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2 **Female age, serum anti-Mullerian Hormone (AMH) and the number of oocytes affect the rate**  
3 **and number of euploid blastocysts in IVF/ICSI cycles**

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7  
8 Running Title: AMH and blastocyst euploidy in IVF/ICSI cycles

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25  
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27 **Abstract**

28 **Objective:** To study the relative role of female age and ovarian reserve, measured through serum  
29 anti-Mullerian Hormone (AMH) in determining the rate and number of euploid blastocysts in  
30 IVF/ICSI cycles

31 **Design:** Retrospective analysis of cycles performed in 2014-15

32 **Setting:** A tertiary referral IVF center

33 **Patients:** 578 infertile couples undergoing IVF/ICSI and PGS analysis

34 **Interventions:** All embryos were cultured and biopsied at the blastocyst stage. The method  
35 involved whole genome amplification followed by array-CGH. Serum AMH was measured with  
36 the modified AMH generation II assay.

37 **Main Outcome Measures:** The rate and number of euploid blastocysts and their correlation with  
38 ovarian reserve and response to stimulation

39 **Results:** The mean ( $\pm$ SD) age of patients was  $37.6\pm 4.1$  years and the mean number of blastocyst  
40 per patient was  $3.1\pm 2$ . The total number of blastocysts available to the analysis was 1814 and 36%  
41 of them were euploid after PGS. Age and serum AMH were significantly and independently related  
42 to the rate of euploid blastocysts available for patients. As an effect of the cohort size, the number  
43 of mature oocytes positively affected the total number of euploid blastocysts per patient.

44 **Conclusions** In this study a strong positive age-independent relationship between AMH and the  
45 rate of euploid blastocysts has been found. This confirms that the measurement of ovarian reserve  
46 by means of AMH has high relevance when counselling infertile patients.

47 **Key words:** AMH, mature oocytes, female age, IVF/ICSI, euploid blastocyst

48

49 **Introduction**

50

51 The age associated decline in female reproductive function due to the reduction of the ovarian  
52 reserve and the quality of oocytes has been well established. The age related reduction in ovarian  
53 reserve has been well established and very clearly reflected by the continuous decrease in the ovarian  
54 primordial follicle pool with ageing through atresia or due to ovulatory recruitment. As a  
55 consequence the pool of follicles becomes exhausted, and the reproductive function is considered  
56 ultimate (1,2).

57 Oocyte quality is a more complex characteristic of ovarian reserve to be defined and measured  
58 rather than quantity. Nowadays the association between female age and declining fertility is thought  
59 to be the result of abnormalities in the oocyte in the first place. The association between advancing  
60 maternal age and increased risk of chromosomal abnormality in the embryos is well known; the  
61 association is mainly due to chromosomal abnormalities occurring in the egg. The abnormalities  
62 include modifications in the mechanism for assembly of the meiotic spindle, leading to errors in  
63 chromosome alignment and the microtubule matrix (3), increased rates of chromosome  
64 degeneration into unassociated chromatids (4) and increased rates of chromosome non-disjunction  
65 (5). Aneuploidy affects more than half of human embryos and it is the main reason for implantation  
66 failure and miscarriages in IVF/ICSI cycles (6-8). In a previous study performed in our centre (9),  
67 we found that the aneuploidy rate increases by about 10% per year of female age, with 48.1%,  
68 41.3%, 29.7% and 10.3% of euploid blastocysts in patients with mean female age  $\leq 32$ , 33-36, 37-  
69 41 and  $\geq 42$  years old, respectively. This was in line with what reported by Franasiak and  
70 collaborators (10), who performed a study on 15169 consecutive trophoctoderm biopsies from 2701  
71 patients with female age ranging from 22 to 49 years (10). They found that the rate of aneuploidy  
72 rose steadily with age, showing the lowest risk for embryonic aneuploidy between ages 26 and 30,  
73 while older women had an increased risk for aneuploidy (10).

