Age-specific anti-Mullerian hormone (AMH) levels poorly affects cumulative live birth rate after intra-uterine insemination

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Objective: To evaluate the impact of age-specific anti-Müllerian (AMH) levels on the cumulative live birth rate after 4 intra uterine inseminations (IUI).

Study Design: The retrospective study involved 509 couples who underwent their first IUI between January 2011 and July 2017 in the Toulouse University Hospital. All IUI were performed after an ovarian stimulation combining recombinant FSH and GnRH antagonist. The main measure outcome was the cumulative live birth rate (CLBR) defined as the number of deliveries with at least one live birth resulting from a maximum of 4 IUI attempts.

Results: When compared to normal or high levels, low age-specific AMH (<25th of the AMH in each age group) was associated to a non-significant lower live birth rate (31%, 38% and 42% respectively for low, normal and high age-specific groups; P=0.170) and non-significant higher miscarriage rate (26%, 19% and 14% respectively for low, normal and high age-specific groups; P=0.209). However, it must be pointed out that in low age-specific AMH the initial FSH doses used for stimulation were higher than in the other groups.

Conclusion: This study shows that the age-specific levels of AMH have only a slight effect on IUI outcome when adapting the stimulation protocols to their level.

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Introduction

Intrauterine inseminations (IUI) with fresh husband sperm is one of the first line treatments in case of subfertility. They can be used in mild male abnormality [1,2], ovulation disorders [3] and unexplained infertility (UIU) [4].

Numerous factors have been reported to have a great influence on the success rate of IUI. The number of inseminated motile spermatozoa, as well as the number of mature follicles and the use of GnRH antagonist have been widely reported to be significantly linked to the chance to obtain a pregnancy after IUI [5–8]. Conflicting results have been reported concerning female age. Indeed, if some authors have shown that advanced age was associated with a lower pregnancy rate [3,8–10] or with an increased miscarriage rate [11], others found no correlation [12,13].

The influence of the ovarian reserve parameters has been studied by different authors with major discrepancies since some have found that high levels of ovarian reserve as measured by anti-Müllerian hormone (AMH) and/or antral follicle count (AFC) are good predictors of the pregnancy rate [11,14–18] while other have found that AMH was not a useful tool to predict IUI outcome [19,20]. These differences can be explained by the fact that ovarian reserve is closely correlated to age [21] making difficult to differentiate the respective part of age and of ovarian reserve on the ability of motherhood.

To try to answer this question, the present study aimed to evaluate the ability of the age-specific AMH levels [22,23] to predict the cumulative live birth rate after 4 IUI.

Materials and methods

Patients

Five hundred and nine couples who underwent their first IUI between January 2011 and July 2017 in the Toulouse University Hospital entered the study. The indications of IUI were: unexplained infertility (252; 49.5%), ovulation disorder (151; 29.7%), moderate oligo-asthenospermia allowing to inseminate at least 10⁶ motile spermatozoa (59; 11.6%), stage 1 endometriosis

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examination in injection. (Origio, Nidacon, Mölndal, Sweden). After preparation spermatozoa were incubated in 400 μl universal IVF medium (Origo, Versailles, France) at 37 °C in a 6% CO2 atmosphere. The number of recovered spermatozoa and their progressive motility were assessed in the medium to allow the measurement of the number of recovered motile spermatozoa.

IUI procedures

Ovarian stimulation used a combination of recombinant FSH (Gonal-F, Merck, Lyon, France or Puregon, MSD, Paris, France) and GnRH antagonist (Cetrotide 0.25 mg, Merck, Lyon, France or Orgalutran, MSD, Paris, France). The initial dose of FSH was chosen according the female age and the score described by Chalumau et al. [26]. Ovulation was triggered with recombinant hCG (Ovitrelle, Merck, Lyon, France) when at least one follicle ≥ 18 mm was obtained. Insemination was performed 36 h after hCG injection. A luteal support of 400 mg per day of intra-vaginal progesterone was administrated during 15 days, starting on the day of insemination.

Clinical pregnancy was defined as the presence of a fetal heartbeat evaluated during the transvaginal ultrasonographic examination seven weeks after insemination. Live birth was defined as the delivery of at least one live born infant after a 22 weeks or more pregnancy [27].

