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FERTILITY PRESERVATION

Fertility preservation in women with malignancies: the accuracy of antral follicle count collected randomly during the menstrual cycle in predicting the number of oocytes retrieved

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Abstract

Purpose To evaluate the capacity of random antral follicle count (AFC), i.e., AFC recorded at any time during the menstrual cycle, to predict the number of retrieved mature oocytes in women with malignancies undergoing random start ovarian hyperstimulation

Methods A consecutive series of 72 women with malignancies who underwent ovarian hyperstimulation aimed at egg freezing between July 2014 and December 2016 was retrospectively reviewed. A standardized random start protocol was used for all women. AFC and serum AMH were systematically assessed prior to initiating ovarian hyperstimulation. The main outcome was the retrieval of ≥ 10 mature oocytes. The accuracy of random AFC was tested with the *c*-statistics (area under the ROC curve).

Results For the whole cohort, the *c*-statistics for the prediction of ≥ 10 mature oocytes using AFC and serum AMH were similar. Specifically, the areas under the curve were 0.76 (95%CI 0.66–0.87) and 0.82 (95%CI 0.72–0.92), respectively (p = ns). Moreover, when considering the subgroup of women recruited after day 5 of the cycle (proper random start, $n = 52$), the areas under the curve did not also differ. Specifically, they resulted in 0.77 (95%CI 0.64–0.89) and 0.83 (95%CI 0.72–0.95), respectively (p = ns).

Conclusions AFC collected at any time during the menstrual cycle can provide valuable information for the counseling of women with malignancies scheduled for oocyte cryopreservation. Its reliability appears to be non-inferior to that of serum AMH.

Keywords Antral follicle count · AMH · Oocyte · Fertility preservation · Random start

Introduction

Young women with malignancies are generally counseled on the potential detrimental effects of cancer treatments on future fertility and on the possible benefits of fertility preservation techniques [1–5]. In most cases, this counseling occurs in an urgent setting because delay in the initiation of treatments is generally perceived as potentially detrimental for survival [2] and the techniques for fertility preservation need some time to be done (from days to weeks). In addition, albeit conflicting, there is some evidence that ovarian reserve may be already compromised prior to initiating oncologic treatment in women with cancer [6–8]. There is thus the need to provide prompt counseling on future fertility issues including a reliable estimate of the possible benefits of fertility preservation techniques.

In this context, estimating ovarian reserve prior to initiating cancer treatments represents a relevant information in the decision-making process. The higher the ovarian reserve, the higher the number of oocytes that could be retrieved after ovarian hyperstimulation [9], and thus the chances of future pregnancies [10]. Unfortunately, the available and validated tests of ovarian reserve do not satisfy the peculiar requirements for an urgent counseling [9]. The assessment of serum AMH and inhibin B still requires several days in most settings

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[11, 12]. Serum dosage of FSH is specific only for compromised ovarian reserve and needs to be performed on day 2–3 of the cycle [10, 13]. In contrast, transvaginal ultrasound assessment of the number of antral follicle count (AFC) [14] can be easily obtained regardless of the phase of the menstrual cycle and may thus fulfill the requirements for urgent counseling. However, there is some contrasting evidence suggesting significant fluctuations of AFC during the menstrual cycle [15–17]. Therefore, as for serum FSH, this assessment is claimed to be reliable only if performed in the early follicular phase [18].

In this study, we aimed to investigate the capacity of random AFC, i.e., AFC recorded at any time during the menstrual cycle, to predict ovarian responsiveness in women with malignancies undergoing a random start protocol of hyperstimulation [19, 20]. Specifically, we hypothesized that random AFC was not inferior to serum AMH. To address this issue, we compared the accuracy of these two biomarkers in predicting the number of retrieved mature oocytes.

Materials and methods

This cross-sectional retrospective study was conducted following the STARD guidelines for diagnostic studies [21]. Specifically, we reviewed women with a diagnosis of malignancy referring to the Infertility Unit of the Fondazione Ca′ Granda, Ospedale Maggiore Policlinico of Milan, Italy, between July 2014 and December 2016 for fertility preservation. We selected consecutive women who accepted to undergo ovarian hyperstimulation aimed at egg freezing. The use of oral contraceptives, a history of ovarian surgery, and the presence of organic or functional ovarian cysts were not exclusion criteria. If women underwent more than one cycle, only the first one was included in the analysis. Women were excluded if they did not have previous sexual intercourse because they were assessed using transabdominal rather than transvaginal ultrasound. Moreover, we excluded women who had to interrupt ovarian hyperstimulation because of the sudden worsening of the clinical conditions requiring immediate initiation of oncologic treatments. The study was approved by the local Institutional Review Board. An informed consent was not required since this is a retrospective study. However, all women in our Unit provided an informed consent for their data to be used for research purposes and those denying this consent were excluded.