74 Both quantitative and qualitative aspects of the ovarian reserve are inversely related to age, hence  
75 the relationship existing between quantity and quality may only be indirect and due to their strong  
76 relationship with the third variable, namely female age. However there is the possibility that they  
77 may also be directly related; hence independently of age, a reduced ovarian reserve may lead to  
78 reduced oocyte quality. Infact some evidence in literature indicates a direct link between quantity  
79 and quality of ovarian reserve. Mothers of children with trisomy 21 had significantly higher serum  
80 levels of FSH (indicating low ovarian reserve) than age-matched controls (11,12). Similarly women  
81 with aneuploid spontaneous miscarriage had a reduced ovarian reserve compared to women with  
82 euploid miscarriage (13). Thus, we can make the assumption that a reduced ovarian reserve may be  
83 *per se* associated to an accelerated onset of oocyte aneuploidy leading to increase in the aneuploidy  
84 rate in embryos.

85 Ovarian reserve can now be accurately measured in vivo by measuring circulating levels of Anti-  
86 Mullerian Hormone (AMH). This hormone is produced and secreted by granulosa cells of primary  
87 and mainly secondary follicles up to the stage of 6-8 millimeters (14). Serum AMH concentrations  
88 are strongly related to the pool of primordial follicles and decline with advancing female age  
89 becoming undetectable some years before menopause. Due to the strong relationship with ovarian  
90 reserve, serum AMH measurement may allow to predict the age at menopause and the number of  
91 retrieved oocytes (15, 16).

92 In the present study we aimed to investigate the relative role of female age and ovarian reserve, as  
93 measured by serum AMH, in determining the rate of euploid embryos and the probability of having  
94 at least one euploid blastocyst in patients undergoing IVF/ICSI cycles.

95

## 96 **Materials and Methods**

### 97 *Study subjects*

98 This is a retrospective analysis of ART cycles performed at the European Hospital, Rome, Italy  
99 during 2014 and 2015. Only patients with AMH measured before the treatment and with complete  
100 patient records on clinical, IVF/ICSI cycle characteristics, Preimplantation Genetic Screening  
101 (PGS) analysis could be included in the retrospective analysis. PGS was proposed to the couples  
102 for the following reasons: advanced maternal age, recurrent miscarriage, repeated implantation  
103 failure, severe male infertility and finally to all good prognosis patients who desire information  
104 regarding the health status of their embryos. Data were obtained without applying any particular  
105 exclusion criteria regarding baseline characteristics of patients such as age, main cause of infertility,  
106 ovarian reserve or body mass index (BMI). In total data from 578 patients were obtained for the  
107 analysis. Data are reported in table 1.

108

### 109 *IVF/ICSI treatment protocol*

110 Both the standard GnRH agonist and antagonist protocol were used for controlled ovarian  
111 stimulation. Briefly, the long GnRH agonist protocol was based on the administration of daily  
112 leuprorelin or triptorelin (Enantone die, Takeda, Italy; Fertipeptil, Ferring, Italy) on the mid-luteal  
113 phase before the stimulation cycle. The administration of recombinant FSH (rFSH; GonalF Merck  
114 Serono, Italy and Puregon, MSD, Italy) or human menopausal gonadotropin (hMG, Meropur,  
115 Ferring, Italy) or urinary FSH (Fostimon, IBSA, Switzerland) was started when pituitary  
116 desensitization was achieved (~14 days after the initiation of GnRH agonists), as evidenced by the  
117 absence of ovarian follicles >10mm and endometrial thickness <4 mm on transvaginal ultrasound  
118 examination. In the GnRH antagonist protocol (Cetrotide, Merck Serono, Italy, Orgalutran, MSD,  
119 Italy) treatment with FSH or hMG was started on day 2-3 of the stimulation cycle and the GnRH  
120 antagonist was added when one or more follicles had reached a diameter  $\geq$  14mm. The starting dose

121 of FSH/hMG was based on age, body weight and ovarian reserve thus ranging between 100 to 225  
122 IU per day as per internal protocol of the clinic.

123 An ovarian ultrasound was performed on stimulation day 5 to 6 and gonadotrophin doses were  
124 adjusted according to the ovarian response. When at least one follicle reached  $\geq 18$  mm, 10000 IU  
125 of hCG or 250 mcg of rhCG were administered and 34-36 hours later follicles were aspirated under  
126 patient sedation.

