulter AMH Gen II ELISA (Beckman-Coulter, Immunotech, Webster, TX, USA) was used between 2009 and 2014 and the ultra-sensitive AMH/MIS ELISA Kit (AnshLabs, Webster, TX, USA) during 2015 and 2016, according to the manufacturer's instructions. The lowest reported detected AMH concentrations were 0.08 and 0.023 ng/ml (equal to 0.57 and 0.16 pmol/l, respectively, according to conversion factor 7.14 (Satwik et al., 2012)) in the Beckman-Coulter AMH Gen II ELISA and the ultra-sensitive AMH/MIS ELISA, respectively. The reported intra-assay coefficient of variation was  $<5.4\%$  and  $<4.0\%$  and the inter-assay coefficient of variation was  $<5.6\%$  and  $<4.79\%$ , respectively. Serum samples were collected from patients, batched and stored at  $-20^{\circ}\text{C}$  according to the manufacturer's instructions. AMH assays were routinely performed once or twice a week on all batched samples. Samples with extremely low values (below 2.2 pmol/l (Satwik et al., 2012)) were re-tested two or three times for validation. There were two validation controls per kit.

### Ovarian stimulation

Controlled ovarian hyperstimulation protocols for IVF included a standard long agonist-based protocol and a standard antagonist-based protocol. In most cases, the GnRH antagonist protocol was the first line of treatment due to its advantages of reducing the risk of OHSS among high responders (Gat et al., 2015), comparable efficacy and favourable patient tolerance (The European Middle East Orgalutran Study Group, 2001). The exact dosing regimen was determined according to pre-stimulation assessment parameters including age, AMH, antral follicle count (AFC), day 3 FSH, BMI, response to previous stimulation, infertility diagnosis (i.e. male factor, tubal, etc.), previous pregnancy history (primary versus secondary infertility), OHSS risk and physician preference. In most cases, the initial gonadotrophin dose in the first cycle was 150–300 units/day, while in repeated cycles adjustments were made such as increasing up to 450 units per day in poor responders or lower doses among high responders anticipated for OHSS. However, a minimal stimulation protocol has been selected for patients with low ovarian reserve as previously described (Lazer et al., 2014). After oocyte retrieval, fertilization was achieved through ICSI of mature (metaphase II) oocytes. Fertilization rate was calculated as the number of oocytes exhibiting two visible pronuclei (2PN) divided by the number of mature oocytes injected

during ICSI. The decision to culture embryos to day 5 versus day 3 transfer was made by the attending physician after detailed discussion with the patient and embryologist. A major consideration in this decision was the number of viable embryos on day 3. All morphologically viable blastocysts were biopsied on day 5 or 6 depending on stage of development, as described previously (Balakier et al., 2016). Because eventually our population included only blastocysts biopsied and transferred, data regarding day 3 embryos were beyond the scope of the study.

### Pre-implantation genetic screening

PGS analysis was performed by array comparative genomic hybridization (aCGH). In particular the 24sure microarray (BlueGnome Ltd, Illumina) was used, which is a bacterial artificial chromosome (BAC) array with >5000 DNA clones not in disease or known copy number variant (CNV) regions. From 2009 to the end of 2013, samples were sent to Genesis Genetics (Plymouth, MI, USA) for analysis using BlueGnome technology and protocols followed by BlueFuse Multi™ software (Illumina Inc., San Diego, CA, USA) for data analysis. Since January 2014, samples have been analysed by the laboratory at the CREaTe Fertility Centre using the same technology, protocols and software.

### Embryo transfers (ET)

Until December 2014 euploid blastocysts were transferred in either fresh or frozen (FET) cycles according to clinical decision. Fresh ET were performed on the day after biopsy accompanied by progesterone supplementation for luteal phase support [progesterone suppositories 200 mg three times daily (MEDISCA Pharmaceuticals, Quebec, Canada, compounded by a licenced pharmacist)]. Since January 2015 all transfers were FET after endometrial preparation by exogenous oestrogen supplementation [6–12 mg daily, taken orally (Acerus Pharmaceuticals Corp., Mississauga, Canada) or 100 mg every other day by dermal patches (Sandoz)] followed by progesterone [100 mg intramuscularly once a day or suppositories 200 mg two to three times a day (Medisca Pharmaceuticals, compounded by a licenced pharmacist)]. The number of embryos transferred was determined by the number available as well as by patient age and clinical history.