According to the policy of our Unit, women with a diagnosis of malignancy received a detailed counseling on the potential detrimental effects of chemotherapy on the ovarian reserve. They were also informed of the possible impact of aging considering that a 5-year follow-up is commonly requested after the end of chemotherapy prior to initiating pregnancy seeking. From a therapeutic point of view, they were informed about the possible benefits of the assumption of GnRH analogues during chemotherapy and on the possibility of cryopreserving ovarian tissue or oocytes. The latter is generally recommended in adult women because of the local higher know-how [22, 23] and because it is no more considered experimental [24]. All selected women underwent transvaginal sonography to assess AFC at the time of referral regardless of the phase of the cycle. All ultrasound tests were performed by two expert technicians who used a unique instrument (EUB 6000 HITACHI) equipped with a 6-MHz curvilinear color Doppler probe. A 2D US in real-time was used to identify antral follicles 2–10 mm in diameter [14, 18]. Precise measurement of specific follicles was done only if classification was doubtful (thus for follicles with a diameter close to the thresholds of 2 and 10 mm). Finally, women provided a blood sample for AMH assessment. The latter was systematically prescribed even if not immediately available (sera were stored and tested once every 2 weeks in our hospital) in order to provide a complete overview of the situation of the women at the end of the procedure. Serum AMH concentration was tested by independent blinded biologists of our hospital using the commercially available Beckman Coulter Gen II ELISA assay on the automated GEMINI platform (STRATEC Biomedical AG, Germany) after dilution with assay buffer as previously reported [25]. Serum AMH was chosen as the reference standard because it is poorly influenced by the cycle phase and its use for predicting ovarian responsiveness has been validated [9, 11, 26].

Women opting for oocyte cryopreservation were treated according to a random start protocol as previously reported [23]. Briefly, women initiated gonadotropin treatment on the day of referral, irrespective of their menstrual cycle date. They received long-acting recombinant FSH 100 or 150 mcg according to body weight (< or ≥ 60 kg) (Elonva®, Merck Sharp & Dohme, UK) followed by recombinant FSH daily (Gonal-F®, Merck-Serono, Italy) if needed. Women were monitored with serial transvaginal ultrasound and if required serum estrogen assessment. The dosage could be adjusted during the stimulation according to response, including the possibility to add daily recombinant FSH before the end of the 7-day duration of the effect of long-acting recombinant FSH. Daily GnRH antagonists (Orgalutran® 0.25 mg Merck Sharp & Dohme, UK) were added when a follicle reached the diameter of 13–14 mm up to the time of ovulation trigger. Women who were diagnosed hormone-sensitive cancers were also given Letrozole 5 mg (Letrozolo®, TEVA, Italy) daily for the whole duration of ovarian hyperstimulation and continued for 7 days after ovum retrieval. Final oocyte maturation was triggered with GnRH agonists (Fertipeptyl® 0.2 mg, Ferring, Switzerland) when at least three follicles had a mean diameter ≥ 18 mm. Oocyte retrieval was performed under transvaginal ultrasound