127

### 128 *Oocyte insemination, Embryo culture and biopsy*

129 All biological procedures were performed as described elsewhere (9). Briefly, Cumulus Corona  
130 Complex (COCCs) after retrieval were incubated in fertilization medium (Quinn's Advantage  
131 Protein Plus Fertilization Medium, SAGE) for 2-3 hours at 37°C under the gas phase of 5%O<sub>2</sub> and  
132 6%CO<sub>2</sub> until denudation. For all oocytes, denudation was performed by exposure to 20 IU/ml of  
133 hyaluronidase fraction VIII (Hyaluronidase 80 U/mL in HEPES-HTF, Sage, USA) in hepes-  
134 buffered medium (Quinn's Advantage® Medium with Hepes, Sage, USA). Subsequently, oocytes  
135 were aspirated in and out of a plastic pipette (Flexipet, 170 and 140  $\mu$ m i.d., COOK, Australia) to  
136 allow the removal of cumulus and corona cells. Only oocytes with first polar body extruded  
137 (metaphase II) were treated by ICSI immediately after the denudation procedure. Finally, injected  
138 oocytes were moved to single drops of cleavage medium (Quinn's Advantage Protein Plus  
139 Cleavage Medium, SAGE) under oil at 37°C, 5%O<sub>2</sub> and 6%CO<sub>2</sub>. On day-3, for all the developing  
140 embryos a media change-over (Quinn's Advantage Blastocyst Medium, SAGE) for sequential  
141 culture was performed. On day 3, a non-contact 1.48  $\mu$  diode laser (17) was used to create a circular  
142 6-9 $\mu$  diameter opening in the zona pellucida in cleavage stage embryos, in order to allow the  
143 trophectoderm cells to herniate. Depending on the embryo's development, the blastocyst stage can  
144 be reached on day 5, 6 or 7. On the day of biopsy, 5-10 trophectoderm cells were gently aspirated  
145 into the biopsy pipette (COOK, Ireland Ltd, Limerick, Ireland) followed by a laser assisted removal

146 from the rest of the blastocyst. The obtained trophoctoderm cells were washed in sterile phosphate-  
147 buffered saline solution (PBS) and then placed in microcentrifuge tubes containing 2  $\mu$ L of PBS,  
148 spinned down for few seconds and sent to GENOMA laboratory for analysis (18).

149

#### 150 *Preimplantation genetic analysis*

151 For whole genome amplification (WGA), trophoctoderm cells and negative controls were first lysed  
152 and genomic DNA was randomly fragmented and amplified using the SurePlex DNA Amplification  
153 System (BlueGnome, Cambridge, UK), according to the manufacturer's instructions. WGA  
154 products were processed as reported elsewhere (18,19) according to the BlueGnome 24sure V3  
155 protocol (available at [www.cytochip.com](http://www.cytochip.com)). Briefly, WGA products were fluorescently labelled and  
156 competitively hybridized to 24sure V3 arrays (BlueGnome, Cambridge, UK) with a matched  
157 control in an array-CGH experiment format. A laser scanner InnoScanw 710 AL (INNOPSYS,  
158 Carbonne, France) was used to excite the hybridized fluorophores and read and store the resulting  
159 images of the hybridization. Scanned images were then analyzed and quantified by algorithm fixed  
160 settings in BlueFuse Multi Software (BlueGnome, Cambridge, UK), a software package that  
161 performed the steps of grid placement, quantification, normalization and post-processing  
162 automatically. The software combines in an automatic way the data from the single channel sample  
163 experiments with both male and female references from the hybridized reference subarrays, to  
164 produce a single fused result compared with a sex matched and a mismatched reference. Once a  
165 specific amplification was observed (i.e. low autosomal noise), autosomal profiles were analyzed  
166 for gain or loss whole chromosomal ratios using a 3 x SD assessment, greater than  $\pm 0.3 \log_2$  ratio  
167 call, or both. To pass hybridization quality control, female samples hybridized with a male  
168 reference DNA (sex mismatch) had to show a consistent gain on chromosome X and a consistent  
169 loss of chromosome Y (Gutierrez-Mateo et al., 2011).



170 *Assays*

171 The modified Beckman Coulter AMH Gen II assay was used for all AMH analyses in this study.  
172 The AMH Gen II assay is a two-step, sandwich-type enzymatic, microplated assay. Problems with  
173 the robustness of the Gen II assay were solved with a modified version of the AMH Gen II assay kit  
174 (reference A79765), including an additional assay step before calibrators were added (premixing).  
175 This additional step eliminates the complement and thereby overcomes the not optimal assay  
176 reproducibility of the original AMH Gen II assay. The standards cover a range of 0–22 ng/mL. The  
177 sensitivity is reported to be 0.1–0.21 ng/mL. Reported intra- and interassay CVs were <2% and  
178 <12%, respectively.

