Predictive value of anti-Müllerian hormone (AMH) in the fertile population: The Donor Insemination Model
ABSTRACT

Objective: To investigate whether anti-Müllerian hormone (AMH) is related to the chance of a live birth in non-stimulated donor insemination cycles.

Design: A retrospective cohort study.

Setting: University-based tertiary center.

Patient(s): Two hundred and ninety five women with a regular cycle with AMH values measured before intrauterine insemination (IUI) with donor sperm.

Intervention(s): Intrauterine inseminations in non-stimulated cycles with human chorionic gonadotropin (hCG) triggering.

Main Outcome Measure(s): Cumulative live birth rates.

Result(s): The live birth rate after intra-uterine donor insemination in non-stimulated cycles does not relate to the AMH values. We included 295 women undergoing a total of 854 IUI cycles. These IUI cycles resulted in a total of 214 pregnancies (25.1 % per cycle). A total of 155 pregnancies resulted in a live birth while the other pregnancies were either biochemical (n = 24), miscarriages (n=32) or an extra-uterine pregnancy (n=1). A total of 2 pregnancies were lost to follow-up. The overall cumulative clinical pregnancy rate was 54.9% and the overall cumulative live birth rate was 52.5%. No difference was found in terms of treatment outcome between the four different AMH quartiles. After regression analysis, only the age (p = 0.004) and not the AMH levels (p = 0.944), appeared to be significantly related to clinical outcome.

Conclusion(s): The donor insemination model demonstrates that AMH is not a predictor of reproductive success in fertile women. Basic research studies are needed to confirm the absence of correlation of AMH with oocyte quality.

Key Words: AMH, intrauterine insemination, donor insemination, age, ovarian reserve, age related infertility.
INTRODUCTION

Anti-Müllerian hormone (AMH) is produced by granulosa cells of preantral and small antral follicles. The plasma levels of AMH demonstrate a relationship with this non-growing follicle (NGF) pool and it has been shown that AMH relates to the age of menopause (1, 2, 3). AMH can therefore be considered as an important biomarker for ovarian ageing (2).

Next to this association of AMH values with the NGF pool, AMH levels have been shown to relate the oocyte yield after ovarian stimulation (4). The association appears not to be subject to intra- and intercycle variability and appears to be not affected by a time interval of up to 12 months between assessment and actual treatment (5, 6). This makes the assessment of AMH clinically important prior to ovarian stimulation as the ovarian response is significantly related with the live birth chances after ovarian stimulation and in vitro fertilization cycles (7, 8). Moreover, AMH has been shown to be a better predictor of the number of oocytes retrieved than age (9).

Although, it has been demonstrated in several studies that AMH is an independent variable associated with live birth in in vitro fertilisation (IVF) (4, 10, 11), meta-analysis of cohort studies has shown that AMH cannot predict live birth rates in women undergoing ovarian stimulation for IVF or intracytoplasmic sperm injection (ICSI) (12). Brodin et al., (2013) found that age, oocyte yield and AMH are all of importance and have a statistical impact on live-birth rates after IVF-ICSI (10). Multivariate analysis had shown that after controlling for age and for oocyte yield, the impact of AMH with regard to the IVF-ICSI outcome was still 42% (10). These findings are however challenged by the findings of a meta-analysis of individual patient data (IPD) (12). This study confirms the added value of AMH and the antral follicle count (AFC) next to the age for predicting poor ovarian response but found a limited capacity for these tests to predict an ongoing pregnancy after IVF.

Several authors have postulated that AMH is also related to oocyte and embryo quality in IVF-ICSI (13, 14, 15). This interplay of oocyte quantity and quality is also expressed by the finding that AMH appears to predict twin versus singleton pregnancies after IVF (16). However, it remains debatable whether such a correlation actually indicates that the AMH value predicts the spontaneous fertility chances of women. Although previous studies demonstrated an association between AMH and live birth rates in intrauterine insemination (IUI) cycles, ovarian stimulation has been used in these cycles. Moreover, male factor infertility was not an exclusion criterion for the patients recruited (17). These variables may have indeed caused potential bias, mainly due to the possible confounding effect related to ovarian stimulation and/or andrological factor.
Taking into account the above-mentioned evidence, we decided to utilize the donor-insemination model in order to evaluate whether AMH in women undergoing natural cycle IUI with donor sperm may actually predict live birth rates.