Biochemical, clinical and ongoing pregnancies were defined as positive human chorionic gonadotrophin (HCG) in urine/serum, fetal heart pulse on ultrasound at 7–8 gestation weeks and viable pregnancy on nuchal translucency performed between 11 and 13 weeks of gestation, respectively. Pregnancy loss was defined as loss of pregnancy after diagnosis of a biochemical pregnancy.

### Statistical analysis

Continuous variables were expressed as mean values  $\pm$  standard deviation, or median values with interquartile ranges for non-normally distributed variables, while categorical variables were expressed as frequencies (percentages). Continuous variables were compared between age groups using analysis of variance (ANOVA), and categorical variables were compared using Fisher's exact test. The primary outcome was the proportion of euploid/total biopsied embryos, accompanied by the calculation of simple and complex aneuploidy rates. Secondary endpoints included stimulation outcome (such as number of oocytes retrieved, fertilization rate and number of embryos

available for biopsy) and clinical outcome (biochemical, clinical and ongoing pregnancies as well as pregnancy loss).

The proportion of euploid embryos was calculated as the number of embryos reported as euploid divided by the number of embryos with ploidy status. Cases of degraded DNA or unclear euploidy status were excluded. Simple and complex aneuploidy rates were defined as the ratio between blastocysts with single or multiple chromosomal abnormalities, respectively, divided by total aneuploid embryos per patient.

To investigate the relationship between AMH and the primary outcome, logistic regression analysis was used adjusting for the following covariates: age, BMI, AFC, parity and infertility duration. We repeated the analysis stratifying by age group:  $\leq 36$  years, 37–40 years, and  $\geq 41$  years including 91, 81 and 44 patients in each group, respectively, with a total of 216 patients. These categories were generated based on the number of patients in each age group and age relevance on reproductive function. All statistical tests were two-sided and evaluated at the 0.05 level of significance. Data were analysed using the R statistical software (CRAN, Version 3.2.2).

In order to determine whether the sample size was large enough to detect significant differences, we performed a power calculation as follows. First, we verified the lack of age variance significant differences between the age groups, so the ANOVA power test could be used. Second, assuming a power of 80% and significance level of 5% the required size of each group was 84.

### Ethical approval

The study was approved by the University of Toronto Research Ethics Board on 11 August 2014 (reference number 30444).

## Results

The study included the first IVF-PGS cycle of 216 infertile couples, resulting in a total of 911 blastocysts examined by PGS-aCGH. The average age of female patients was 37.2 (SD 3.8) and median AMH was 20 pmol/L. The  $\leq 36$ -year-old and 37–40 year old groups (Groups 1 and 2, respectively) included 91 and 81 patients, respectively, and the cohort of  $\geq 41$ -year-old patients (Group 3) included 44 couples. Both ovarian reserve markers (AMH and AFC) decreased significantly with age ( $P = 0.05$  and  $P = 0.0001$ , respectively). The most notable demographical significant difference ( $P < 0.0001$ ) was the increased incidence of reduced ovarian reserve among Group 3 compared with Groups 1 and 2 (Table 1).

Ovarian responses to stimulation differed significantly between the groups. Group 3 ( $\geq 41$  years) was characterized by significantly higher gonadotrophin consumption ( $3899 \pm 1472$  units,  $P = 0.01$ ), as well as lower number of oocytes retrieved and developed blastocysts ( $12.4 \pm 7.5$  and  $2.7 \pm 1.7$ , respectively) compared with Group 1 ( $18.6 \pm 10.1$  and  $5 \pm 3$ , respectively) and Group 2 ( $16.7 \pm 11$  and  $4.1 \pm 2.8$ ,  $P = 0.004$  and  $P < 0.001$ , respectively, Table 2).

PGS outcome was characterized by significant inverse association between euploidy rate and age. While the total euploidy rate was 38.3%, it was highest among Group 1 (49.2%), decreased to 34.6% in Group 2, while the lowest rate was 22.6% in Group 3 ( $P < 0.05$ ). Of all aneuploid embryos in Groups 1 and 2, 40.4% and 49.5%, respectively, had complex aneuploidy, compared with 69.9% in Group 3 ( $P = 0.013$ , Figure 1). Using multivariate regression controlling for

Table 1 – Demographic parameters.