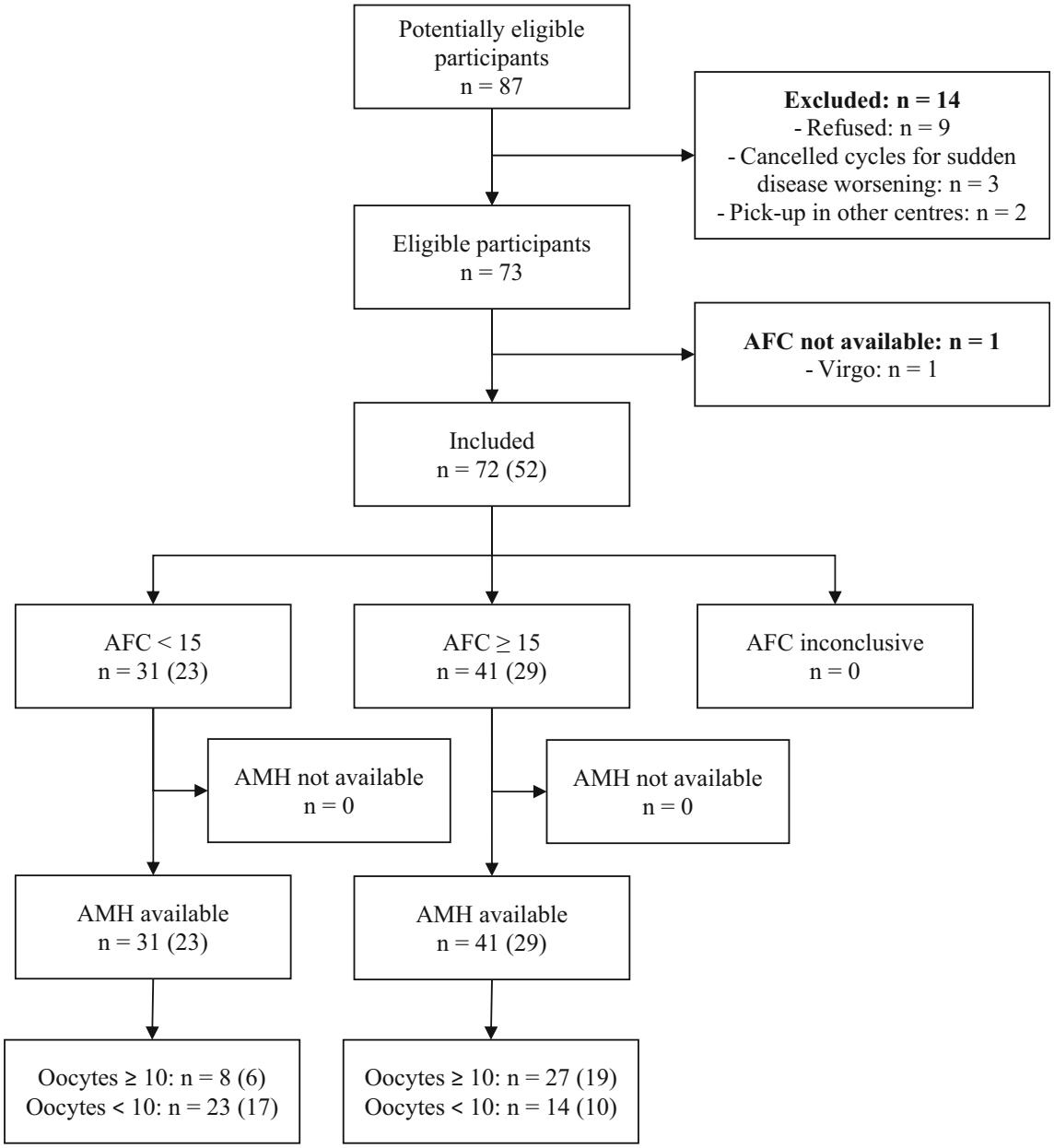

Fig. 1 Flowchart of the study. Seventy-two women were ultimately included. The cutoff value of 15 for AFC was chosen a posteriori based on the Youden index identified with the *c*-statistics. Specific data for the subgroup of women undergoing proper random start ($n = 52$) are reported in parentheses (this information was added only for the included women)

guidance 36 h after the trigger. Cycles were canceled if less than 3 follicles developed (poor response) since we deemed exposing women to the risks of the oocyte retrieval unfair in this situation. However, they were included in the analysis comparing women from whom more and less than 10 mature oocytes were retrieved. Specifically, they were included in the latter group because, even if they had continued the stimulation, we confidently infer that they could not retrieve more than 10 oocytes. Oocyte cryopreservation was performed on the day of retrieval using a vitrification procedure as described in detail elsewhere [22]. Post-oocyte retrieval active monitoring for the detection of ovarian hyperstimulation syndrome (OHSS) [27] was performed in women deemed at risk.

Analyses were performed for the whole cohort and for the subgroup of "proper" random start cycles, i.e., cycles that were initiated in a non-early follicular phase (thus excluding women who were assuming contraceptives and those who were by chance recruited before day 5 of the cycle). We indeed wanted to not only evaluate the accuracy of a systematic policy of random AFC in all women regardless of the phase of the cycle or the assumption of oral contraceptives (pragmatic

Table 1 Baseline characteristics of the studied women

Characteristics	Whole cohort $n = 72$	Proper random start $n = 52$
Age (years)	31.4 ± 5.6	32.0 ± 5.3
BMI (kg/m^2)	21.7 ± 3.6	21.6 ± 3.4
Previous deliveries	7 (10%)	5 (10%)
Menstrual cycle duration (days)		
24–27	10 (14%)	7 (14%)
28–32	51 (71%)	36 (69%)
> 32	11 (15%)	9 (17%)
Serum AMH (ng/ml)	2.3 (1.3–4.2)	2.2 (1.3–4.4)
Total AFC	18.7 ± 10.7	19.4 ± 11.3
Indication to oocyte cryopreservation		
Breast cancer	42 (58%)	32 (62%)
Lymphoma	20 (28%)	14 (27%)
Others	10 (14%)	6 (11%)
Ovarian cysts[a]	4 (6%)	3 (6%)
Cycle phase at referral		
Estroprogestins	6 (8%)	0 (0%)
Follicular phase [b]	34 (47%)	22 (42%)
Luteal phase [c]	32 (45%)	30 (58%)

Data is reported as number (%) or mean ± SD or median (interquartile range)

IQR, interquartile range

[a] Included unilateral endometriomas ($n = 3$) and bilateral endometriomas ($n = 1$)

[b] A dominant follicle (diameter ≥ 11 mm) was visualized in 16 (22%) cases (whole cohort) and 14 (27%) (proper random start)

[c] A corpus luteum was visualized in 27 (38%) cases (whole cohort) and 25 (48%) (proper random start)

approach) but also to specifically report on women who were actually treated starting in a random (non-early follicular) phase of the cycle.

