Scientific Research Publishing

Advances in Reproductive Sciences, 2025, 13(4), 207-224
https://www.scirp.org/journal/arsci
ISSN Online: 2330-0752
ISSN Print: 2330-0744

Association of Anti-Mullerian Hormone on the Quality of Human Oocytes in Some Patients Accessing *in Vitro* Fertilization (IVF) Treatment in a Private Fertility Center in Ghana

Dickson Mawusi[1,2*], Muller Makafui Mawusi[2,3], Gideon Kwesi Nakotey[4]

[1]School of Basic and Biomedical Sciences, Department of Biomedical Sciences, University of Health and Allied Sciences, Ho, Ghana
[2]Assisted Conception Unit, Airport Women's Hospital, Accra, Ghana
[3]Department of Anatomy, School of Medical Sciences, College of Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[4]Department of Microbiology and Immunology, School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana
Email: *mdickson@uhas.edu.gh

How to cite this paper: Mawusi, D., Mawusi, M.M. and Nakotey, G.K. (2025) Association of Anti-Mullerian Hormone on the Quality of Human Oocytes in Some Patients Accessing *in Vitro* Fertilization (IVF) Treatment in a Private Fertility Center in Ghana. *Advances in Reproductive Sciences*, **13**, 207-224.
https://doi.org/10.4236/arsci.2025.134018

Received: August 3, 2025
Accepted: September 25, 2025
Published: September 28, 2025

Abstract

The quantity and quality of oocytes obtained after egg harvesting or retrieval have been regarded as a variable that influences fertilization and the development of viable embryos, determining the number of good embryos required for embryo transfer to achieve implantation (biochemical and clinical pregnancies), as well as the freezing of remaining embryos for future use. This study determined the effects of AMH on oocyte quantity and quality in response to controlled ovarian stimulation (COS) in women undergoing IVF treatment in Ghana. We performed a cohort prospective study at an IVF center in Ghana. Data analysis was performed according to five AMH ranges: group 1 ≤ 0.3 ng/mL (probably negligible response); Group 2 > 0.3 ≤ 2.19 ng/mL (expected lower response); Group 3 > 2.19 ≤ 4.00 ng/mL (possibly intermediate response); Group 4 > 4.00 and ≤6.79 ng/mL (normal response); and Group 5: ≥ 6.79 ng/mL (high response). The number of metaphase II oocytes obtained in each group was recorded. 426 patients were admitted. The data presented were limited to the first IVF cycles to minimize repeated-measures bias. Eight cycles were cancelled due to poor ovarian response. The remaining 418 retrieval cycles were divided into five subgroups according to AMH categories: group 1 ≤ 0.30 (n = 20), group 2 > 0.30 ≤ 2.19 (n = 132), group 3 > 2.19 ≤ 4.00 (n = 158), group 4 > 4.00 ≤ 6.79 (n = 73), and group 5 ≥ 6.79 (n = 35). AMH is the best ovarian reserve test (ORT) for predicting and

individualizing gonadotropin dosing to achieve appropriate ovarian responsiveness and minimize both hyper- and poor-ovarian response. This study also demonstrated that anti-Müllerian hormone is a fairly robust metric for predicting cancellation and the number of oocytes that may be retrieved after stimulation in some Ghanaian women undergoing IVF treatment; however, it is a relatively poor predictor of oocyte quality.

Keywords

Anti Mullerian Hormone, Fertility Centers Ghana, Women, Oocytes, IVF

1. Introduction

Human oocytes play an important role in determining embryo competence and, therefore, *in vitro* fertilization (IVF) results [1]-[3]. Oocyte quality is not only influenced by the nuclear and mitochondrial genome but also by the microenvironment provided by the ovary and the pre-ovulatory follicle that influences transcription and translation as a consequence of cytoplasmic maturity [3]-[5]. In contrast to *in vivo* processes, the application of ovarian hormone stimulation protocols for IVF bypasses the complicated selection procedure that usually occurs during oocyte development and maturation of a single oocyte for ovulation and allows for the maturation of many oocytes, often with compromised quality [3] [6]. It has been speculated by Iussig *et al*. that some morphological irregularities that can easily be assessed at the light microscopy level may reflect a compromised developmental ability of the oocytes and could therefore represent a useful tool for selecting competent oocytes before fertilization [1]. Oocyte morphological assessment in the laboratory is first based on the presentation of the cumulus-corona cells [1]. The cumulus-corona mass should appear as an expanded and mucified layer for mature oocytes due to the active secretion of hyaluronic acid [3] [7]. This extracellular matrix molecule interposes between the cumulus cells (CCs), separating them and conferring on the cumulus-corona mass a fluffy 'cloud-like' appearance [3]. However, stimulated cycles may be characterized by asynchrony between the nuclear maturation status of the oocyte and the expansion of the cumulus-corona cell mass. This has been suggested to be caused by a different sensitivity of the oocyte and the cumulus-corona mass to the stimulants [1]-[3].

