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Original article

Specific ranges of anti-Mullerian hormone and antral follicle count correlate to provide a prognostic indicator for IVF outcome

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ABSTRACT

Advanced female age correlates with reduced ovarian reserve (OR) and is the primary factor underlying the limitation of success rates in Assisted Reproductive Technology (ART). Currently, predicted ovarian response to gonadotrophin stimulation is determined using transvaginal ultrasound to measure antral follicle count (AFC), an indirect measure of OR. However, assessing the level of anti-Mullerian hormone (AMH) has more recently been shown to correlate with OR, and its application has been adopted widely. This retrospective study was designed to determine the relationship between novel ranges of AMH and AFC in patients undergoing ART. There was a positive correlation between AMH and AFC category ($r = 0.458$, $P < 0.01$), with an 87% linear concordance observed for specific AFC ranges and mean serum AMH levels. Both OR markers were inversely correlated with female age ($r = -0.428$ and $r = -0.392$, respectively). Pregnancy and live birth rates were influenced by both AMH ($P < 0.05$) and AFC categories ($P < 0.05$). Conversely, miscarriage rates appeared to be more dependent on AFC categories ($P > 0.05$), but even more reliant on female age. Finally, the number of oocytes collected was positively correlated with AMH and AFC grading, while oocyte and embryo utilization rates were negatively correlated. Overall, both OR markers were positively and strongly related with each other, and when individual AMH readings were categorised into specific novel ranges, they demonstrated a more robust correlation with AFC groupings. Taken together, applying patient AMH within specific ranges may lead to a better estimation of OR and IVF outcomes.

1. Introduction

Anti-Mullerian hormone (AMH), also known as Mullerian inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor beta-family. AMH is secreted by granulosa cells [1,2] and promotes the regression of Mullerian ducts during the formation of the male foetus [3]. In the female foetus, the Mullerian duct develops into the uterus, Fallopian tubes and upper part of the vagina during gestation [4]. AMH is strongly and constantly produced by pre-antral and small antral follicles, rather than the larger follicles selected for ovulation. Consequently, AMH is considered a marker for the ovarian reserve (OR) as it is mainly expressed by those follicles that have recently advanced from the primordial follicle pool [4]. Around 60% of serum AMH is derived from follicles approximately 5–8 mm in diameter [5]. The serum concentration depends on the constituent cellular population of developing follicles, and in turn this controls the recruitment and growth of primary follicles [1]. Therefore, serum AMH levels are representative of the quantity and quality of the ovarian follicular pool [7]. While AMH is highly expressed during the reproductive life span [6], it is undetectable after menopause [4].

The association between women's age, their reproductive capacity and Assisted-Reproductive Technologies (ART) are well defined [8]. Female age plays a primary role in the success rate of In Vitro-Fertilization (IVF), where younger women tend to be more successful [2]. The reproductive potential of the ovary was traditionally evaluated by determining the basal serum Follicle

Abbreviations: AMH, anti-Mullerian hormone; AFC, antral follicle count; OR, ovarian reserve; ART, assisted Reproductive Technologies; IVF, In Vitro Fertilisation.

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Stimulating hormone (FSH), serum Inhibin B or estradiol (E2) levels [9]. However, these factors are dependent on the number of developing eggs, and fluctuate throughout the menstrual cycle. In addition, they are also affected by artificial hormonal variations including those arising from the use of oral contraceptives [10], which influence the pituitary ovarian feedback system. Consequently, using these factors to determine OR can be inaccurate and the serum levels can be measured only in the early follicular phase [4,2]. Intuitively, the identification of an appropriate ovarian marker or method that is performed at a particularly precise time in the cycle, can lead to a more definitive estimation of OR, which is important for ART success. Therefore, these methods have been mostly replaced by alternative techniques such as AMH measurement in association with Antral Follicle Counts (AFC) [11], which are more robust. The technological development of more sensitive assays has facilitated increased accuracy for AMH measurement [12–15].

There are distinct differences between AFC and AMH methods of OR estimation. The advantages of AMH include a less invasive procedure (simple blood test), and lower intra- and inter-cycle variability, which suggests that it can be predictive at any time during the menstrual cycle [16–18]. However, its sole use has not been fully adopted by the entire ART community due to variations in measurement systems including equipment and kits. On the other hand, AFC is much more invasive as it is determined by counting the resting follicles using transvaginal ultrasound. However, it is a very accurate technique and follicles less than 1 mM can be observed, but measurements of those greater than this size are generally recorded ($\geq$2 mm). AFC is determined in both ovaries at the starting point of the proliferative phase of the menstrual cycle [11]. Today, the combination of both AMH levels and AFC by ultrasound, provide IVF clinics with a sufficient method to predict potential ovarian response to rFSH, oocyte yield, and the possibility of pregnancy outcome in ART [8]. This is not only helping in IVF success rates, but is also reducing the financial and emotional burden on couples seeking ART treatments, as they are provided with a more realistic prognostic indicator of the chance to conceive. Consequently, failure of IVF and other ART procedures are largely accredited to progressive decline in OR, which is mostly associated with increasing age.

Even though the AFC and serum AMH have been shown to correlate previously [1,11], recent approaches assessing the level of AMH and its subsequent correlation with OR are still unclear [3]. Furthermore, little is known about whether AMH can also provide predictive information regarding IVF outcome [3]. Many studies have identified the supremacy of one method over the other [19,20], but no clear consensus remains. Therefore, the objective of this study was to establish whether a close correlation between AFC and AMH existed within our clinic, and to examine the predictive value of each in relation to IVF outcome.