**MATERIALS AND METHODS**

A retrospective analysis of the live birth outcome of intra-uterine inseminations with the use of donor sperm between January 2009 and December 2012 has been performed. It is a single centre study performed at a tertiary referral centre and was approved by the institutional ethical commission. The indications for donor insemination treatment were severe male factor infertility, requests from lesbian couples and single-parent requests. All women underwent a routine fertility work-up including either hysterosonography, hysterosalpingography or laparoscopy and were therefore considered normally fertile.

During this observation period, 625 women out of a total of 2066 treated women had an AMH assessment performed at the fertility centre. This corresponded to 2368 insemination cycles. The AMH tests were performed using enzyme linked immunosorbent assay (ELISA) at no specific time in the menstrual cycle. Due to an in-house change of immunoassay on the 23th April 2012, a total of 58 women (192 cycles) were excluded from further analysis as they only had an AMH test performed with the new assay. AMH values were assessed at variable times within the menstrual cycle. If multiple AMH values were available, the value closest to the initiation of the first IUI cycle was considered. Cycles ranked as >6th attempt were also excluded (n = 117). Only patients with all their intra-uterine insemination cycles performed in a natural cycle were considered for this study. Therefore, women of whom the treatment included at least one cycle stimulated with either clomiphene citrate or gonadotrophins were excluded (n = 272) (1160 cycles). Finally, IUI cycles after previous result of live-born were also excluded (n = 45). An overview of patient selection is shown in figure 1.

All patients received one single IUI performed 36–44 hours after injecting 5000 U human chorionic gonadotrophin (hCG) (Pregnyl, MSD, Belgium) whenever ultrasound showed the presence of one follicle measuring at least 17 mm in diameter (18). IUI was performed using frozen-thawed donor sperm with a minimum of 1 x 10^6 progressively motile spermatozoa being inseminated using a Frydman catheter (Laboratories CCD, Paris, France). Sperm for insemination was prepared by a two-layer density gradient (Pure sperm™, Nidacon, Mölndal, Sweden) after thawing.
Pregnancies were diagnosed by a standard serum assays for hCG showing levels of >10 IU/l on at least two consecutive occasions. A clinical pregnancy is defined as pregnancies evidenced by the presence of an intrauterine gestational sac at ultrasound (18). Pregnancy follow-up was done by sending questionnaires to patients and their doctor or by telephone queries whenever questionnaires were incomplete (7). Live birth delivery after 25 weeks of gestation was the main outcome measure of this study.

Measurement of anti-Müllerian hormone
Blood was drawn in plain serum tubes, centrifugation was performed within 1 hour and serum was separated and immediately stored at −80 °C until analysis. The AMH assay demonstrated stable intra- and inter-assay coefficients of variation < 9.5% and a functional sensitivity of 0.35 ng/ml. AMH was measured with the Immunotech Beckmann-Coulter assay. AMH values were obtained at a time interval less than 12 months before the initiation of the IUI cycle, given that a previous trial by our group has demonstrated that AMH values can predict the level of ovarian response up to 12 months from the initial AMH assessment (5).

Statistical analysis
To allow the assessment of baseline characteristics between different levels of AMH, the sample was divided into quartiles according to the patient’s AMH (AMH percentile <25%, 25-50%, 50-75% or >75%). The demographic characteristics were compared amongst these groups using either the chi-square test (for categorical variables) or analysis of variance (ANOVA, for continuous variables, followed by a post-hoc pairwise analysis when significant). To assess if there was a relationship between the number of IUI the patient underwent until live-birth and the patient’s AMH, survival analysis using Cox regression was performed. In this analysis, IUI rank was considered the time variable while AMH, age and indication for IUI were added in the model as independent continuous variables. A p-value was considered significant if below 0.05, adjusted for multiple comparisons (using the Bonferroni correction) when performed. The statistical analysis was performed using Stata software version 13 (StataCorp, Texas, USA).
RESULTS
The study included a total of 295 women performing a total of 854 IUI cycles. The majority of donor inseminations were performed as a fertility treatment for lesbian couples (61.0%). Single-parent requests accounted for 26.1% of the indications while 12.9% of the treatments were performed in heterosexual couples. These IUI cycles resulted in a total of 214 pregnancies (25.1 % per cycle). A total of 155 pregnancies resulted in a live birth while the other pregnancies were either biochemical (n = 24), miscarriages (n = 32) or an extra-uterine pregnancy (n = 1). A total of 2 pregnancies were lost to follow-up.