Statistical analyses were performed using the Statistics Package for Social Sciences (SPSS 18.0, Chicago, IL, USA). Data are reported as number (%), and mean ± SD or median (interquartile range, IQR), as appropriate. A p value ≤0.05 was considered statistically significant. The total number of retrieved mature oocytes was the primary outcome. Women who were canceled for poor response were not excluded: they were arbitrarily associated a number of oocytes retrieved of 0 (intention to treat analysis). The Kolmogorov-Smirnov test was used to assess the normality of the distribution. For non-normal variables, the logarithmic and quadratic transformations were used to obtain normality for positively and negatively skewed distributions, respectively. Correlations between the total number of mature oocytes retrieved and AFC or AMH were tested using the Pearson coefficient of correlation (R) after transformation of non-normal variables if required. A linear regression model was used to evaluate the crude B coefficient and to adjust for age and BMI. The predictive capacity of AFC and AMH was evaluated using receiver operating characteristic (ROC) curves and data are presented as the area under the curve (AUC) and 95% confidence interval (95%CI). A good response was defined as ≥ 10 retrieved mature oocytes because this number was shown to ensure a reasonable chance of subsequent pregnancy [9, 22]. The best threshold was identified using the Youden index and was used to calculate the odds ratio (OR). A logistic regression model was used to provide ORs adjusted for age and BMI. We did not use pre-specified cutoffs for AFC or AMH because those available in the literature were validated for other

Table 2 Treatment cycle characteristics of the studied women ($n = 72$)

Characteristics	Whole cohort $n = 72$	Proper random start $n = 52$
Canceled cycles (poor response)	3 (4%)	3 (6%)
Drugs used [a]		
Corrifollitropin 100–150 mcg	69 (100%)	49 (100%)
Total dose of recombinant FSH (IU)	1651 ± 1118	1778 ± 1200
Total N of GnRH antagonist ampoules	5.3 ± 1.2	5.4 ± 1.4
Duration of stimulation (days) [a]	11.4 ± 1.9	11.5 ± 1.8
N of developed follicles (diameter ≥ 11 mm) [a]	19.4 ± 10.1	19.8 ± 10.5
N of oocytes retrieved	14.5 ± 9.7	14.7 ± 10.1
N of mature oocytes retrieved (frozen)	10.6 ± 7.9	10.9 ± 8.1
< 10	37 (51%)	27 (52%)
≥ 10	35 (49%)	25 (48%)