2. Methods

2.1. Patient selection, stimulation and management

This retrospective study conducted at PIVET Medical Centre examined pregnancy and birth outcomes of 1425 IVF treatment cycles with AFC and AMH measurements along with a fresh transfer, from a total of 3505 initiated cycles conducted over a period of approximately 7.5 years (01 April 2008–31 December 2015) (Fig. 1). Women were stimulated using standard ovarian stimulation methods, the majority of which were GnRH antagonist regimen (60.7%), gonadotrophin flare/GnRH agonist (28.8%), the AACEP (antagonist, agonist conversion with estrogen priming) for 5.7% and the Long GnRH agonist down regulation (4.8%). The type of treatment protocol chosen was dependent on clinical data from

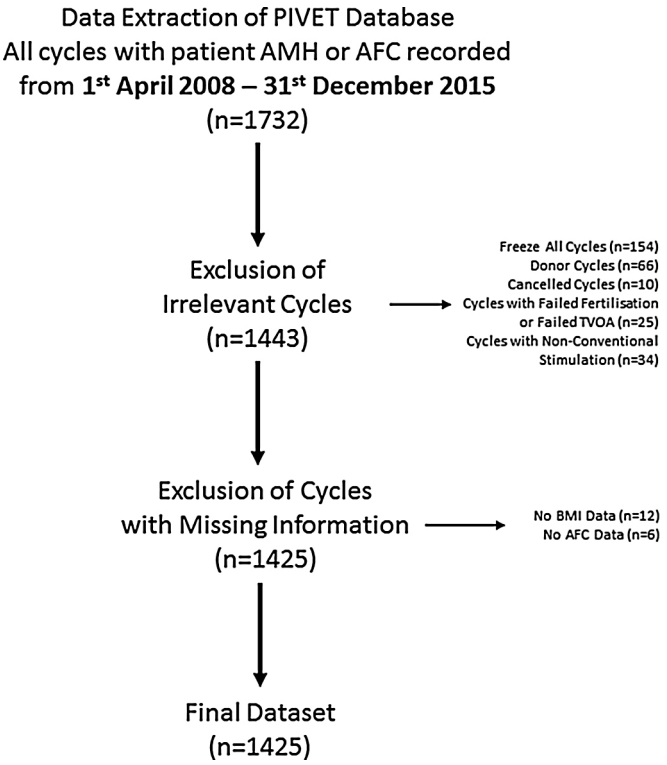

Fig. 1. Flow Chart Demonstrating the Inclusion and Exclusion of Treatment Cycles. This retrospective study included an initial 1732 treatment cycles with AMH and AFC recorded from a total 3505 initiated cycles conducted at PIVET Medical Centre from 1 April 2008–31 December 2015. Cycles that were cancelled (n = 10), or setup for donor/recipient patients were excluded (n = 66). Freeze all cycles (n = 154), or cycles resulting in failed TVOA or fertilisation (n = 25) were also excluded. Cycles receiving unconventional stimulation (e.g. Shanghai protocol) (n = 34), and cycles with no BMI or AFC information were also excluded (n = 18). A total of 1425 IVF cycles in women aged 22–48 years and which included a fresh embryo transfer were included.

the assessment cycle and any previous treatment outcomes such as proportion of poor embryos retrieved, or previous failure. At transvaginal oocyte aspiration (TVOA), the number of oocytes collected was recorded along with the number of subsequent embryos generated. Utilisation rates of these oocytes and embryos were calculated as previously published and as follows [21].

Oocyte Utilization rate was calculated by:

$$\frac{(\text{Number of Embryos Transfered and Cryopreserved}) \times 100}{\text{Total number of Oocytes Collected at TVOA}}$$

Embryo utilization rate was calculated by:

$$\frac{(\text{Number of Embryos Transfered and Cryopreserved}) \times 100}{\text{Total number of 2PNs}}$$

(2PNs reflects fertilised eggs; which in turn reflects number of mature M-II eggs)

2.2. Pregnancy

Serum beta-HCG levels above 25 IU/L were used for pregnancy diagnosis and were checked each week thereafter until week 7. Clinical pregnancy was defined by the detection of an intrauterine gestational sac along with an appropriately rising beta-HCG level at week 7. A measurable crown rump length and foetal heart beat detection at gestational week 7 defined a viable clinical pregnancy. Those pregnancies fading before this stage or diagnosed as PUL (pregnancy of unknown location, including possible ectopic gestations) were excluded from analysis. Clinical pregnancy loss

refers only to those pregnancies showing an identifiable intrauterine gestational sac, but which do not proceed beyond 20 weeks gestation; mostly designated as spontaneous miscarriage or delayed miscarriage and submitted to curettage. A live birth was diagnosed when delivery of a live infant occurred after 20 weeks gestation and cases of monozygotic twins were classified as a single live birth event.