The patient characteristics and outcome on a per patient basis are represented in table 1. When comparing the 4 quartiles according to AMH levels, no difference in the mean number of cycles performed. There was however a difference in mean age (p < 0.0001): women in the AMH quartile 1 category had significantly lower mean age compared to women in quartile 2 (p<0.001), 3 (p < 0.0001) and 4 (p < 0.0001). The overall cumulative clinical pregnancy rate was 54.9% and the overall cumulative live birth rate was 52.5%. No difference was found in terms of treatment outcome between the four different AMH quartiles. There was a significant difference between indication groups with regard to AMH (p = 0.002). Table 2 shows that single women who underwent IUI have a higher mean age then both hetero (p < 0.0001) and lesbian (p < 0.0001) women. AMH was lower in single women compared to lesbian women (p < 0.018). The Kaplan-Meier failure estimates up to six insemination cycles are presented on figure 2. In order to assess which variables affected cumulative live-birth, Cox proportion hazard regression was performed including age, AMH levels and indication for IUI treatment in the model as independent variables to adjust for potential confounding. Age was the only variable which significantly (p = 0.004) influenced cumulative live-birth after IUI (table 3).

DISCUSSION
Our study is the first to examine the ability of serum AMH to predict live birth in women undergoing natural cycle IUI with the use of donor sperm. According to our results serum AMH levels cannot predict live birth rates in a healthy women without a clear female factor or infertility and with the presence of normal sperm parameters. This finding suggests that although in women undergoing ART reduced AMH level and reduced ovarian reserve may actually reduce live birth chances, cumulative live birth rates are not
compromised with diminishing AMH levels in healthy regularly cycling women undergoing intrauterine insemination.

This finding is also of clinical relevance for women that consider oocyte cryopreservation for the prevention of age related fertility decline or AGE banking. Initial assessment of the feasibility of preventive banking requires an assessment of the ovarian reserve (19). These findings indicate that single women with an expected low ovarian response and therefore less efficient for oocyte banking, do not have lower chances when turning to their only alternative being donor sperm insemination.

The findings of this study also suggest that AMH values are not predictive for the natural fertility in the general population. This may be contradicting the findings of Steiner et al. (2011), whose study concluded that early-follicular phase AMH did however appear to be associated with natural fertility in the general population (20). Their findings were based on a limited sample size of 100 women and was, unlike our study, not adjusted for the important male factor covariate. Our findings appear to confirm the findings of a recent study in women who conceive naturally by Hagen et al. (2012) and Streuli et al. (2014) (21, 22). The latter study by was a relatively small observational study (n=87) that could not find a relation between AMH values and the time to pregnancy (21). The former by Hagen et al., found that low AMH values in young healthy women are not predictive for a reduced fecundability (22). As opposed to our study, this prospective cohort study was performed on women in their mid-20s. Although this study was adjusted for important covariates, it studied natural conception on a cohort of a smaller sample size compared to this this study.

Our findings may open the debate on the necessity to routinely perform an AMH assessment prior to non-stimulated IUI insemination cycles in regularly cycling women. An argument in favour of such test could be the identification of women with a reduced ovarian reserve. For those patients, a sense of urgency may lead towards the initiation of stimulated IUI insemination cycles. Although stimulation prior to IUI insemination does increases the outcome (23), it remains unclear of this effect is also beneficial for those with a limited ovarian reserve (17). However, if AMH has not been assessed prior to insemination, it would become recommended after repeated failed insemination in deciding whether or not to proceed towards ART.

The analysis of the relation between AMH and fecundity in a donor insemination model in non-stimulated cycles is the major strength of this study. The donor sperm insemination guarantees in minimum sperm quality and an optimal timing which cannot be guaranteed in studies performed with natural intercourse. Secondly, other studies studying the relation between AMH and insemination
outcome included stimulated cycles (17). Moreover, the patient population in the present study allows better generalization to the general population.