179

180

181 *Outcomes and statistical analysis*

182 Data are presented as mean  $\pm$  SD when they had a Gaussian distribution or median (25th–75th  
183 range) when they were not normally distributed. Parametric and non-parametric tests were used to  
184 compare baseline characteristics and outcomes as appropriate. To identify characteristics that may  
185 be associated with the rate and number of euploid blastocysts, multivariate logistic regression  
186 analysis was performed. Univariate regression analyses were performed to identify factors that  
187 predict the rate and number of euploid blastocysts. Variables found to have tendency of  
188 association with the primary outcome ( $P < 0.25$ ) in the univariate analysis were included in the  
189 multivariate analysis. All independent variables were simultaneously entered into the logistic  
190 regression model. The Hosmer–Lemeshow goodness-of-fit test, assessed the goodness-of-fit of the  
191 normal regression models. All statistical tests used a two-tailed  $\alpha$  of 0.05. Statistical analysis was  
192 done using Stata 12.

193 *Ethics approval*

194 Full ethics committee approval was not required because of the retrospective design of the study  
195 and the anonymized handling of the data. The synopsis and objective of the study were  
196 communicated to local Institution review board. All women were treated at the European Hospital,  
197 Rome and routinely they provided informed consent for their clinical data as anonymous records to  
198 be used for research purposes.

199

200 **Results**

201 In total, data from 578 patients were available for analysis. Characteristics of the patients are  
202 reported in table 1. Briefly, the mean ( $\pm$ SD) age was  $37.6\pm 4.1$  years (range 27-43) and the mean  
203 number of blastocysts per patient was  $3.1\pm 2$  (range 1-11) . The total number of blastocysts available  
204 for the analysis was 1814 and 654 (36%) of them were euploid after PGS. The mean number of  
205 euploid blastocyst per patient was  $1.1\pm 1.2$  (Table 1)

206 The mean ( $\pm$ SD) rate of euploid blastocysts per patient was 38% ( $\pm 36.2\%$ ), and as expected was  
207 negatively associated to female age (Fig.1). The rate of euploid blastocysts was also positively and  
208 significantly related to serum AMH and to the number of retrieved mature oocytes in univariate  
209 analysis (Fig.1). Multiple regression analysis showed that female age and serum AMH, but not MII  
210 oocytes number were independent predictors of the rate of euploid embryos in IVF cycles (Table 2).  
211 For the two variables (age and AMH), ANOVA analysis showed a statistically significant effect in  
212 determining the rate of euploid blastocysts per patient (female age: F ratio=30,  $p<0.001$ ; serum  
213 AMH: F ratio=65,  $p<0.001$ ) (supplemental figure 1). No significant association was found between  
214 the rate of euploidy and other patient's or cycle characteristics such as type and duration of  
215 infertility, body mass index, the protocol, type and dose of drugs for ovarian stimulation, the  
216 indication to the PGS and the day of the blastocyst biopsy (data not shown).

217 While the total number of mature oocytes did not affect the rate of euploid blastocysts, it strongly  
218 and expectedly influenced the total number of euploid blastocysts available for each single patient  
219 (supplemental figure 2). This was due to a positive size-cohort effect of the number of oocytes.

220 When using the regression analysis the total number of euploid blastocyst per patient was related to  
221 age, AMH and to the number of mature oocytes. The relationship between the different variables  
222 with the total number of euploid blastocysts was then examined using univariate and multivariate  
223 regression analysis (Table 2).