2.3. AMH assay and AFC determination

Blood was collected by venipuncture into serum separation gel tubes on day 5 ± 1 of a preceding cycle (1–3 months prior to the IVF procedure). Following clotting (~30 mins) the sample was centrifuged at 1100–1300 × *g* for 10–15 min. The serum sample was then referred to Repromed Laboratory (Australia) and AMH determined using the Beckman Coulter Immunotech AMH Enzyme Immunoassay. The lowest amount of AMH that could be detected with a 95% probability was 1.0 pmol/L. The estimated minimum concentration achieved at 20% total imprecision was 1.2 pmol/L. The maximum inter- and intra-assay imprecision (CV%) was 14.2% and 12.3% respectively. The measured value of AMH was categorised into specific ranges and defined with a letter from grade A indicating the highest range of values, to E with the lowest range of values, i.e., A (≥20.0 pmol/L), B (15.0–19.9 pmol/L), C (10.0–14.9 pmol/L), D (5.0–9.9 pmol/L) and E (≤4.9 pmol/L). Similarly, the AFC was determined in both ovaries using transvaginal ultrasound (Voluson 730 Expert, General Electric Australia), and the total AFC was graded as follows: A (≥20 follicles), B (13–19 follicles), C (9–12 follicles), D (5–8 follicles) and E (≤4 follicles).

2.4. Ethical consideration

PIVET is accredited with both the self-regulatory National Australian Reproductive Technology Committee (RTAC) as well as the Reproductive Technology Council (RTC) of Western Australia. Reporting of the data was approved under Curtin University Ethics Committee approval no. RD_25–10 general approval for retrospective data analysis 2011, updated 2015.

2.5. Statistical analysis

Statistical data analysis was performed using SPSS software version 22.0, and figures were prepared using Graph Pad Prism version 6.0. The relationship between different parameters were analysed using either Pearson correlation, one way ANOVA (with Tukey's test) or chi-square analysis. For a test of significance, a P value < 0.05 was considered significant.

Table 1
Pearson correlation between AMH and AFC with various patient parameters.

	AFC category	Age (year)	BMI (Kg/m^2)	Number of Oocytes
AMH (pmol/L)	0.458[a]	−0.392[a]	−0.01	0.355[a]
AFC category		−0.428[a]	−0.01	0.502[a]
Age (year)			0.046	−0.282[a]
BMI (Kg/m^2)				−0.017

[a] Indicates correlation is significant at the <0.01 level (2-tailed).

3. Results

3.1. Correlation between AFC category and serum AMH level

The concordance between the individually measured serum AMH values and the corresponding AFC patient grading demonstrated a 21.0% linear correlation ($R^2 = 0.210$) (Fig. 2A). However, when the mean AMH was calculated for all of the patients within each AFC grouping, the linear correlation increased to 87.0% ($R^2 = 0.870$) (Fig. 2B). Fig. 2C illustrated a statistically significant difference between mean AMH levels and specific AFC categories. It is clear that the mean AMH value rises almost proportionally with the elevation of the AFC category and there was a highly significant Pearson correlation between serum AMH and AFC ($r = 0.458$, $P < 0.01$) (Table 1).

3.2. Associations among serum AMH, AFC category, BMI and age

There was a statistically significant decrease in mean age as the AMH level increased ($r = -0.392$, $P < 0.01$) (Table 1). This was also observed when AMH was categorised into ranges (Fig. 3A). Likewise, a similar statistical and graphical pattern was found between age and AFC category ($r = -0.428$, $P < 0.01$) (Table 1 & Fig. 3B). Conversely, the mean BMI did not change according to AMH or AFC grouping (Fig. 3C & D).

3.3. Correlation between AMH and AFC categories with rates of clinical pregnancy, live birth and miscarriage

Analysis of clinical pregnancy and live birth data according to serum AMH and AFC category showed that the rates were somewhat dependent on these OR markers. In general, clinical pregnancy rates were significantly elevated in the higher AMH ($P < 0.05$) and AFC groupings ($P < 0.05$), corresponding to increased levels of serum AMH (grade D to A; i.e. ≥5.0 pmol/L) or a larger number of developing follicles (grade B to A; i.e. ≥13 follicles) (Fig. 4A & B, respectively). In the AMH groupings, the clinical pregnancy rate was 18.4% in category E and increased to 26.6% and 30.1% for category D and C, respectively, which increased

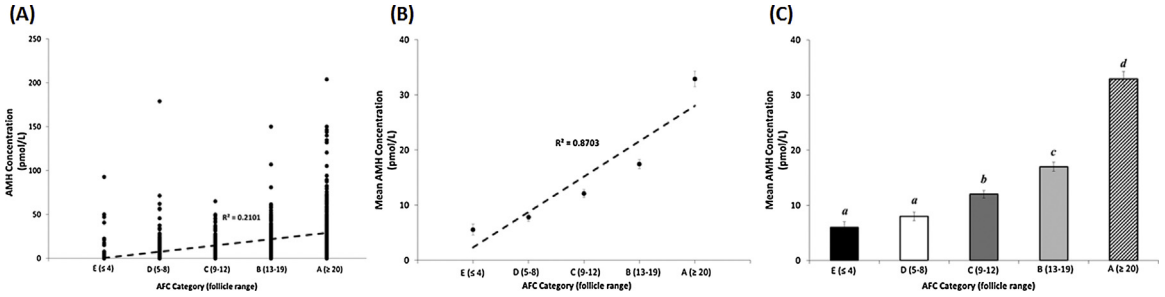

Fig. 2. Correlation Between AFC Category and Serum AMH Level. (A) Association between all serum AMH measurements and AFC category ($R^2 = 0.210$). (B) Association between mean AMH level and AFC category ($R^2 = 0.870$). (C) Relationship between mean AMH recorded for specific AFC categories. Results are presented as mean ± SEM. Column comparisons with the same letters are not statistically different from each other. Consequently, different letters above the columns are significantly different ($P < 0.05$).