	Total	≤36 years	37–40 years	≥41 years	P-value
Number of patients	216	91	81	44	
Number of biopsied embryos	911	459	332	120	
Age (years)	37.2 ± 3.8	33.5 ± 2.4	38.7 ± 1.0	42 ± 1.1	<0.0001
AFC	20 ± 11.3	24 ± 13.4	20.9 ± 10.5	14.5 ± 6.7	0.0001
AMH (pmol/L, median and range) <sup>a</sup>	20 [2.4–178.2]	24 [3.6–178.2]	21.5 [2.4–150]	16.1 [2.8–33.3]	0.05
BMI	23.2 ± 4.4	23.0 ± 3.2	23.3 ± 4.2	23.8 ± 6.4	NS
Infertility duration (months)	29.4 ± 24.8	29.0 ± 21.3	30.8 ± 27.8	27.5 ± 26.7	NS
Diagnosis (% of patients)					
Decreased ovarian reserve <sup>b</sup>	68 (32.7)	12 (13.8)	28 (36.4)	28 (63.6)	<0.0001
Ovulation dysfunction	35 (16.8)	19 (21.8)	12 (15.6)	4 (9.1)	
Male factor	35 (16.8)	17 (19.5)	10 (13.0)	8 (18.2)	
Tubal factor	9 (4.3)	3 (3.4)	5 (6.5)	1 (2.3)	
Uterine	15 (7.2)	5 (5.7)	9 (11.7)	1 (2.3)	
Other	46 (22.1)	31 (35.6)	13 (16.9)	2 (4.5)	

<sup>a</sup> AMH is reported as median and range due to high variability.  
<sup>b</sup> AMH <10 pmol/L.  
Values expressed as mean ± SD unless stated otherwise.  
AMH = anti-Müllerian hormone; AFC = antral follicle count; BMI = body mass index; NS = not statistically significant.

Table 2 – Clinical parameters.

	Total	≤36 years	37–40 years	≥41 years	P-value
Days of stimulation	10.3 ± 1.8	10.5 ± 1.9	10.0 ± 1.8	10.3 ± 1.6	NS
Total dosage of gonadotrophins	3404 ± 1272	3233 ± 1207	3326 ± 1173	3899 ± 1472	0.01
Oestradiol on trigger day (pmol/L)	11836 ± 9022	12703 ± 7998	12100 ± 8471	9559 ± 6709	NS
Retrieved oocytes	16.6 ± 10.2	18.6 ± 10.1	16.7 ± 11.0	12.4 ± 7.5	0.004
Mature oocytes (MII)	10.8 ± 6.4	12.1 ± 6.7	11.0 ± 6.9	7.9 ± 3.7	0.001
Fertilized oocytes (2PN)	7.8 ± 4.9	8.7 ± 4.8	7.8 ± 5.5	6.1 ± 3.2	0.013
No. of blastocysts	4.2 ± 2.8	5.0 ± 3.0	4.1 ± 2.8	2.7 ± 1.7	<0.001

Values expressed as mean ± SD.  
NS = not statistically significant.

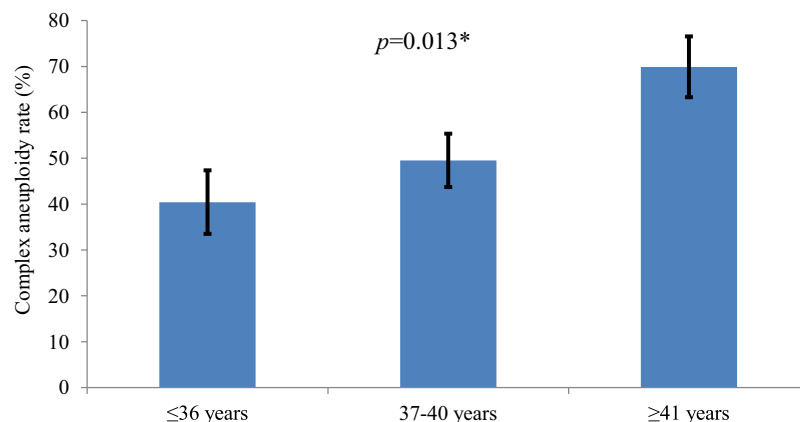


Figure 1 – Complex aneuploidy rate among the three age subgroups. Values are expressed as average ± standard error.

age, AFC, BMI and parity, there was a statistical association between serum AMH and proportion of euploid embryos (OR = 1.08; 95% CI: 1.01–1.15;  $P = 0.02$ ) (Table 3). After stratifying by age, we observed an association between AMH and proportion of euploid embryos only in the ≤36-year-old age group (OR = 1.09; 95% CI: 1.01–1.17;  $P = 0.02$ ). In other words, an increased AMH of 10 pmol/L in this patient group is correlated with an improved euploidy rate of 9%.