[a] Excluding canceled cycles

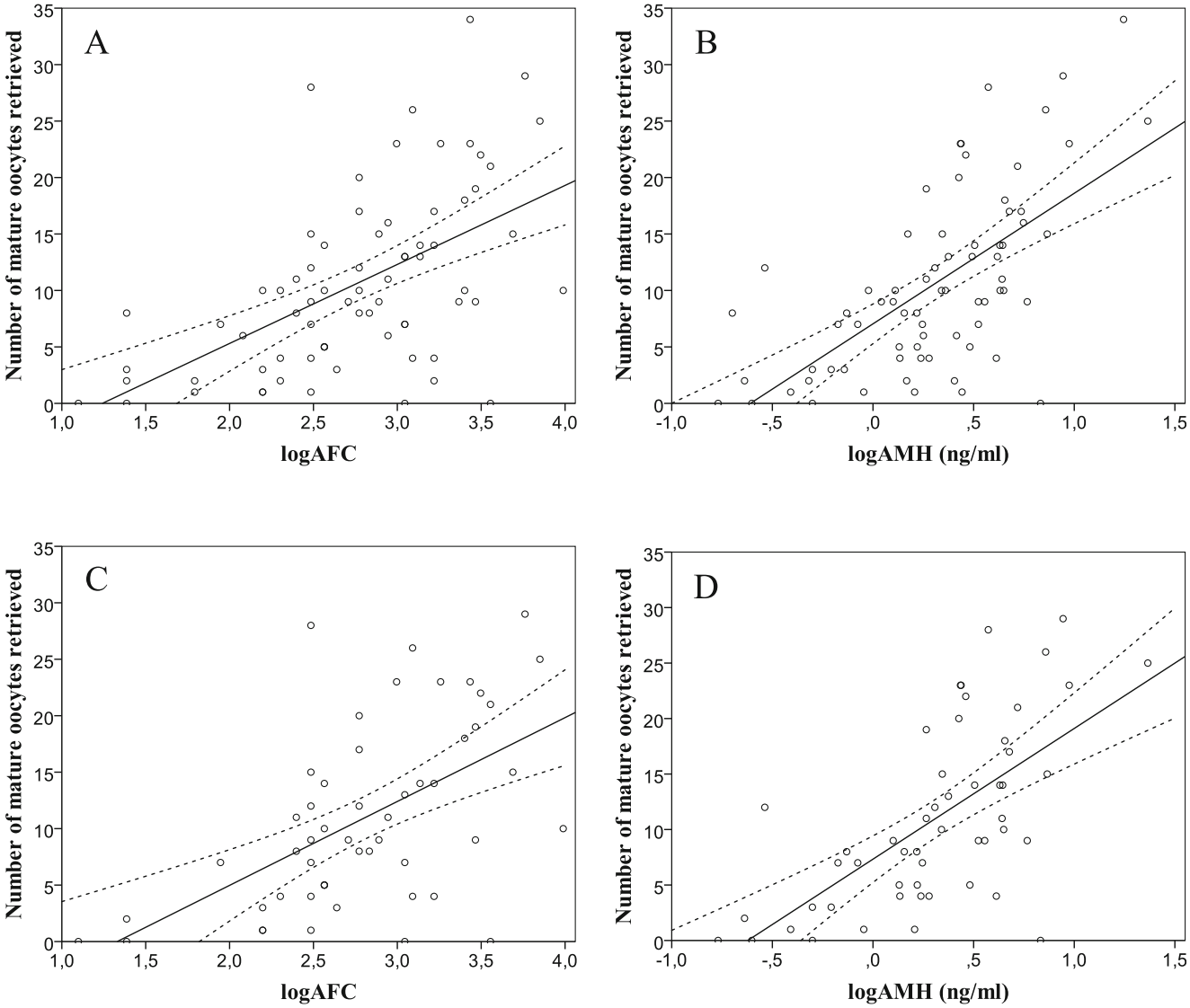

Fig. 2 Correlations between the number of mature oocytes retrieved and AFC or AMH. Data is presented separately for the whole cohort (Panel A for AFC and Panel B for AMH) and for women undergoing proper random start (Panel C for AFC and Panel D for AMH). The coefficients of correlation are 0.55 (p<0.001), 0.64 ($p < 0.001$), 0.53 ($p < 0.001$) and 0.62 ($p < 0.001$), respectively. AFC and AMH are reported in a logarithmic scale. The straight lines represent the mean correlation whereas the dot lines represent the corresponding 95%CIs

outcomes (such as poor or hyper response). Women with indeterminate or missed tests were excluded. The required sample size was calculated on the AUC for a good response. Based on previous data from our Center, the expected AUC (± SD) for both AFC and serum AMH was about 0.80 (± 0.15). Setting type I and II errors at 0.05 and 0.10 and stating as clinically relevant a reduction of the AUC of 0.10 with random AFC compared to serum AMH, the number of women to be included was at least 50. We estimated that recruiting women over a 30-month period could allow us to achieve the required sample size without difficulties, for both the intention to treat analysis (whole cohort) and for the subgroup of women undergoing a proper random start cycle.

Results

Seventy-two women were ultimately included. The flowchart of the study is summarized in Fig. 1. Baseline characteristics and cycle outcome of these women are shown in Tables 1 and 2, respectively. The mean ± SD age for the whole cohort was 31.4 ± 5.6 years. Thirty-four (47%) were in the follicular phase, 32 (45%) in the luteal phase, and the remaining six (8%) were assuming oral contraceptives. A dominant follicle or a corpus luteum was visualized in 43 cases (60%). Thirty-five women (49%) retrieved ≥ 10 mature oocytes. Eleven (15%) retrieved ≥ 20 mature oocytes. All selected women initiated the stimulation within 2 days. Since the distribution of AFC and AMH was

Table 3 *B* coefficients between AFC or serum AMH and the total number of mature oocytes retrieved

Study group	Number	Crude analysis		Adjusted analysis[a]	
		B (95%CI)	*p*	*B* (95%CI)	*p*
Whole cohort					
AFC	72	0.39 (0.24–0.54)	< 0.001	0.37 (0.21–0.52)	< 0.001
$\log_{10}$ AMH	72	11.56 (8.27–14.85)	< 0.001	11.12 (7.89–14.35)	< 0.001
Proper random start [b]					
AFC	52	0.37 (0.20–0.55)	< 0.001	0.34 (0.16–0.53)	< 0.001
$\log_{10}$ AMH	52	11.78 (7.89–15.67)	< 0.001	11.16 (7.31–15.00)	< 0.001

B, coefficient of regression

[a] Data were adjusted for age and BMI

[b] Excluding women who were assuming oral contraceptives and those who were causally recruited at the beginning of the cycle

positively skewed and age was negatively skewed, we used for the analyses LogAFC, LogAMH, and $(age)^2$, respectively. As expected, AFC and AMH strongly correlated with each other in both the whole cohort ($R = 0.76$, $p < 0.001$) and in the subgroup of proper random start cases ($R = 0.76$, $p < 0.001$).