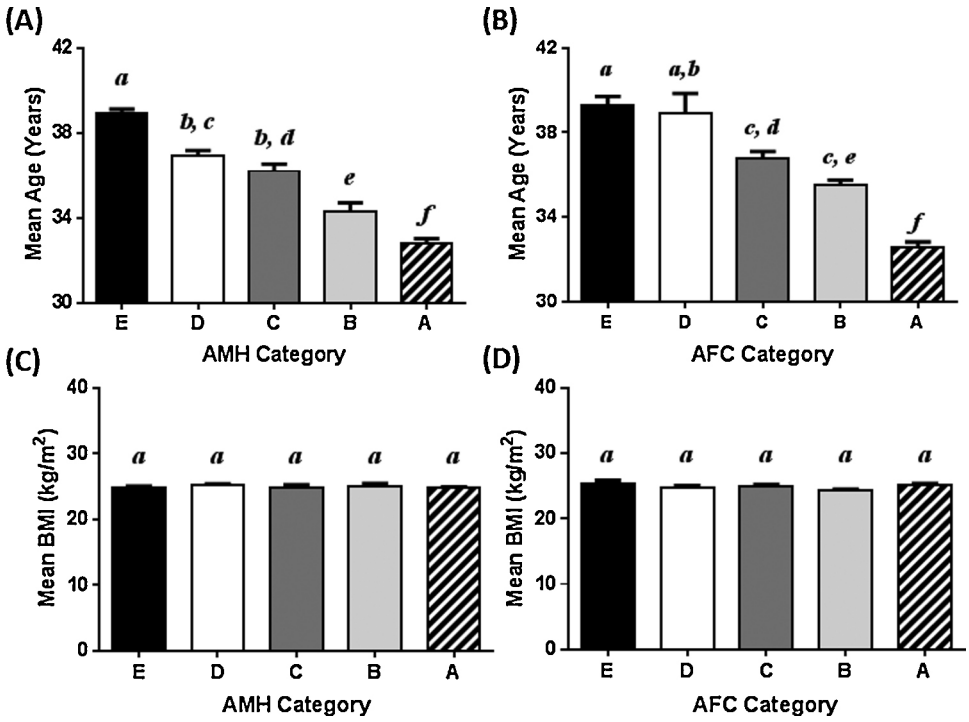

Fig. 3. Relationships Among Serum AMH, AFC Category, BMI and Age. (A) & (B) Association between mean female age and AMH and AFC categories, respectively. (C) & (D) Association between mean BMI and AMH and AFC categories, respectively. Results are presented as mean ± SEM. Column comparisons with the same letters are not statistically different from each other. Consequently, different letters above the columns are significantly different ($P < 0.05$).

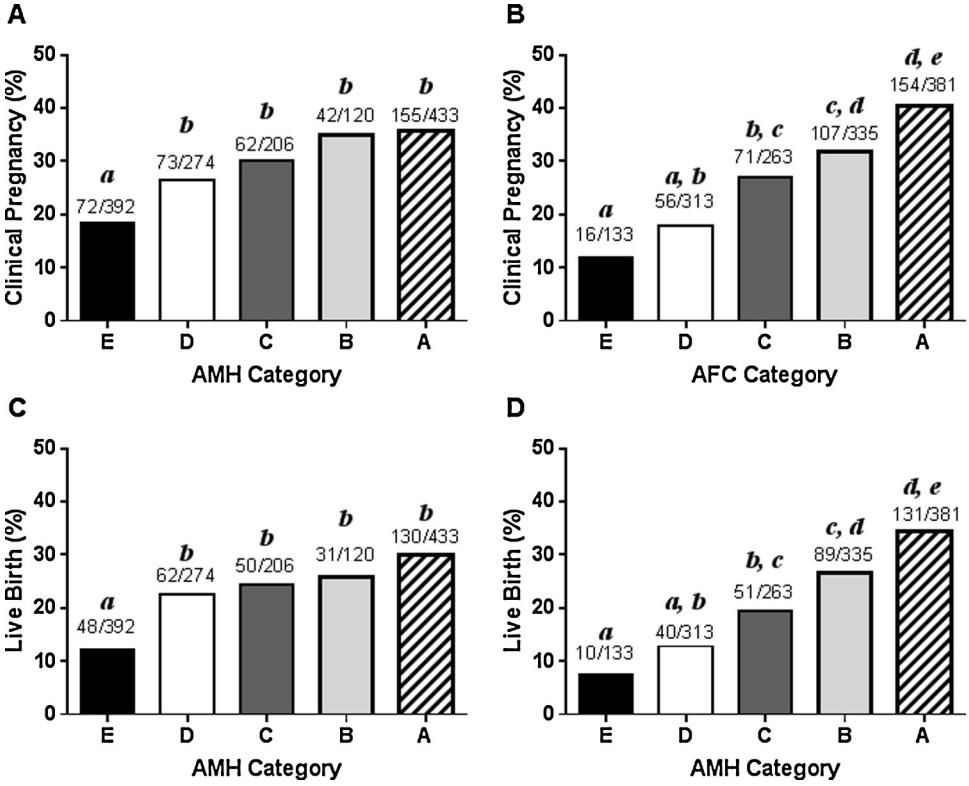

Fig. 4. Correlation Between AMH and AFC Categories with Clinical Pregnancy and Live Birth Rates. (A) & (B) Clinical pregnancy rates were higher according to rising AMH and AFC categories, whilst higher rates of pregnancies occurred for AFC category "B" and "A" ($P < 0.05$). Conversely, rates plateaued and were at a maximum for AMH category D through to A (AMH ≥ 5.0 pmol/L). (C) & (D) Similar trends were observed for live birth rates, with live birth rate plateauing from AMH grade D (AMH ≥ 5.0 pmol/L). Significantly higher live birth rates occurred in the highest AFC categories "B" and "A" ($P < 0.05$). Column comparisons with the same letters are not statistically different from each other. Consequently, different letters above the columns are significantly different ($P < 0.05$).