In order to assess the clinical relevance of AMH correlation with blastocyst euploidy, we consequently evaluated the clinical outcome of the IVF-PGS cycles that resulted in ET. Not surprisingly, patients younger than 37 had significantly higher ET per oocyte retrieval (OR) (72.5%) compared with Groups 2 and 3 (55.6% and 40.9%, respectively,  $P = 0.001$ ). Sixty-six ET were performed in Group 1 compared with 45 and 18 in Groups 2 and 3, respectively. The average number



**Table 3 – Regression coefficients for the binomial regression of the proportion of euploid embryos with AMH as covariate, overall and by age group, controlling for age, AFC, BMI and parity.**

Model/subset	Increase in odds of euploid embryos <sup>a</sup>	P-value
Total	1.08 (1.01–1.15)	0.02
≤36 years	1.09 (1.01–1.17)	0.02
37–40 years	1.15 (0.94–1.40)	NS
≥41 years	0.82 (0.33–1.89)	NS

<sup>a</sup> Per 10 pmol/l increase in AMH.

AMH = anti-Müllerian hormone; AFC = antral follicle count; BMI = body mass index; NS = not statistically significant.

**Table 4 – Clinical outcome.**

	Total	≤36 years	37–40 years	≥41 years
Total transfers	129	66	45	18
ET per OR (%) <sup>a</sup>	60.0	72.5	55.6	40.9
Average embryos per transfer	1.33	1.35	1.38	1.11
Biochemical PR per transfer (%)	47.0	48.5	42.2	50.0
Clinical PR per transfer (%)	43.0	44.0	40.0	50.0
Ongoing PR per transfer (%)	39.4	42.4	40.0	44.4
Pregnancy loss (%)	10	12.5	5.3	11.1

<sup>a</sup>  $P = 0.001$ .

ET = embryo transfers; OR = oocyte retrieval; PR = .

of transferred embryos was 1.33 without significant differences between the groups. Pregnancy rate was calculated for the first ET per OR only, rather than cumulative pregnancy rate, to prevent age-related differences in total ET per group. We observed overall similar biochemical, clinical or ongoing pregnancy rates per transfer as well as pregnancy loss between the three subgroups (Table 4).

## Discussion

Fetal aneuploidy, mainly resulting from oocyte aneuploidy, is the most common cause of spontaneous loss, particularly in the first trimester of pregnancy (Holubcova et al., 2015; Hyde and Schust, 2015). Therefore, it is not surprising that ovum euploidy is crucial to achieving and maintaining viable pregnancy. While the association between female age with both AMH (Van Rooij et al., 2002) and egg quality (Hourvitz et al., 2009) is well known, and although AMH reliability as ovarian reserve biomarker has been confirmed (Gat et al., 2015), the specific and direct association between AMH and egg quality is in doubt. To the best of our knowledge, this is the first reported correlation between AMH and blastocyst euploidy rate specifically among patients younger than 37 years old undergoing IVF–PGS.

Previous researchers have tried to investigate the possible association between AMH and egg quality in various methodologies such as FISH and biopsies performed on day 3 embryos, leading to conflicting results. The current research included 911 biopsied embryos in 216 IVF/ICSI cycles. As opposed to previous reports, which focused on different populations such as pregnant patients (Atasever et al., 2016; Irez et al., 2011; Plante et al., 2010; Shim et al., 2015), our conclusions are based on an infertile cohort. The high sample size enabled

detailed age-independent evaluation. Furthermore, our PGS–aCGH allowed us not only to perform blastocyst stage biopsy, which today represents the safest approach (Cimadomo et al., 2016), but also included comprehensive 24 chromosomal evaluation.

Lie Fong et al. (2008) reported contradictory results in similar age groups (younger than 38 years old) by examination of day 3 embryos with only nine chromosomes by FISH. On the contrary, the current report included blastocyst biopsies with 24 chromosome evaluation by the more advanced aCGH methodology. Moreover, euploidy rate comparison between day 3 embryos and blastocysts is far from being adequate because aneuploid day 3 embryos have an increased risk of development arrest prior to achieving blastocyst stage (Magli et al., 2000). Therefore, this study is relevant and important regarding the specific correlation between blastocyst euploidy and AMH. Embryo euploidy rate declined continuously among all three groups accompanied by increasing complex aneuploidy rate (Figure 1), confirming the well-known association between age and the risk of embryo aneuploidy.