Following the removal of the cumulus-corona cells in preparation for intracytoplasmic sperm injection (ICSI) [3] [8], oocyte evaluation is more accurate and is based on the nuclear maturation status, the morphology of the cytoplasm, and the appearance of the extracytoplasmic structures [2] [3] [6] [9]. The presence of the first polar body (PB) is normally considered to be a marker of oocyte nuclear maturity [2]. However, studies using polarized light microscopy have shown that oocytes displaying a polar body may still be immature [10]-[12]. Only those displaying a meiotic spindle (MS) can be considered as true, mature, Metaphase II (MII)

stage oocytes [11]. The presence, position, and retardance of MS have been suggested to be related to the developmental competence of the oocyte [11]. In a meta-analysis, however, only *in vitro* development can be related to the morphology of the MS. Analysis of *in vivo* development is relatively rare in the literature, and most meta-analyses failed to show significant differences in implantation rates between embryos derived from oocytes displaying detectable MS and those without [3] [13]. Nuclear maturity alone is, in fact, not enough to determine the quality of an oocyte. Nuclear and cytoplasmic maturation should be completed in a coordinated manner to ensure optimal conditions for subsequent fertilization [13].

Considering that both extreme forms of ovarian response (low and hyper-response) may be associated with diminished oocyte quality [14]-[16], it is hypothesized that lower or higher levels of AMH might negatively affect the quality of oocytes and embryo development. *In Vitro* Fertilization (IVF) treatments involve the application of sophisticated ovarian stimulation protocols, continuous patient monitoring, and delicate follicle aspiration, whereby mature oocytes are harvested and fertilized.

Available evidence mostly relates to reported outcomes from patients in the developed world, including Europe and the United States. The present study aimed to assess various limiting factors, including ovarian reserve testing, to serve as a guide for controlled ovarian stimulation to obtain high-quality oocytes. AMH, despite being a good marker of ovarian response to Controlled Ovarian Stimulation (COS) that predicts oocyte quantity, has failed to predict the quality of oocytes in ART programs. Previously published reports have focused on the relation between AMH and oocyte quantity but not the nuclear maturity of oocytes [17] [18]. Hence, the importance of conducting this research.

2. Materials and Methods

2.1. Study Design and Site

This prospective cohort study was carried out at the Airport Women's Hospital (AWH) and Fertility Centre in Ghana between January 2017 and December 2021. The AWH is one of the renowned and leading assisted reproductive technology (ART) private fertility hospitals in Ghana and conducts 50 to 60 IVF treatments annually. It was established in 2012, and attendance at the hospital is generally high by local standards, with patients mostly from across Ghana and the West African sub-region.

2.2. Study Population

This study included women accessing IVF treatment at AWH in Accra, Ghana. The inclusion criteria comprised Ghanaian women of good health, with regular menstrual cycles, between 20 and 55 years of age, the presence of both ovaries on transvaginal ultrasound scan, and undergoing their first cycle of ovarian stimulation (COS) with exogenous gonadotropins. The specific exclusion criteria were

women with previous exposure to cytotoxic drugs or pelvic radiation therapy (radiotherapy), and a previous history of ovarian surgery. In addition, women with confirmed diagnoses of PCOS and known chronic medical conditions were excluded.

2.3. Controlled Ovarian Stimulation (COS)

Pituitary down-regulation was achieved with the use of gonadotropin-releasing hormone (GnRH) agonist goserelin (Zoladex 3.6 mg depot injection, AstraZeneca, Zug, Switzerland) on day 18 or 21. Ovarian stimulation was carried out with recombinant follicle-stimulating hormone (FSH) [Fostimon, IBSA, Switzerland]. Stimulation was initiated with a starting dose of 300 IU (4 ampoules) or 450 IU (6 ampoules), depending on the AMH value and age of the patient, and adapted/adjusted to the follicle response ascertained by follicle review using transvaginal ultrasound and assessment of blood estradiol levels. When follicle diameter of 18 - 20 mm was detected after monitoring with ultrasound, ovulation was induced with 10,000 IU human chorionic gonadotropin (hCG) [choriomon /Pregnyl, IBSA, Switzerland, Aesca Pharma, Vienna, Austria or Organon, Pfaffikon, Switzerland] and vaginal oocyte harvesting was carried out under anesthesia and ultrasound guidance 34 -36 hours after hCG administration at the IVF theatre.