A limitation of our study is its retrospective design. We can therefore not exclude that the AMH values may have influenced the decision of the clinician. One may argue that women with a limited reserve may have been counselled to proceed towards stimulated IUI or IVF causing an underrepresentation of women with a lower reserve.

Another possible limitation of this study is the absence of other established ovarian reserve tests such as the AFC or serum follicle stimulating hormone (FSH) levels. However, several studies have indicated AMH as the reserve tests most related to ovarian response and outcome after ART (10, 20). Moreover, studies that did find a strong and significant association between AMH and natural fertility did not find such an association with other ovarian reserve tests such as FSH or AFC (20, 24).

In the presence of optimal fecundation through cycle monitoring of the mono-follicular growth, ovulation triggering and intra-uterine donor sperm insemination, live birth appears not to relate to the AMH value. Therefore, AMH does not appear to correlate with the live birth rate and suggests therefore that AMH only expresses the ovarian reserve and not the oocyte quality as such.

**CONCLUSION**

The donor insemination model demonstrates that AMH is not a predictor of reproductive success in healthy women without a clear female factor or infertility and with the presence of normal sperm parameters. Further, our findings suggests that cumulative live birth rates are not compromised with diminishing AMH levels in healthy regularly cycling women undergoing intra-uterine insemination. These findings indicate that single women with an expected low ovarian response do not have lower chances in terms of live births. AMH only appears to reflect the ovarian reserve and not the oocyte quality. Additional research is needed to confirm the absence of correlation of AMH with oocyte quality.

**ACKNOWLEDGEMENTS**

Foremost, I would like to express my gratitude to my advisor Professor Dominic Stoop. His knowledge and patience were invaluable throughout the year. I will be graduating this academic year in part
due to his mentoring and guidance in conducting and finishing my thesis. Next, I would like to thank Doctor Nikolaos Polyzos and Doctor Samuel Santos-Ribeiro for their help in the analysis of the dataset and on the interpretation of the results. Additional gratitude is expressed towards Professor Herman Tournaye, general overseer of my dissertation and possibly subsequent publication. Furthermore, I also wish to thank Walter Meul, staff member, for his help in the data collection and abstraction. Finally, I like to mention my student colleagues Seher and Melissa, who’s support and encouragement has meant a great deal to me throughout the year.

**FIGURES AND TABLES**

### Table 1: Outcome per patient per AMH quartile

<table>
<thead>
<tr>
<th>DATA PER PATIENT</th>
<th>Total</th>
<th>≤25% (≤2.26)</th>
<th>25%-50% (2.26-3.60)</th>
<th>50%-75% (3.60-5.44)</th>
<th>&gt;75% (&gt;5.44)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n patients</td>
<td>295</td>
<td>74</td>
<td>74</td>
<td>74</td>
<td>73</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cycles (mean ±SD)</td>
<td>2.9 ±1.8</td>
<td>3.0 ±1.7</td>
<td>2.8 ±1.8</td>
<td>2.7 ±1.8</td>
<td>3.0 ±1.9</td>
<td>0.6881</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>32.0 ±4.4</td>
<td>34.4 ±4.3</td>
<td>31.7 ±4.2</td>
<td>31.2 ±4.4</td>
<td>31.0 ±4.0</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>AMH (mean ±SD)</td>
<td>4.20 ±2.69</td>
<td>1.54 ±0.55</td>
<td>2.88 ±0.38</td>
<td>4.53 ±0.56</td>
<td>7.92 ±2.37</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Cumulative PR (%)</td>
<td>60.3%</td>
<td>63.5%</td>
<td>58.1%</td>
<td>58.1%</td>
<td>61.6%</td>
<td>0.880</td>
</tr>
<tr>
<td>Cumulative CPR (%)</td>
<td>54.9%</td>
<td>54.1%</td>
<td>52.7%</td>
<td>55.4%</td>
<td>57.5%</td>
<td>0.945</td>
</tr>
<tr>
<td>Cumulative LBR (%)</td>
<td>52.5%</td>
<td>51.4%</td>
<td>51.4%</td>
<td>54.1%</td>
<td>53.4%</td>
<td>0.982</td>
</tr>
<tr>
<td>Single/Hetero/Lesbian</td>
<td>77 / 38 / 180</td>
<td>31 / 12 / 31</td>
<td>20 / 9 / 45</td>
<td>10 / 11 / 53</td>
<td>16 / 6 / 51</td>
<td>0.002</td>
</tr>
<tr>
<td>%severe male factor infertility</td>
<td>12.9%</td>
<td>16.2%</td>
<td>12.2%</td>
<td>14.9%</td>
<td>8.2%</td>
<td>0.486</td>
</tr>
</tbody>
</table>