Correlations between the total number of mature oocytes retrieved and AFC or AMH are represented in Fig. 2. Data are reported separately for the cohort of women reaching oocyte retrieval ($n = 72$) and for the subgroup of women initiating in a non-early follicular phase (proper random start, $n = 52$). Table 3 shows the crude and adjusted regression coefficients *B* between AFC or AMH and the total number of mature oocytes. Again, data are reported separately for the whole cohort of women reaching oocyte retrieval and for the subgroup of proper random start. All these analyses were repeated excluding the three women who were canceled because of poor response and results were extremely similar (data not shown).

Baseline characteristics of women who retrieved less and more than 10 oocytes are shown in Table 4. The *c*-statistics in the whole cohort for the prediction of ≥ 10 mature oocytes using AFC and serum AMH were similar. Specifically, the AUC was 0.76 (95%CI 0.66–0.87; $p < 0.001$) and 0.82 (95%CI 0.72–0.92; $p < 0.001$), respectively (Fig. 3, upper panel). The two AUCs did not significantly differ (p = ns). When considering the subgroup of proper random start, the *c*-statistics resulted as 0.77 (95%CI 0.64–0.89; $p = 0.001$) and 0.83 (95%CI 0.72–0.95; $p < 0.001$), respectively (Fig. 3, lower panel). Again, the two AUCs did not significantly differ (p = ns). The Youden index for AFC for the whole cohort was 15. The OR for ≥ 10 mature oocytes in women with AFC ≥ 15 was 5.5 (95%CI 2.0–15.5). The OR adjusted for $(age)^2$ and BMI was 4.5 (95%CI 1.6–13.2). When considering the subgroup of proper random start, the crude and adjusted ORs were 5.1 (95%CI 1.5–17.0) and 4.8 (95%CI 1.3–17.4), respectively.

Discussion

Random AFC, i.e., AFC collected at any time during the menstrual cycle, is a valuable tool for predicting the number of mature oocytes that can be retrieved in women with malignancies undergoing a random start protocol of hyperstimulation. Women whose random AFC exceeded 15 had a more than fourfold increased chances to store ≥ 10 mature oocytes (good retrieval). We failed to document relevant differences in the predictive capacity of a good retrieval between AFC and AMH. Noteworthy, the *c*-statistics observed in our cohort for both AFC and AMH are similar to what are observed in previous studies in non-cancer infertile women evaluated in the early follicular phase [28]. Our findings support and extend previous preliminary findings from Cakmak et al. who failed to observe statistically significant differences in the ratio between the number of retrieved oocytes and AFC when comparing random start and conventional stimulations [19].

Providing to women with malignancy a personalized counseling on the potential benefits of egg banking is important. It may play a role in the decision-making process. These women are overwhelmed by the situation and commonly reluctant to delay the initiation of chemotherapy and to undergo additional non-oncologic treatments. Providing an estimate of the potential benefits of egg banking may thus be clinically useful for a shared and informed decision. Noteworthy, data on the ovarian reserve may be particularly relevant for women with breast cancer (the most common indication for fertility preservation) considering the ongoing debate on the integrity of the ovarian reserve in affected cases [29]. On the other hand, information from random AFC has to be handled with caution. The *c*-statistics is statistically significant but the AUC (0.76) is far from optimal. Firmly denying treatment exclusively based on a random AFC < 15 cannot be done because of the relatively modest prediction capacity of AFC and because of the arbitrary decision to set the threshold for a good

Table 4 Baseline characteristics according to the number of mature oocytes retrieved (≥ 10 vs < 10) in the whole cohort and in the subgroup of women undergoing proper random start