224 Finally, in order to measure the effect size of this association, the probability of having at least one  
225 euploid blastocyst available for transfer was chosen as outcome. The odds ratio for female age (1  
226 year) was 0.84 (CI 95% 0.79-0.89), while for AMH (1ng/ml) and MII oocytes (1 oocyte) it was  
227 1.27 (CI 95% 1.14-1.41) and 1.09 (95%CI 1.04-1.14), respectively ( $y=5.3+0.24AMH-$   
228  $0.16Age+0.08MII$ ;  $R^2: 0.24$ ; model fit: chi-square 170.9, df 3,  $p<0.0001$ ). This indicates that for  
229 two women of similar age, higher serum AMH and more oocytes lead to increased probability of  
230 having at least one euploid blastocyst for the embryo transfer. In figure 3 the effect of female age,  
231 serum AMH and number of retrieved oocytes on the rate of euploidy, the number of euploid  
232 blastocysts per patient and probability of having at least one euploid blastocyst is clearly reported.

233

234 **Discussion**

235 To our knowledge, the present study is the first one to clearly investigate the independent  
236 relationship between female age, ovarian reserve (as assessed by circulating AMH) and the rate of  
237 embryo euploidy in ART. We found that independently of age, the increase in ovarian reserve is  
238 associated to increased rate of euploid blastocysts .

239 The results of our study at least in part support the hypothesis that quality and quantity of the  
240 follicular pool are directly related. Qualitative and quantitative aspects of the ovarian reserve are  
241 inversely related to women's age. However the decrease in oocyte quantity and quality could  
242 progress regardless of female ageing (20). Independently of age, selection of oocytes may become  
243 impaired when few oocytes are available or physiological modifications secondary to follicle loss  
244 may affect oocyte competence (20,21, 22, 23). The fact that serum AMH is related to the euploidy  
245 of blastocysts in IVF cycles independently of female age, could explain our previous observation  
246 that AMH was a weak but statistically significant age-independent predictor of live birth in IVF  
247 (24,25). This once again highlights the possible role of serum AMH as a qualitative other than  
248 quantitative marker of ovarian reserve.

249 According to our findings, in women with the same ovarian reserve, young age may increase the  
250 rate of euploidy in blastocysts. From this point of view young female age at least in part may be a  
251 protective factor in women with low ovarian reserve that generally have a low prognosis in IVF.  
252 Indeed it is has generally been assumed that younger women with poor response still have a good  
253 prognosis for pregnancy while older women with poor response have an extremely poor outcome  
254 with IVF. In the present study we had a low incidence of poor response and could not address this  
255 point, hence a specific investigation should be designed to address this relevant question.

256

257

258 In the clinical practice, the availability of euploid blastocysts for patients is extremely important  
259 considering that the rate of cumulative success of IVF itself is strictly dependent on this figure.  
260 Very recent studies clearly confirmed that the number of cryopreserved embryos following the fresh  
261 embryo transfer is directly related to the cumulative live birth rate that results from the success of  
262 the fresh and successive frozen embryo transfers (26). In the present study we showed that the total  
263 number of euploid blastocysts is dependent on female age, serum AMH and number of retrieved  
264 mature oocytes. Of course this may be expected and is secondary to the fact that for any given rate  
265 of blastocyst euploidy, the higher the number of oocytes, the higher the number of euploid embryos  
266 that will be obtained. This confirms previous findings by Ata and coll (27), that found that the  
267 number of euploid embryos in IVF/ICSI cycles was significantly decreased by increasing female  
268 age and was significantly increased by every additional embryo available for analysis. In the present  
269 study we calculated that the probability of having at least one euploid blastocyst available at  
270 transfer in order to measure the effect size of the association of the predictors and found it was  
271 reduced by 16% per every year of female age, raised by 27% for every increase in AMH by 1 ng/ml  
272 and increased by 9% for each additional mature oocyte.

273 The strength of our study is that observations are made on a very large number of blastocysts  
274 (n=1814) and that AMH was centrally measured. Another strong point is related to the fact that the  
275 preimplantation aneuploidy testing was based on the array comparative genomic hybridization  
276 (array-CGH) technology that has been sufficiently validated using cells of a known genotype (27)  
277 and is now used worldwide. At the same time we recognize the retrospective design as the main  
278 limitation of the study.

279 In conclusion in the present study we could confirm the very well known negative effect of female  
280 age on euploidy of embryos in IVF/ICSI cycles and also reported the original finding of the  
281 independent and positive role of ovarian reserve. Serum AMH, independently of age, significantly  
282 affects a very relevant outcome in ART, namely the rate of euploid blastocysts per patient. The

283 probability of having at least one euploid blastocyst is finally dependent on female age, serum  
284 AMH and the number of mature oocytes.