*The Bonferroni-corrected pairwise comparisons were significant only for the following comparisons: 1 vs 2 (p=0.001); 1 vs 3 and 1 vs 4 (p<0.0001)
† The Bonferroni-corrected pairwise comparison was significant (p<0.0001) for all group-by-group comparisons

Notes: AMH = anti-Müllerian hormone, PR = pregnancy rate, CPR = clinical pregnancy rate, LBR = livebirth rate

### Table 2: Data according to indication

<table>
<thead>
<tr>
<th>DATA PER PATIENT</th>
<th>Total</th>
<th>Single</th>
<th>Hetero</th>
<th>Lesbian</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n patients</td>
<td>295</td>
<td>77</td>
<td>38</td>
<td>180</td>
<td>N.A.</td>
</tr>
<tr>
<td>Cycles (mean ±SD)</td>
<td>2.9 ±1.8</td>
<td>3.0 ±1.7</td>
<td>2.7 ±1.8</td>
<td>2.9 ±1.8</td>
<td>0.7755</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>32.0 ±4.4</td>
<td>35.8 ±2.9</td>
<td>30.5 ±5.2</td>
<td>30.8 ±3.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>AMH (mean ±SD)</td>
<td>4.20 ±2.69</td>
<td>3.55 ±2.47</td>
<td>3.87 ±2.54</td>
<td>4.55 ±2.77</td>
<td>0.0165†</td>
</tr>
<tr>
<td>Cumulative PR (%)</td>
<td>60.3%</td>
<td>67.5%</td>
<td>47.4%</td>
<td>60.0%</td>
<td>0.114</td>
</tr>
<tr>
<td>Cumulative CPR (%)</td>
<td>54.9%</td>
<td>59.7%</td>
<td>42.1%</td>
<td>55.6%</td>
<td>0.195</td>
</tr>
<tr>
<td>Cumulative LBR (%)</td>
<td>52.5%</td>
<td>51.9%</td>
<td>42.1%</td>
<td>55.0%</td>
<td>0.349</td>
</tr>
</tbody>
</table>

* The Bonferroni-corrected pairwise comparison was significant (<0.0001) for the group-by-group comparisons 1 vs 2 and 1 vs 3
† The Bonferroni-corrected pairwise comparison was significant (<0.018) for the group-by-group comparisons 1 vs 3

Notes: AMH = anti-Müllerian hormone, PR = pregnancy rate, CPR = clinical pregnancy rate, LBR = livebirth rate
Table 3: Cox proportion regression of cumulative live-birth

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.94 (0.90;0.98)</td>
<td>0.004</td>
</tr>
<tr>
<td>AMH</td>
<td>1.00 (0.94;1.06)</td>
<td>0.944</td>
</tr>
<tr>
<td>Indication: severe male factor vs single</td>
<td>1.58 (0.84;2.96)</td>
<td>0.158</td>
</tr>
<tr>
<td>Indication: severe male factor vs lesbian</td>
<td>1.28 (0.75;2.18)</td>
<td>0.368</td>
</tr>
</tbody>
</table>

IUI rank was considered the time variable while AMH, age and indication for treatment (severe male factor infertility, requests from lesbian couples or single-parent, using male factor as the indicator variable) were added in the model as independent variables.

Figure 1: Flowchart of patient selection

Flowchart showing step-by-step exclusion criteria of the selected sample.

Notes: Only gen 2 AMH essay performed = AMH measurements after 23th April 2012
Figure 2: Live birth rate in function of the treatment cycle

Kaplan-Meier curves showing cumulative proportion of pregnancy by serum level of anti-Müllerian hormone (AMH). AMH was divided into four quartiles: ≤25% ; 25-50% ; 50-75% ; >75%
REFERENCES