Characteristics	Whole cohort			Proper random start		
	≥ 10	< 10	*p*	≥ 10	< 10	*p*
Number	35	37		25	27	
Age (years)	30.1 ± 5.1	32.6 ± 5.8	0.06	30.2 ± 4.6	33.6 ± 5.5	0.02
BMI (kg/m^2)	22.4 ± 3.9	21.0 ± 3.2	0.09	22.3 ± 3.7	20.9 ± 3.0	0.14
Previous deliveries	4 (11%)	3 (8%)	0.71	2 (8%)	3 (11%)	1.00
Menstrual cycle duration (days)			0.30			0.31
24–27	3 (9%)	7 (19%)		2 (8%)	5 (19%)	
28–32	25 (71%)	26 (70%)		17 (68%)	19 (70%)	
> 32	7 (20%)	4 (11%)		6 (24%)	3 (11%)	
Serum AMH (ng/ml)	3.7 (2.2–5.2)	1.4 (0.6–2.6)	< 0.001	3.7 (2.3–5.0)	1.4 (0.6–1.9)	< 0.001
Total AFC	23.5 ± 11.0	14.1 ± 8.1	< 0.001	24.9 ± 12.0	14.4 ± 7.8	< 0.001
Indication to oocyte cryopreservation			0.73			0.98
Breast cancer	20 (57%)	22 (60%)		15 (60%)	17 (63%)	
Lymphoma	11 (31%)	9 (24%)		7 (28%)	7 (26%)	
Others	4 (11%)	6 (16%)		3 (12%)	3 (11%)	
Ovarian cysts	2 (6%)	2 (5%)	1.00	1 (4%)	2 (7%)	1.00
Cycle phase at referral			0.45			1.00
Estroprogestins	2 (6%)	4 (11%)		0 (0%)	0 (0%)	
Follicular phase	19 (54%)	15 (40%)		11 (44%)	11 (41%)	
Luteal phase	14 (40%)	18 (49%)		14 (56%)	16 (59%)	
Canceled cycles (poor response)	0 (0%)	3 (8%)	0.24	0 (0%)	3 (11%)	0.24
Drugs used [a]						
Corrifollitropin 100–150 mcg	35 (100%)	34 (100%)		25 (100%)	24 (100%)	
Total dose of recombinant FSH (IU)	1423 ± 1092	1924 ± 1109	0.10	1530 ± 1198	2038 ± 1175	0.18
Total *N* of GnRH antagonist ampoules	5.6 ± 1.2	5.0 ± 1.4	0.06	5.8 ± 1.1	5.0 ± 1.5	0.05
Duration of stimulation (days)[a]	11.3 ± 1.5	11.6 ± 2.1	0.49	11.4 ± 1.2	11.7 ± 2.2	0.49
N of developed follicles (diameter ≥ 11 mm)[a]	25.6 ± 9.9	13.1 ± 5.2	< 0.001	26.4 ± 10.0	12.9 ± 5.1	< 0.001
N of oocytes retrieved	21.8 ± 8.1	7.5 ± 4.6	< 0.001	22.8 ± 7.7	7.2 ± 4.8	< 0.001
N of mature oocytes retrieved (frozen)	16.9 ± 6.3	4.6 ± 3.1	< 0.001	17.8 ± 5.9	4.6 ± 3.2	< 0.001

[a] Excluding canceled cycles

retrieval at 10 oocytes. Albeit lower, good chances of pregnancy can be ensured also with less than 10 oocytes. Moreover, age independently and markedly impacts on the success of oocyte preservation program [4]. Finally, the considerations on effectiveness should not hide from view the additional important psychological benefits of fertility preservation [30].

Some limitations and strengths of the study deserve to be commented. Firstly, the study is retrospective and thus exposes our data to some potential inaccuracies, in particular for the AFC evaluation [14, 28]. On the other hand, it has to be underlined that all the assessments were made by only two expert physicians and in a standardized manner. Secondly, we did not exclude women on oral contraceptives, those with organic ovarian cysts, and those who were assessed in the early follicular phase by chance. The main reason for this choice was to provide pragmatic data reflecting real-life situations. Noteworthy, whereas oral contraceptives may alter AFC [31], there is evidence that the presence of small ovarian cysts do not have an impact [32, 33]. Moreover, to overcome this potential criticism, we repeated the analyses excluding oral contraceptives users and including only women who were