To the best of our knowledge, the current findings regarding the age-related selective AMH correlation with egg quality have never been described. Interestingly, Al-Edani et al. (2014) have reported a similar age-selectivity effect on AMH expression in human cumulus cells. Expression of AMH and other proteins involved in the TGF- $\beta$  signalling pathways were significantly reduced among patients older than 37 compared with younger ages. These proteins are essential for follicular development and oocyte maturation (Knight and Glistner, 2003). Therefore, the preserved AMH expression in cumulus cells among patients younger than 37 years old may contribute to better egg quality compared with older patients. Our clinical observation emphasizes the role of AMH among patients of these ages. In other words, the Al-Edani et al. (2014) report may supply the molecular explanation for the age selectivity observed in the current clinical study.

Aggressive gonadotrophin stimulations have been suggested to increase the risk of embryo aneuploidy among infertile patients younger than 37 years old (Baart et al., 2007) and egg donors (Rubio et al., 2010). On the other hand, a similar incidence of chromosomally abnormal embryos was reported, independent of the number of retrieved oocytes, in both patients with advanced maternal age and recurrent implantation failure (Gianaroli et al., 2000). We found increasing stimulation doses due to lower ovarian reserve accompanied by decreased euploidy rate and increased complex aneuploidy among older patients. However, specific investigation of the association between stimulation doses and euploidy rate was beyond the scope of the current study.

AMH is considered as an indicator for fertility and pregnancy outcome in assisted reproductive technology cycles (Meczekalski et al., 2016). The traditional explanation is that the higher number of retrieved oocytes leads to more developing embryos, resulting in a higher number of ET per oocyte retrieval with the inclusion of cryopreserved ET, leading to a higher cumulative ongoing pregnancy rate (Nelson et al., 2015). Our results demonstrate that AMH is not solely related to oocyte quantity, but also has some significant correlation with egg quality among patients younger than 37 years old. Importantly, we found comparable clinical outcome regardless of a patient's age once euploid ET has been performed. These findings emphasize that clinical outcome after euploid ET is not necessarily related to maternal age.

A prominent limitation of the current research arises from the high AMH, especially among Group 3 (median 16.1 pmol/l). This may be related to selection bias because patients with high AMH levels may

be favoured towards extended cultures to blastocysts and PGS compared with day 3 ET in patients with lower AMH values and lower number of embryos. This assumption is supported by Kushnir et al. [2016], who reported a higher rate of ET in IVF cycles that included PGS compared with non-PGS cycles. They reported that patients older than 37 with PGS had significantly higher transfers per oocyte retrieval (53.1%) compared with the non-PGS population (41.9%,  $P < 0.0001$ ) [Kushnir et al., 2016]. Therefore, patients referred to PGS may have preliminary higher ovarian reserve, which may explain the relatively high AMH in our cohort. A second limitation arises from the relatively small number of ET in Group 3 (including only 44 observations) which prevented regression analysis assessment being performed for the possible correlation between AMH and clinical outcome. However, we feel that the focus on pregnancy rates after single ET rather than cumulative pregnancy rate is a convincing demonstration of the lack of age impact on clinical outcome once euploid ET has been performed. Because no significant correlation has been detected between AMH and euploidy rate in Group 2, it is reasonable to assume that significant correlation may not be found among the older patients in Group 3. However, a multi-centre study is required to recruit such a large sample size of patients with euploid blastocysts in that age group. Lastly, the AMH measurement assay was changed within the long study period. The importance of AMH among various clinical settings such as infertility, pregnancy and menopause [Kedem et al., 2014; La Marca et al., 2004, 2005a, 2005b, 2006] has led to enormous efforts to improve the quality and accuracy of AMH assays [Pigny et al., 2016], especially because AMH may not be stable under some storage or assay conditions [Rustamov et al., 2012]. For example, Tadros et al. [2016] have recently reported that fully automated AMH assays have better correlation with AFC, specifically in patients with reduced ovarian reserve. Therefore, although measurement by consistent single assay may seem favourable, we believe that the adoption of the more reliable ultra-sensitive kit encompasses benefits that overcome the negative effect of assay switching within the study time period. Similarly, the change of software analysis during the study period should be considered as a confounder to our data.

In conclusion, the importance and role of AMH continues to be elucidated. To our knowledge, this is the first report of significant correlation between AMH and aneuploidy rate using comprehensive chromosome screening for 24 chromosome analysis. Herein we demonstrate a positive correlation between serum AMH and euploidy rate among blastocysts of infertile patients younger than 37 years of age. The explicit mechanism by which AMH is associated with egg quality specifically in that age group should be investigated.

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