Evaluation of the ovarian reserve

Evaluations were done in the year before the first IUI. The hormonal measurements (FSH, LH, AMH and E2) were performed between cycle days 2 and 5 in the biochemistry laboratory of the Toulouse University Hospital with the same kits and the antral follicle count (AFC) at the same time, through 2D transvaginal ultrasonography by several different physicians in the department of obstetrics of the Toulouse University Hospital. Every follicle within 2–9 mm mean diameter (after 2 measures on orthogonal plans) was count. Serum LH, FSH, and E2 levels were assayed by an automated electrochemiluminescent based-assay (Elecsys® e602 Roche Diagnostics, Meylan, France) The interassay coefficient of variation (CV) were respectively 3.4% (around 11 UI/L) for LH, 2.2% (around 17 UI/L) for FSH and 3.4% (around 95 pg/mL) for E2. AMH was measured by ELISA GENII Beckman essay (Beckman Coulter Inc, Brea, CA) with an interassay CV of 4.4% (around 3.8 ng/mL) and a 0.08 ng/mL low limit of detection.

Statistical analysis

Data were extracted from the Gynelog clinical database used in our department. This database is approved by the French National Commission for Information Technology and Civil Liberties (CNIL) to be used for clinical research. According to French law (2012–300), patients are aware that their data can be used for anonymous clinical studies unless they specifically state otherwise. This information is detailed in posters in the rooms of the centre, and patients can inform the centre through a letter if they do not want to participate in clinical studies.

The measured primary outcome was the cumulative live birth rate (CLBR) after a maximum of 4 attempts. Pregnancy loss was defined as the outcome of any pregnancy that does not result in at least one live birth [27].

Statistical analyses were performed using StatView software (Abacus Concepts Inc., Berkeley, CA). Data are means ± SD or median (range) according to the normality of the data. Percentages were compared by the χ² test. Means were compared using the Student’s t-test and medians using the Mann-Whitney test according to the normality of data distribution.

Groups of age were defined by the 25th, 50th and 75th percentiles. Groups of age-specific AMH were defined by the 25th and 75th percentiles of AMH inside each group of age. The age-specific AMH was called “low” when it was lower than the 25th percentile, “normal” between the 25th and the 75th percentile and “high” when it was higher than the 75th percentile, to allow easy reading.

The demographic data of different groups are described in Table1.

Results

Table 2 shows the results of IUI as a function of age-specific AMH. There was a trend, but not statistically significant, for a lower live birth rate in all low age-specific AMH groups. This was also true after considering age-specific AMH groups (low, normal and high).

Table 1
Demographic data of the different groups of age-specific AMH levels.

| Age     | ≤ 29 | 30–33 | 33–37 | ≥ 37 |
|---------|------|-------|-------|------|
| AMH (ng/ml) |      |       |       |      |
| n       | 34   | 67    | 43    | 32   |
| 0.16    | 1.6  | 1.6–4.6 | 4.6 | 6.4 |
| 1.2    | 33   | 33–58 | 58    | 32   |
| 3.8    | 33   | 33–63 | 63    | 32   |
| 7.6    | 35   | 35–59 | 59    | 29   |

Groups of age were defined as the 25th, 50th and 75th percentile. Groups of AMH were defined as the 25th and the 75th percentile in each group of age.
### Table 2

Results of IUI as a function of age and age-specific AMH levels.

| Age | AMH (ng/ml) | n | 0.1 – 1.6 | 1.6 – 4.5 | ≥ 4.5 | Total |
|-----|-------------|---|-----------|----------|------|-------|
| 25  | N ULI       | 25| 23.2      | 13.6     | 0.3  | 39.1  |
| 30  | Initial FSH dose | 23.2 | 13.6 | 0.3 | 39.1 |
| 35  | N follicles ≤ 15 mm | 23.2 | 13.6 | 0.3 | 39.1 |
| 40  | Cumulative live birth rate (4 IUI) (%) | 23.2 | 13.6 | 0.3 | 39.1 |
| 45  | Ratio of the number of newborns to the cumulative number of follicles ≥ 15 mm | 23.2 | 13.6 | 0.3 | 39.1 |

*Note: Groups of AMH were defined as the 25th, 50th, and 75th percentile in each group of age.*

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whatever the age (Table 3), the CLBR rate was not significantly different but showed a trend for a decrease in the low group. It must be pointed out that patients in the low AMH group had significantly higher initial FSH administrated doses and more mature follicles. Therefore, we have calculated the ratio of the number of newborns to the cumulative number of mature follicles. This ratio appeared significantly different among the groups of age-specific AMH: it appeared that, to obtain a newborn, twice more mature follicles were needed in the low than in the high AMH group (Table 3). There was a non-significant trend for a higher miscarriage rate per pregnancy in the low AMH group.