Table 2
Cumulative pregnancy and live birth rates per TVOA (initiated cycle) for specific AMH and AFC categories.

	AMH/AFC category *AMH (pmol/L)* *AFC (follicle #)*	E *≤ 4.9* *≤ 4*	D *5.0–9.9* *5–8*	C *10.0–14.9* *9–12*	B *15.0–19.9* *13–19*	A *≥20.0* *≥20*
Cumulative Pregnancy Rate (CPR)	AMH Grouping	25.8% (101/392)^{a,b,c,d}	46.0% (126/274)^{a,b,e}	53.4% (110/206)^{a,b,e}	69.2% (83/120)^{c,d,e}	66.5% (288/433)^{c,d,e}
	AFC Grouping	22.6% (30/133)^{a,b,c}	26.8% (84/313)^{a,b,c}	43.0% (113/263)^{a,b,d,e}	58.5% (196/335)^{a,c,d,e}	74.8% (285/381)^{b,c,d,e}
Cumulative Live Birth Rate (CLBR)	AMH Grouping	18.1% (71/392)^{a,b,c,d}	35.4% (97/274)^{a,b,e}	44.2% (91/206)^{a,e}	48.3% (58/120)^{d,e}	52.9% (229/433)^{c,d,e}
	AFC Grouping	13.5% (18/133)^{a,b,c}	19.5% (61/313)^{a,b,c}	31.2% (82/263)^{a,b,d,e}	45.4% (152/335)^{a,c,d,e}	61.2 (233/381)^{b,c,d,e}

Rate percentage = (total pregnancies or live births from fresh and frozen cycles divided by total cycles reaching TVOA).
"a", "b", "c", "d" and "e" indicate significant difference from Grade A, B, C, D and E in same row respectively ($P < 0.05$).

Table 3
Cumulative pregnancy and live birth rates per Embryo Transfer (ET) for specific AMH and AFC categories.

	AMH/AFC category *AMH (pmol/L)* *AFC (follicle #)*	E *≤4.9* *≤4*	D *5.0–9.9* *5–8*	C *10.0–14.9* *9–12*	B *15.0–19.9* *13–19*	A *≥20.0* *≥20*
Cumulative Pregnancy Rate (CPR)	AMH Grouping	15.6% (101/647)^{a,b,c,d}	25.1% (126/502)^{a,b,e}	26.4% (110/417)^{a,b,e}	34.6% (83/240)^{c,d,e}	35.6% (288/810)^{c,d,e}
	AFC Grouping	14.1% (30/213)^{a,b,c}	15.4% (84/547)^{a,b,c}	23.5% (113/481)^{a,b,d,e}	29.7% (196/660)^{a,c,d,e}	39.9% (285/715)^{b,c,d,e}
Cumulative Live Birth Rate (CLBR)	AMH Grouping	11.0% (71/647)^{a,b,c,d}	19.3% (97/502)^{a,e}	21.8% (91/417)^{a,e}	24.2% (58/240)^{a,e}	28.3% (229/810)^{b,c,d,e}
	AFC Grouping	8.5% (18/213)^{a,b,c}	11.2% (61/547)^{a,b,c}	17.0% (82/481)^{a,b,d,e}	23.0% (152/660)^{a,c,d,e}	32.6% (233/715)^{b,c,d,e}

Rate percentage = (total pregnancies or live births from fresh and frozen cycles divided by total cycles reaching TVOA).
"a", "b", "c", "d" and "e" indicate significant difference from Grade A, B, C, D and E in same row respectively ($P < 0.05$).

further to approximately 35% in category B and A (Fig. 4A). Likewise, a similar pattern and pregnancy rate was observed for AFC classification, where 40.4% and 31.9% were pregnant for group A and B, respectively. Rates decreased to 27.0%, 17.9% and 12.0% for groups C, D and E, respectively (Fig. 4B). Live birth rates also displayed a similar trend for both AMH and AFC (Fig. 4C & D) and were 12.2%, 22.6%, 24.3%, 25.8%, and 30.0% for AMH groups E, D, C, B and A, respectively, and 7.5%, 12.8%, 19.4%, 26.6% and 34.4% for AFC groups E, D, C, B and A, respectively. The data indicated higher rates according to higher AMH or AFC grouping, but the difference in proportions were not significant ($P > 0.05$) above AMH group D (≥5.0 pmol/L). However, a more obvious stepwise and significant increase was found when cumulative pregnancy (CPR) and cumulative live birth rates (CLBR) were calculated according to AMH and AFC category (Tables 2 and 3). In addition, we investigated whether miscarriage rates for the same dataset were statistically different (Fig. 5). Miscarriage rate appeared to decrease according to increasing AFC. While this was loosely replicated for AMH, the rates appeared to be inconsistent according to AMH group and did not demonstrate a clear trend (Fig. 5A & B). Conversely, we found the miscarriage rate to be significantly reliant upon age group and increased with the older women (Fig. 5C).