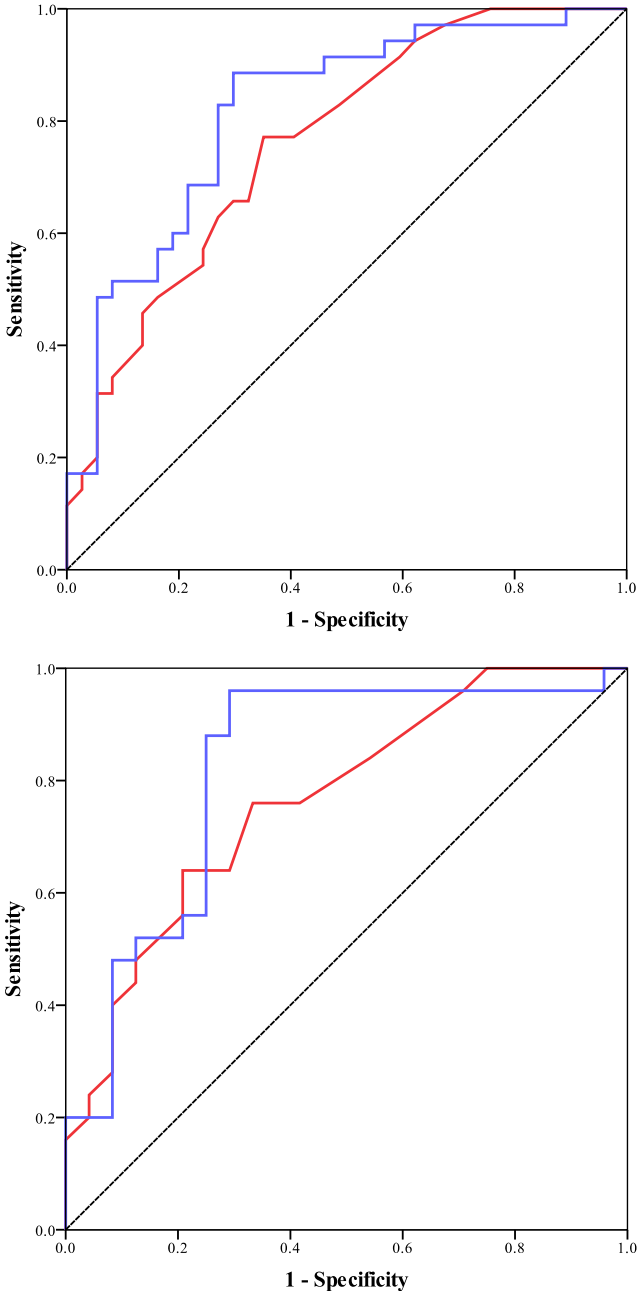

Fig. 3 ROC curves on the capacity of AFC (red line) and AMH (blue line) to predict the retrieval of ≥ 10 mature oocytes. The *upper panel* refers to the whole cohort of women ($n = 72$, 35 from whom ≥ 10 mature oocytes were retrieved). The *lower panel* refers to the subgroup of proper random start ($n = 52$, 25 from whom ≥ 10 mature oocytes were retrieved)

recruited after day 5 of the cycle. Results were extremely similar, thus supporting the robustness of our conclusions. The further exclusion of the four women with ovarian cysts did not modify the results (data not shown). Thirdly, we presented data exclusively on AFC and AMH. We did not have information on other biomarkers such as inhibin B. Even if this assessment was shown to be less predictive of ovarian response in normal settings [34], one cannot definitely exclude that it might be conversely more reliable in the specific group of women with malignancies. Fourthly, the peculiar policy adopted in our Unit of a systematic use of long-acting recombinant FSH may hamper inferences, in particular in contexts where this drug is not yet available. In this regard, it has however to be noted that we failed to highlight relevant differences between long-acting recombinant FSH and daily FSH in a previous comparative study of our group [23]. Finally, albeit relatively large, we have to recognize that our sample size is insufficient to draw firm conclusions. In particular, our study does not allow us to exclude a milder but potentially still clinically interesting advantage of serum AMH. This is potentially relevant considering that AMH will be more and more promptly available in the next future [11, 12]. Evidence from independent group and larger series is thus required. To note, independent evidence is also required to validate the AFC threshold of 15. Indeed, this value was based on the studied sample and cannot be used routinely without external validation.

Some possible clinical inferences of our results merit to be commented. Firstly, even if in our unit we opted for a unique protocol of stimulation and a standardized starting dose, other groups may decide for a personalized approach and may use random AFC to adapt the starting dose of gonadotropins to be administered [14, 30]. Secondly, our findings may be of interest also for non-malignant cases. In fact, AFC varies during the menstrual cycles but these variations may not be significant for clinical practice [35], i.e., they may only mildly influence counseling and clinical decisions. Van Disseldorp et al. [15] examined the intracycle variability of AFC and showed an up to 30% variability while Mavrelos et al. [16] and Rombauts et al. [17] showed that this variability may not be clinically significant. On the other hand, the arbitrary indication to strictly perform AFC on day 3–5 of the cycle [18] has significant drawbacks. It is difficult to schedule (a consistent proportion of women has irregular cycles) and the evaluation of the endometrium may be hampered by the still ongoing menstrual flow. A second assessment some days later may thus be required in several cases. In fact, the definitive demonstration that AFC is reliable regardless of the phase of the menstrual cycle may ultimately simplify the ultrasound assessment of the infertile woman and reduce costs and general burden. Further studies specifically designed to ascertain the reliability of random AFC in the general population are however required prior to drawing recommendations. Finally, it is intriguing noting that AFC and AMH appear similarly predictive while the latter is known to better inform on the ovarian reserve [36]. To explain this finding, it may be speculated that AFC could reflect the number of follicles that will respond to ovarian hyperstimulation (rather than ovarian reserve). Therefore, in the particular setting of random start protocols (when women initiate the ovarian hyperstimulation

immediately after the assessment), the clinical utility of AFC could be enhanced.

In conclusion, random AFC can be used to predict the number of retrievable oocytes in women with cancer. Its reliability appears to be similar to that of serum AMH and, therefore, its use deserves particular consideration in settings not providing prompt serum AMH assessment. On the other hand, it should be emphasized that measurement of the ovarian reserve should not be the unique element to guide the decision on the opportunity to perform egg freezing. Other variables obviously require utmost consideration. They include age, parity, prognosis, type of scheduled therapies, and duration of the planned post-treatment follow-up without seeking pregnancy.

Compliance with ethical standards

Conflict of interest Edgardo Somigliana received during the last 3 years grants of research from Ferring and Merck-Serono. All the other authors do not have any financial disclosure to declare.

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