### Discussion

Even if low age-specific AMH tend to be associated with a lower birth rate and with a higher risk of miscarriage, its predictive value remained poor. The impact of AMH levels on the chances of success in ART highly varies among the studies.

The ability of AMH to predict the ovarian response to stimulation has been widely reported and numerous studies have shown good correlations between AMH and the ovarian stimulation index [28] or the number of collected oocytes in IVF [28] and thus has a good ability to diagnose high and poor responders [29,30]. J. Moro et al. using the same FSH dose (75 IU) for all patients, have shown that the number of recruited mature follicles (18 mm) was dependent on the AMH levels [19]. We found opposite data but this can be explained by the fact that we have adapted FSH dose to age-specific AMH in order to compensate the effect in ovarian responsiveness of patients with low AMH, as attested by the significantly higher follicles≥ 15 mm obtained when age-specific AMH is low.

Concerning the relations between AMH and pregnancy rate in IUI, several authors have reported a significantly higher AMH in patients who achieved a clinical pregnancy [15,17,18]. In the same way, Moro et al. found a threshold of 2.3 ng/ml allowing to discriminate women according their chances of success [16] and Li et al. found a similar threshold at 1.8 ng/ml [14]. However, other authors found a modest [14] even a non-significant [20] correlation between the AMH level and the chances of ongoing pregnancy. Similar discrepancies have been reported in IVF with a significant impact on the results for some authors [31–35] and no predictive value for others [18,36–38].

These discrepancies could be due to the fact that these studies were focused on the sole AMH level. While AMH and age are closely linked, wide variations exist inside a year of age [39], thus the use of age-specific AMH allows to better discriminate the effect of age and of diminished ovarian reserve [40].

In our study, there were a non-significantly higher miscarriage rate when age-specific AMH was low. Low AMH has been shown to be linked, independently of age, to increased pregnancy loss as well in naturally conceived pregnancies [41] as after IVF [42], probably due to a higher embryonic aneuploidy [43,44] These data suggest that the diminution of the ovarian reserve; notably when unexplained, may not only be a quantitative problem but also a qualitative one. Our observation of a significant decrease of the efficiency of the stimulation, which we have estimated through the ratio of the number of newborns to the cumulative number of follicles ≥ 15 mm, with the age-specific AMH levels, is in line with this hypothesis. For example, the mechanisms by which some molecules, such as environmental pollutants, can alter the pool of follicles, can also impair oocyte quality [45].

The main limitation of this study is the relatively low number of subjects in each group of age and AMH, which decrease the statistical power of the analyses.

In conclusion, these data show that correct live birth rates can be obtained by IUI in case of low age-specific AMH if higher doses
of FSH are used. Indeed, our results have shown that, to obtain a newborn, patients with low AMH required 1.5 more follicles than those with normal AMH and twice more than those with high AMH.

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Erdem M, Erdem A, Mutlu MF, Ozsik S, Yildiz S, Guler I, et al. The impact of sperm morphology on the outcome of intrauterine insemination cycles with gonadotropins in unexplained and male subfertility. Eur J Obstet Gynecol Reprod Biol 2016;197:120–4.

[2] Ounbelet W, Dhont N, Thijssen A, Bosmans E, Kruger T. Sperm quality and prediction of IUI success in male subfertility: a systematic review. Reprod Biomed Online 2014;28(3):300–9.

[3] Dinelli L, Courbiere B, Achard V, Jouve E, Deveze C, Gusci A, et al. Prognosis factors of pregnancy after intrauterine insemination with the husband’s sperm: conclusions of an analysis of 2,019 cycles. Fertil Steril 2014;101(4):994–1000.

[4] Erdem M, Erdem A, Guler I, Armaca S. Role of antral follicle count in controlled ovarian hyperstimulation and intrauterine insemination cycles in patients with unexplained subfertility. Fertil Steril 2008;90(2):360–6.

[5] Wainer R, Albert M, Doron A, Baily M, Bemgare M, Lombrros R, et al. Influence of the number of motile spermatozoa inseminated and of their morphology on the success of intrauterine insemination. Hum Reprod 2004;19(9):2060–5.

[6] Monraissin O, Chansel-Debordeaux L, Chiron A, Floret S, Cens S, Bourrinet S, et al. Evaluation of intrauterine insemination practices: a 1-year prospective study in seven French assisted reproduction technology centers. Fertil Steril 2016;105(6):1589–93.

[7] Gomez-Palomo JA, Acevedo-Martin B, Chavez M, Manzanares M, Ricciarello E, et al. Multifollicular recruitment in combination with gonadotropin-releasing hormone antagonist increased pregnancy rates in intrauterine insemination cycles. Fertil Steril 2008;90(3):620–4.