3.4. The impact of AMH and AFC groupings on the number of oocytes collected, and the rates of oocyte and embryo utilization

The mean number of oocytes harvested tended to increase with increasing AMH and AFC (Fig. 6). Using Pearson correlative analysis, we revealed a statistically significant positive relationship between the mean number of oocytes collected and the AMH category ($r = 0.355$, $P < 0.01$), and the AFC category ($r = 0.502$, $P < 0.01$) (Table 1 and Fig. 6A & B). The number of oocytes retrieved peaked for AMH category C to A (i.e. >10.0 pmol/L), while they peaked in AFC category A (≥20 follicles). The mean number of oocytes retrieved in category A for both AMH and AFC, were almost three times higher than that from group E (Fig. 6A & B). For both measurements, the maximum quantity of oocytes collected was similar in the upper category (approximately 12 oocytes) and reflected our targeted rFSH stimulation protocols for <15 oocytes [22].

Interestingly, the oocyte utilization rates showed a decreasing trend with higher level of AMH ($P < 0.05$) and higher AFC categories ($P < 0.05$) (more follicles), and the trends were very similar for both measurements (Fig. 6C & D). The rates for AMH and AFC groups B and A (approximately 30%), were significantly lower than that of group E (50%). Similarly, the embryo utilization rate

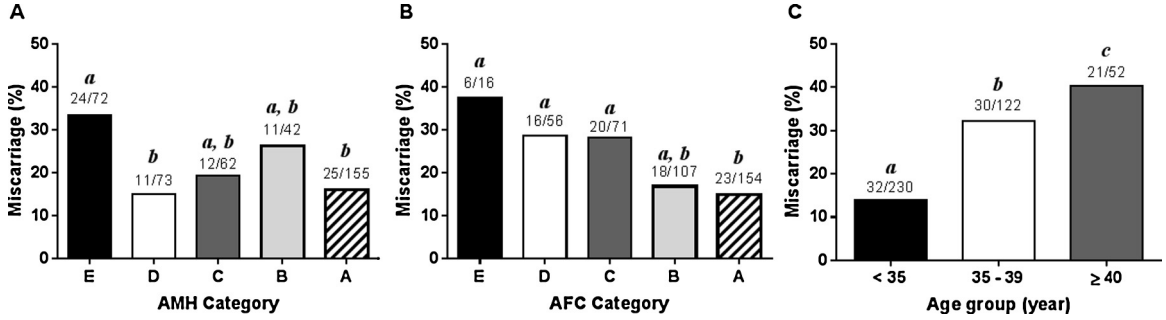

Fig. 5. Association Between Miscarriage Rate and AMH/AFC category and Age Group. (A) & (B) Miscarriage rates were highest in the poor-prognosis category E for both AMH and AFC. While these rates decrease significantly for AFC according to grade, there were more inconsistent for AMH groupings. (C) However, there was an age dependent increase in miscarriage rates with the highest rate observed in females older than 39 years of age. Column comparisons with the same letters are not statistically different from each other. Consequently, different letters above the columns are significantly different ($P < 0.05$).