285

286

287

288• Authors' contributions

289•

290 ALM, EG, MGM.: study design and data analysis; MGM, PG, FF: cohort collection and  
291 characterization; ALM, MGM, GS, PG, CA, VG, FF, EG: preparation and approval of the final  
292 version of the manuscript.

293

294

296 **References**

- 297 1. Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human  
298 ovarian follicles: increase in the disappearance rate of non-growing and early-growing  
299 follicles in aging women. *Biol Reprod.* 1994;50(3):653-63.
- 300 2. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance  
301 of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod.*  
302 199;7(10):1342-6.
- 303 3. Battaglia DE, Goodwin P, Klein NA, Soules MR. Influence of maternal age on meiotic  
304 spindle assembly in oocytes from naturally cycling women. *Hum Reprod.* 1996  
305 ;11(10):2217-22.
- 306 4. Lim AS, Tsakok MF. Age-related decline in fertility: a link to degenerative oocytes? *Fertil*  
307 *Steril.* 1997 ;68(2):265-71.
- 308 5. Pellestor F, Andréo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal  
309 abnormalities: new data drawn from in vitro unfertilized human oocytes. *Hum Genet.* 2003  
310 ;112(2):195-203.
- 311 6. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of  
312 early pregnancy loss. *Hum Reprod Update* 2002;8:333–343.
- 313 7. Fragouli E, Wells D. Aneuploidy screening for embryo selection. *SeminReprod Med*  
314 2012;30:289–301.
- 315 8. Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, et al. The origin and  
316 impact of embryonic aneuploidy. *Hum Genet* 2013;132:1001–1013.
- 317 9. Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F, et al. Correlation  
318 between aneuploidy, standard morphology evaluation and morphokinetic development in  
319 1730 biopsied blastocysts: a consecutive case series study. *Hum Reprod.* 2016;31(10):2245-  
320 54.
- 321 10. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al.. The nature  
322 of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive  
323 trophoctoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril.*  
324 2014;101(3):656-663.
- 325 11. van Montfrans JM, Dorland M, Oosterhuis GJ, van Vugt JM, Rekers-Mombarg LT,  
326 Lambalk CB. Increased concentrations of follicle-stimulating hormone in mothers of  
327 children with Down's syndrome. *Lancet.* 1999;353:1853-4.
- 328 12. van Montfrans JM, van Hooff MH, Martens F, Lambalk CB. Basal FSH, estradiol and  
329 inhibin B concentrations in women with a previous Down's syndrome affected pregnancy.  
330 *Hum Reprod.* 2002;17(1):44-7
- 331 13. Nasser A, Mukherjee T, Grifo JA, Noyes N, Krey L, Copperman AB. Elevated day 3 serum  
332 follicle stimulating hormone and/or estradiol may predict fetal aneuploidy. *Fertil Steril.*  
333 1999;71(4):715-8.
- 334 14. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-  
335 Müllerian hormone expression pattern in the human ovary: potential implications for initial  
336 and cyclic follicle recruitment. *Mol Hum Reprod.* 2004 ;10(2):77-83.