[8] Merviel P, Heraud MA, Grenier N, Lourdel E, Sanguinet P, Copin H. Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature. Fertil Steril 2010;93(1):79–88.

[9] Jeon YE, Jung JA, Kim HY, Seo SK, Cho S, Choi YS, et al. Predictive factors for pregnancy during the first four intrauterine insemination cycles using gonadotropin. Gynecol Endocrinol 2013;29(9):834–8.

[10] Thijssen A, Creerens A, Van der Elst W, Creerens E, Vandormael M, et al. Predictive value of different ovaries influencing pregnancy rate following intrauterine insemination with homologous semen: a prospective cohort study. Reprod Biomed Online 2017;34:463–72.

[11] Speyer BE, Abramov B, Saab W, Doshi A, Sarna U, Harper JC, et al. Factors influencing the outcome of intrauterine insemination (IUI): age, clinical variables and significant thresholds. J Obstet Gynaecol 2013;33(7):697–700.

[12] Iberico G, Vioque J, Ariza N, Lozano JM, Roca M, Llacer J, et al. Analysis of factors influencing pregnancy rates in homologous intrauterine insemination. Fertil Steril 2004;81(5):1308–13.

[13] Aydin Y, Hassa H, Oge T, Topkaz VY. Factors predictive of clinical pregnancy in the first intrauterine insemination cycle of 306 couples with favourable female patient characteristics. Hum Fertil (Camb) 2013;16(4):286–90.

[14] Li HW, Yeung WS, Lau EY, Ho HY, Ng EH. Evaluating the performance of serum antimullerian hormone concentration in predicting the live birth rate of controlled ovarian stimulation and intrauterine insemination. Fertil Steril 2010;94(3):772–7.

[15] Donkink Y, Virji N, Butler TS, Gaskins JT, Pagidas K, Sung L. The value of antimullerian hormone in predicting clinical pregnancy after intrauterine insemination. J Obstet Gynaecol Can 2017;39(10):880–5.

[16] Moro F, Tropea A, Scarnici E, Leoncini E, Boccia S, Federico A, et al. Anti-Mullerian hormone concentrations and antral follicle counts for the prediction of pregnancy outcomes after intrauterine insemination. Int J Gynaecol Obstet 2016;133(1):64–8.

[17] Bakas P, Boutas I, Creatts M, Vlahos N, Gregoriou O, Creatts G, et al. Can anti-Mullerian hormone (AMH) predict the outcome of intrauterine insemination with controlled ovarian stimulation? Gynecol Endocrinol 2015;31(10):765–6.

[18] Wang MH, Chen CH, Wang CW, Hsu ML, Tseng CR. A higher anti-Mullerian hormone level is associated with an increased chance of pregnancy in patients undergoing controlled ovarian stimulation and intrauterine insemination. J Obstet Gynaecol 2015;35(1):64–8.

[19] Freesleven N, Rosenberg M, Johannsen TH, Lossi K, Loft A, Bangsboll S, et al. Prospective investigation of serum anti-Mullerian hormone concentration in ovulatory intrauterine insemination patients: a preliminary study. Reprod Biomed Online 2010;20(5):582–7.

[20] Freesleven K, Kolo M. Does anti-Mullerian hormone is a useful measure of quantitative ovarian reserve but does not predict the chances of live-birth pregnancy. Aust N Z J Obstet Gynaecol 2010;50(6):568–72.

[21] Zhu J, Li T, Xing W, Lin H, Ou J. Chronological age vs biological age: a retrospective analysis on age-specific serum anti-Mullerian hormone levels for 3280 females in reproductive center clinic. Gynecol Endocrinol 2018;34(10):890–4.

[22] Tzian F, Mansouri MA, Solaymani-Dodaran M, Azzizi F. Age-specific serum anti-Mullerian hormone levels: estimates from a large population-based sample. Climacteric 2014;17(5):591–7.

[23] Moreno J, Gatiell N, Cohade C, Parinaud J, Leandri R. Mother’s age at menopause but not own age at menarche has an impact on ovarian reserve. Gynecol Endocrinol 2018;34(8):664–5.

[24] Coclet T, Gatiell N, Moreno J, Cohade C, Fajau C, Lesourd F, et al. Effect of unilateral tubal abnormalities on the results of intrauterine inseminations. Reprod Biomed Online 2017;35(3):314–7.