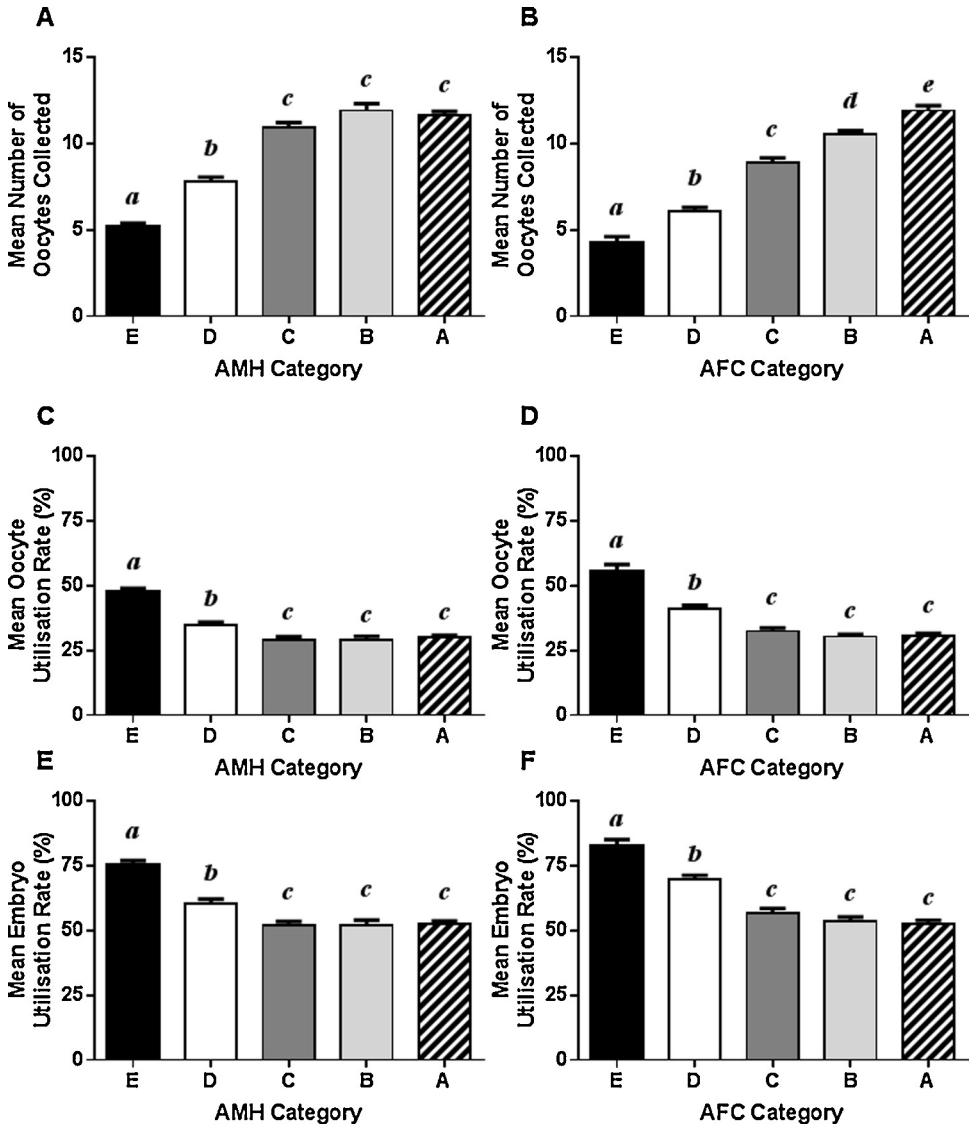

Fig. 6. Impact of AMH and AFC category on the Number of Oocytes Collected at TVOA and Rates of Utilization for Oocytes and Embryos. (A) & (B) Positive association between the number of oocytes collected and AMH and AFC categories, respectively. (C) & (D) Negative association between the oocyte utilization rates and AMH and AFC categories, respectively. (E) & (F) Negative association between the embryo utilization and AMH and AFC categories, respectively. Results are presented as mean ± SEM. Column comparisons with the same letters are not statistically different from each other. Consequently, different letters above the columns are significantly different ($P < 0.05$).

decreased with higher AMH ($P < 0.05$) and AFC ($P < 0.05$), and again the trends were almost identical between the two methods for determining ovarian response (Fig. 6E & F). The embryo utilization rates for AMH and AFC group A, B and C, were significantly lower than that of group E and D. Finally, we demonstrated, as expected, that the number of oocytes retrieved were inversely dependent on female age (years) ($r = -0.282$, $P < 0.01$) (Table 1).

4. Discussion

This study used novel and specific AMH and AFC ranges previously defined and published by our group to determine the relationship between these two independent markers of OR [23,22]. To our knowledge, the current study is the first to simultaneously examine the relationship between AMH and AFC with three clinical parameters: clinical pregnancy, live birth and miscarriage rate in a single investigation. Our results have shown a 21% linear concordance between individual serum AMH measurements and specific AFC categories, but this level of correlation was vastly improved to 87% when mean serum AMH readings were evaluated within each AFC category, indicating a strong dependent relationship. However, because only the mean AMH value appeared to correlate very well with AFC category, this also suggested that there was still a degree of unknown discordance that may possibly be explained by technological and methodological limitations for both measures. In addition, it must also be noted that the AFC was recorded in specific ranges (designated by letters) for each patient, rather than by a specific AFC summation in the form of a continuous variable. Consequently, this did not allow a full correlative analysis to be performed, and it constitutes a limitation of our study. However, while other investigations have shown that mean serum AMH significantly correlated with the number of antral follicles [3,6,24], the approach of the current study using specifically defined AFC and AMH ranges revealed a more robust relationship between these parameters, and may allow a more accurate estimation of OR or response to gonadotrophin stimulation.

It has been routinely suggested that AMH has a comparable level of precision and clinical value to AFC, and in one study it demonstrated nearly identical poor response prediction [25]. Several other studies have reported that AMH and AFC affect clinical pregnancy, live birth and miscarriage rates individually, but limited studies have investigated the impact of both markers with all three clinical outcomes simultaneously [3,26,27]. Importantly, the current study found that the relationship between AMH and AFC categories with clinical pregnancy, live birth and miscarriage rates were comparable. Clinical pregnancy and live births were elevated in patients with higher AMH and AFC ranges and this supported the importance of quantitative measurement of OR. Interestingly, other studies have also revealed a significant positive correlation between the clinical pregnancy rate and AMH or AFC [26–28]. These trends were also reflected in our cumulative pregnancy rate where we incorporated outcomes of directly-related frozen embryo transfers. The highest rates were observed for patients with more than 12 follicles (grade B/A) or more than 20.0 pmol/L AMH (grade A) (Table 2). These results were echoed for cumulative live birth rates (Table 2). Another study showed that cumulative live birth rates for women with higher serum AMH level (median 26.4 pmol/L vs 16.4 pmol/L) and higher AFC (median 11.0 vs 8.0 follicles) was initially greater, but the influence of both markers disappeared following adjustment for the number of available embryos and patient age [3]. Intuitively, this study further demonstrated that live birth rates were significantly higher in younger females (median 34.0 vs 36.0 years) who had a significantly higher number of transferable embryos (median 5 vs 2). Similar to our findings, these data clearly showed that mean AMH and AFC grading decreased with advancing age, which is the primary driver of the reduced ovarian pool. However, some important studies have suggested that the relationship between rising AMH and increasing live birth rate may also be dependent on specific thresholds, with no difference in rates observed above an AMH concentration of 7.8 pmol/L [29], which is the maximum AMH level for the definition of poor ovarian response (POR) patients according to the Bologna Criteria [30]. Although our study was designed differently, we found a comparable plateau effect above 4.9 pmol/L AMH, which is within the POR Bologna range. Nonetheless, these data demonstrated that live births were significantly influenced by serum AMH level and pointed towards a minimum threshold.