2.4. Oocyte Assessment and Grading

Evaluation of the initial maturity of oocytes was based on the expansion and radiance of the cumulus-oocyte-corona complex (COC), which surrounds the retrieved oocyte(s) [19]. With this assessment, oocytes were rapidly categorized as mature (correlated to metaphase II of maturation) when they possessed an expanded and luteinized cumulus matrix and a radiance of *sunburst* corona radiata. A less expanded cumulus-corona complex implies an intermediate stage of maturity (correlated to metaphase I of maturation), and the absence of an expanded cumulus-corona complex and the presence of a germinal vesicle were generally associated with immaturity (correlated to prophase I of maturation). Oocyte maturity was also determined by grading the size of the oocyte, size /cohesiveness of the associated membrane granulosa cells, and color or shape of the oocyte itself if it was visible within the mass surrounding cellular reserves under the stereo zoom microscope. Visualization of the oocyte and first polar body or germinal vesicle (GV) was done by spreading out the cumulus mass. For ICSI procedures, the cumulus mass was removed with the aid of enzymes -Hyaluronidase (Origio, Copenhagen, Denmark). After denudation (removal of cumulus cells), oocytes were classified according to the presence of the first polar body or not and were injected or inseminated accordingly. The following oocyte dysmorphisms summarized were recorded: intra-cytoplasmic defects such as (i) cytoplasm color, (ii) vacuoles in the ooplasm, (iii) aggregates of smooth endoplasmic reticulum clusters (ERC) in the ooplasm and (iv) retractile bodies; and extracytoplasmic defects such as (i) large perivitelline space (PVS), (ii) PVS granularity, (iii) zona pellucida (ZP) ab-

normalities, (iv) shape abnormalities and (v) fragmented polar body (PB).

3. Statistical Analysis

Data analysis was performed using Spearman's rho correlation coefficient. An initial descriptive study was conducted. The correlation between AMH, age, and the quantity of eggs retrieved was determined using Spearman's rank correlation coefficient. Results showed a positive correlation between AMH and the number of metaphase II oocytes retrieved. Higher AMH levels were associated with more Metaphase II oocytes obtained, while lower AMH levels were associated with lower ovarian response and fewer mature (metaphase II) oocytes obtained. However, there was a strong negative correlation between AMH levels and the age of women. All p-values < 0.001 were considered statistically significant. Anti-Müllerian hormone levels and the number of oocytes obtained in the study groups and cycle outcomes according to AMH were plotted as bar charts (Figure 1, Figure 2). Subgroup analysis was performed according to five AMH ranges: group 1: ≤ 0.3 ng/mL (probably negligible response); Group 2: > 0.3 ≤ 2.19 ng/mL (expected lower response); Group 3: > 2.19 ≤ 4.00 ng/mL (possibly intermediate response); Group 4: > 4.00 and ≤ 6.79 ng/mL (normal response); and Group 5: ≥ 6.79 ng/mL (high response) were plotted as bar chart showing specific dysmorphism in the oocytes (Figure 3).

4. Results

Four hundred and twenty-six (426) patients were admitted to IVF/ICSI treatment from January 2017 to December 2019. The data presented were limited to the first