[25] Monraissin O, Chansel-Debordeaux L, Chiron A, Floret S, Cens S, Bourrinet S, et al. Evaluation of intrauterine insemination practices: a 1-year prospective study in seven French assisted reproduction technology centers. Fertil Steril 2016;105(6):1589–93.

[26] Chalumeau C, Moreno J, Gatiell N, Cohade C, Lesourd F, Parinaud J, et al. Establishment and validation of a score to predict ovarian response to stimulation in IVF. Reprod Biomed Online 2018;36(1):26–31.

[27] Zegers-Hochschild F, Adamson GD, Dyer S, Racovsky C, de Mouzon J, et al. The international glossary on infertility and fertility care. 2017. Fertil Steril 2017;108(3):393–406.

[28] Patrelli TS, Gizzo S, Sianesi N, Leviali V, Pezzuto A, Ferrari B, et al. Anti-Mullerian hormone serum values and ovarian reserve: can it predict a decrease in fertility after ovarian stimulation by ART cycles? PLoS One 2012;7(9):e44571.

[29] Broer SL, Dollman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excess response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 2011;17(1):46–54.

[30] Burks HR, Ross L, Oppier N, Paulsson E, Stanczyk FZ, Chung K. Can highly sensitive antimullerian hormone testing predict failed response to ovarian stimulation? Fertil Steril 2015;104(3):643–8.

[31] Ridolfini S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 2012;18(4):460–70.

[32] Merhi Z, Zaptantis A, Berger DS, Jindal SK. Determining an anti-Mullerian hormone cutoff level to predict clinical pregnancy following in vitro fertilization in women with severely diminished ovarian reserve. J Assist Reprod Genet 2013;30(10):1361–5.

[33] Brodin T, Hadzisavovnic N, Berglund L, Olovsson M, Holte J. Antimullerian hormone levels are strongly associated with live-birth rates after assisted reproduction. J Clin Endocrinol Metab 2013;98(3):1107–14.

[34] La Marca A, Sghinolfi G, Raci D, Argento C, Baraldi E, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010;16(2):113–30.

[35] Alson SE, Bungum LJ, Giewerman A, Henic E. Anti-Mullerian hormone levels are associated with live birth rates in ART, but the predictive ability of anti-Mullerian hormone is modest. Eur J Obstet Gynecol Reprod Biol 2018;225:199–204.
[36] Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott Jr RT, Seli E. Diminished ovarian reserve and poor response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in performance. Hum Reprod 2018.

[37] Seifer DB, Tal O, Wantman E, Edul P, Baker VL. Prognostic indicators of assisted reproduction technology outcomes of cycles with ultralow serum antimullerian hormone: a multivariate analysis of over 5,000 autologous cycles from the Society for Assisted Reproductive Technology Clinic Outcome Reporting System database for 2012-2013. Fertil Steril 2016;105(2):185–93 e3.

[38] Hamdine O, Eijkemans MJ, Lentjes EGW, Torrance HL, Macklon NS, Fauser B, et al. Antimullerian hormone: prediction of cumulative live birth in gonadotropin-releasing hormone antagonist treatment for in vitro fertilization. Fertil Steril 2015;104(4):891–8 e2.

[39] Shebl O, Ebner T, Sir A, Schreier-Lechner E, Mayer RB, Tews G, et al. Age-related distribution of basal serum AMH level in women of reproductive age and a presumably healthy cohort. Fertil Steril 2011;95(2):832–4.

[40] Barad DH, Weghofer A, Gleicher N. Utility of age-specific serum anti-Mullerian hormone concentrations. Reprod Biomed Online 2011;22(3):284–91.

[41] Lyttle Schumacher BM, Jukic AMZ, Steiner AZ. Antimullerian hormone as a risk factor for miscarriage in naturally conceived pregnancies. Fertil Steril 2018;109(6):1065–71 e1.

[42] Tarasconi R, Tadros T, Ayoubi JM, Belloc S, de Ziegler D, Fanchin R. Serum antimullerian hormone levels are independently related to miscarriage rates after in vitro fertilization-embryo transfer. Fertil Steril 2017;108(3):518–24.

[43] Jiang X, Yan J, Sheng Y, Sun M, Cui L, Chen ZJ. Low anti-Mullerian hormone concentration is associated with increased risk of embryonic aneuploidy in women of advanced age. Reprod Biomed Online 2018;37(2):178–83.

[44] La Marca A, Minasi MG, Sighinolfi G, Greco P, Argento C, et al. Female age, serum antimullerian hormone level, and number of oocytes affect the rate and number of euploid blastocysts in in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril 2017;108(5):777–83 e2.

[45] Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health 2017;16(1):37.