AMH was reported to show a greater degree of accuracy for prediction of poor ($\leq$3 oocytes) and high ($\geq$15 oocytes) retrievals [31]. Our statistical analysis further demonstrated that AFC and AMH classifications were positively associated with the number of oocytes collected, which has been widely reported [9,11,17,32,33], and is strongly linked to live birth rates [34]. Hypothetically, subjects with higher AFC and AMH value who are likely to be younger, should have high oocyte utilization and embryo utilization rates. However, our outcomes do not maintain this hypothesis and displayed opposite findings with higher utilization rates observed in patients with lower AMH and AFC. This data indicated that more eggs were utilized in the lower AMH and AFC categories (group E and D), possibly because of the desperate nature and prognosis of patients in these groupings. Alternatively, the higher AMH and AFC categories returned more immature eggs that were not used or frozen (i.e. utilized), and thus may be associated with the polycystic ovary syndrome (PCOS) phenotype. These effects reflect a statistical aberration as the utilization rate is dependent on the denominator, being the number of oocytes recovered. Consequently, oocyte maturity and embryo quality play an important role in these clinical IVF outcomes.

While it is reported that there is an association among AMH, AFC and oocyte and embryo quality [8], increasing the number of oocytes and embryos with stronger stimulation may not yield higher live birth rates [35,3,36]. It has been proposed that collection of 15 oocytes will maximize treatment success, while minimizing the risk of OHSS (Ovarian Hyperstimulation Syndrome) [34]. However, OHSS risk can increase dramatically following collection of more than 12 oocytes, and we have recently published unique rFSH dosing algorithms which allow the collection of $<$15 oocytes in normal and hyper-responders (only 11.6% of patients generated >15 oocytes) [22]. Consequently, we are not of the view that high stimulation leads to better results. These dosing algorithms take into account the novel AMH and AFC ranges in the current study. However, we primarily use the AFC range to determine initial rFSH starting dose, and carefully adjust rFSH dose downwards if the AMH is in a higher AMH group than the AFC rating. Clearly, in this complex milieu, there may be other influencing aspects like embryo development and endometrial factors that influence outcomes [3,27]. As a result, it is currently not fully established whether embryo and oocyte quality can be truly predicted by either AMH level or AFC, hence further work is required [35,36], and we suspect many other factors can affect embryo and oocyte utilization rates.

Several studies demonstrated that AMH is a better predictor of OR [18,31,37], and showed less individual intra- and inter-cycle variation [18]. However, recent systematic reviews, meta-analyses and observational studies have suggested that AMH and AFC were the best predictors of OR, with varying degrees of similarity [25,38,39]. This similarity has led to the almost interchangeability of the terms in relation to the measurement of OR [40–42], but there are significant differences with regard to ovarian response and oocyte retrieval [43,31]. The development of a rapid, reliable and precise method to accurately determine OR is completely required by ART clinics, and simplicity, along with high speed of application is extremely beneficial to improve IVF outcomes. Clearly, AMH measurement offers several advantages over AFC, consisting of a simple blood test that is relatively free from operator experience or bias. Moreover, AMH levels only fluctuate by approximately 4% during an individual patient's cycle, dipping slightly at ovulation [44,45]. However, there is a lack of standardization of AMH assay results.

Conversely, AFC is a rapid technique that is widely available in ART clinics and ubiquitously used. However, AFC is largely dependent on the sonographer experience and the device used, which has been moderately improved with the introduction of 3D sonography, and offline image storage and analysis [42]. Nonetheless, this technique is more invasive than a simple AMH blood test, and is subject to significant inter- and intra-operator bias [44]. In addition, the AFC can also modulate vastly during cycle progression and throughout sequential menstrual cycles and thus may be difficult to accurately establish [18]. Finally, the AFC and AMH serum level can be dependent on individual patient characteristics, and both have been reported to be lower in cancer patients [46], while AFC is generally decreased in smoking females [47]. AMH levels appear to be also dependent on ethnicity, with lower serum concentrations reported in age-matched women of Asian, Black African and Hispanic ethnicity [48,49], and this must be considered when estimating OR and potential ovarian responses.

Overall, both AFC and AMH are strongly dependent on patient age as shown here, with a reduction in both observed in older females approaching the end of their child-bearing years. Since it is still not entirely clear which of the two parameters provides the better assessment of ovarian response, or particularly the level of concordance between the two in ageing females, it may be better to approach correlative analysis using specific AMH and AFC ranges, as indicated in this study. In conclusion, an individual AMH reading that is categorised into a specific range, may demonstrate a more robust correlation (up to 87%) with corresponding AFC

groupings, and together may lead to a better estimation of OR and ultimately IVF outcomes, including pregnancy and live birth rates.

Author's roles

KNK, PN and JLY conceived the idea, planned and designed the manuscript. KNK, VFC and SRW analysed all data. KNK, VFC and SRW wrote the first draft, which was revised by NGC, PN and JLY. Figures were designed by KNK, VFC and SRW. All authors participated in manuscript revision, and approved the final submitted version.

Conflict of interest

The authors confirm they have no conflict of interest to declare.

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