- 337 15. La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, et al. Anti-Müllerian  
338 hormone measurement on any day of the menstrual cycle strongly predicts ovarian response  
339 in assisted reproductive technology. *Hum Reprod.* 2007;22:766-71.
- 340 16. La Marca A, Sighinolfi G, Papaleo E, Cagnacci A, Volpe A, Faddy MJ. Prediction of age at  
341 menopause from assessment of ovarian reserve may be improved by using body mass index  
342 and smoking status. *PLoS One.* 2013;8(3):e57005.
- 343 17. Fragouli E, Lenzi M, Ross R, Katz-Jaffe M, Schoolcraft WB, Wells D. Comprehensive  
344 molecular cytogenetic analysis of the human blastocysts stage. *Hum Reprod.* 2008;25:608-618.
- 345 18. Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, et al. Comparative genomic  
346 hybridization selection of blastocysts for repeated implantation failure treatment: a pilot  
347 study. *Biomed Res Int.* 2014;2014:457913.
- 348 19. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, et al. Application of  
349 next-generation sequencing technology for comprehensive aneuploidy screening of  
350 blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod.*  
351 2014;29:2802-13.
- 352 20. Baird DT, Collins J, Egozcue J, Evers LH, Gianaroli L, Leridon H, et al., ESHRE Capri  
353 Workshop Group. Fertility and ageing. *Hum Reprod Update.* 2005 ;11:261-76.
- 354 21. Warburton D. Biological aging and the etiology of aneuploidy. *Cytogenet Genome Res.*  
355 2005;111:266-72.
- 356 22. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. *Nature.*  
357 1968 6;218:22-8.
- 358 23. Kline J, Kinney A, Reuss ML, Kelly A, Levin B, Ferin M, et al. . Trisomic pregnancy and  
359 the oocyte pool. *Hum Reprod.* 2004;19:1633-43.
- 360 24. La Marca A, Nelson SM, Sighinolfi G, Manno M, Baraldi E, Roli L, et al. Anti-Müllerian  
361 hormone-based prediction model for a live birth in assisted reproduction. *Reprod Biomed*  
362 *Online.* 2011;22:341-9
- 363 25. Khader A, Lloyd SM, McConnachie A, Fleming R, Grisendi V, La Marca A, et al. External  
364 validation of anti-Müllerian hormone based prediction of live birth in assisted conception. *J*  
365 *Ovarian Res.* 2013;6:3.
- 366 26. Vaughan DA, Leung A, Resetkova N, Ruthazer R, Penzias AS, Sakkas D, et al. How many  
367 oocytes are optimal to achieve multiple live births with one stimulation cycle? The one-and-  
368 done approach. *Fertil Steril.* 2017;107:397-404
- 369 27. Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, et al. Array CGH analysis shows that  
370 aneuploidy is not related to the number of embryos generated. *Reprod Biomed Online.* 2012;24:614-  
371 20.
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374 **Legends of Figures**

375 **Fig. 1** The relationship between the rate of euploid blastocysts, female age ( $r:-0.326$  [95%CI -0.28, -  
376 0.43],  $p<0.0001$ ), MII ( $r:0.11$  [95%CI 0.03, 0.19]  $p: 0.006$ ), and serum AMH ( $r:0.21$ [95%CI 0.13,  
377 0.29],  $p<0.0001$ ).

378 **Fig2.** Mean number of euploid blastocysts (n, diamonds), mean rate of euploidy (% , circles) and the  
379 probability of having at least one euploid blastocyst (% , squares) in women according to female  
380 age, serum AMH and the number of mature oocytes . Percentiles are for age: 20<sup>th</sup> 34 years, 40<sup>th</sup> 37  
381 years, 60<sup>th</sup> 39 years and 80<sup>th</sup> 41 years. Percentiles are for AMH: 20<sup>th</sup> 1.2 ng/ml, 40<sup>th</sup> 2.2ng/ml, 60<sup>th</sup>  
382 3.6ng/ml and 80<sup>th</sup> 5.5ng/ml. Percentiles are for mature oocytes: 20<sup>th</sup> 4 oocytes, 40<sup>th</sup> 6 oocytes, 60<sup>th</sup>  
383 9 oocytes and 80<sup>th</sup> 12 oocytes.

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385 **Supplemental Figure 1** A. Box and whisker plots of the rate of euploid blastocysts by quintiles of  
386 serum AMH levels. Values are median (lines), 25<sup>th</sup> to 75<sup>th</sup> percentiles (boxes) and 10<sup>th</sup> to 90<sup>th</sup>  
387 percentiles (whiskers). Percentiles are for AMH: 20<sup>th</sup> 1.2 ng/ml, 40<sup>th</sup> 2.2ng/ml, 60<sup>th</sup> 3.6ng/ml and  
388 80<sup>th</sup> 5.5ng/ml. B. Box and whisker plots of the rate of euploid blastocysts by quintiles of female  
389 age. Values are median (lines), 25<sup>th</sup> to 75<sup>th</sup> percentiles (boxes) and 10<sup>th</sup> to 90<sup>th</sup> percentiles  
390 (whiskers). Percentiles are for age: 20<sup>th</sup> 34 years, 40<sup>th</sup> 37 years, 60<sup>th</sup> 39 years and 80<sup>th</sup> 41 years.

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392 **Supplemental Figure 2** The relationship between female age, serum AMH, mature oocytes and  
393 the total number of euploid blastocysts

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