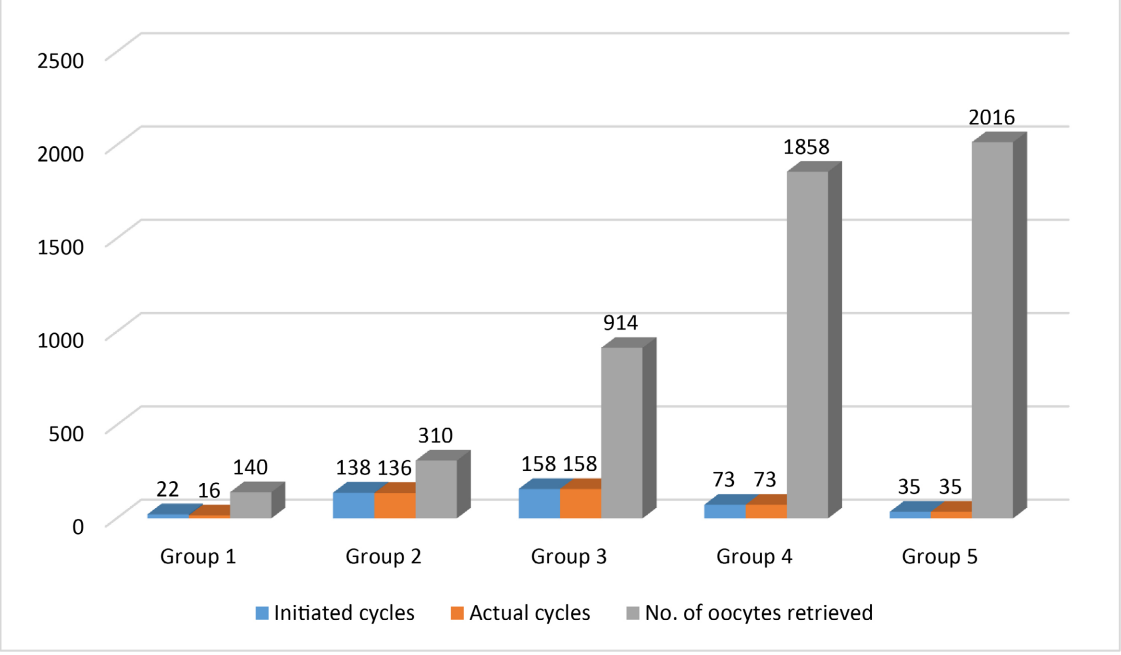

Figure 1. Cycle outcomes according to AMH.

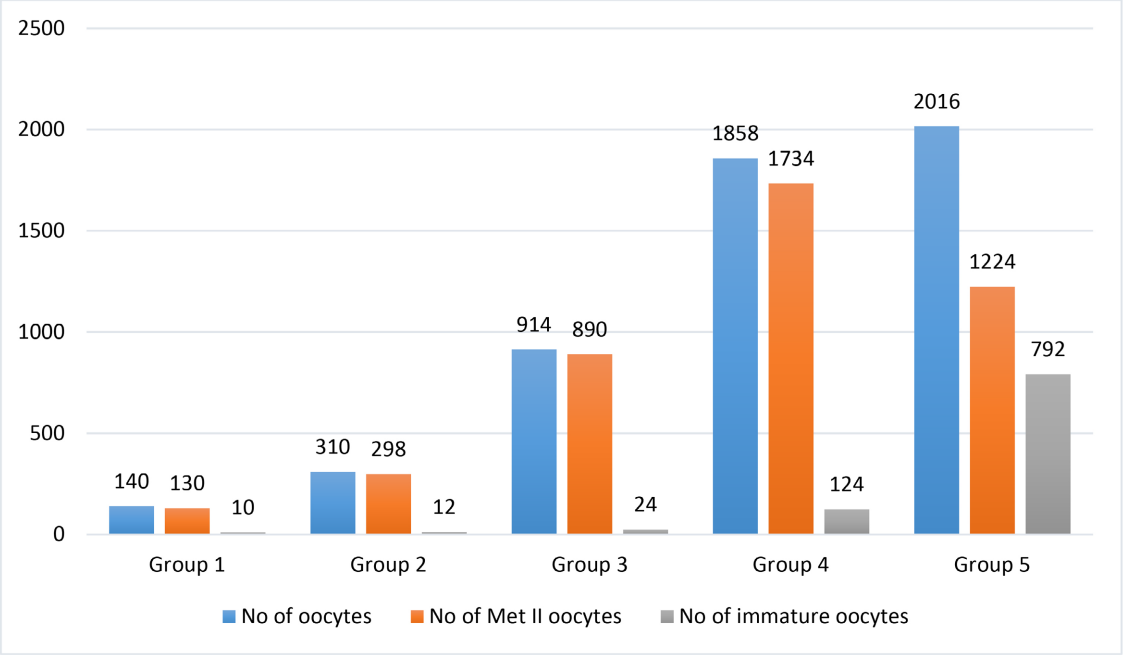

Figure 2. Anti-Müllerian hormone levels and number of Oocytes obtained in the study groups.

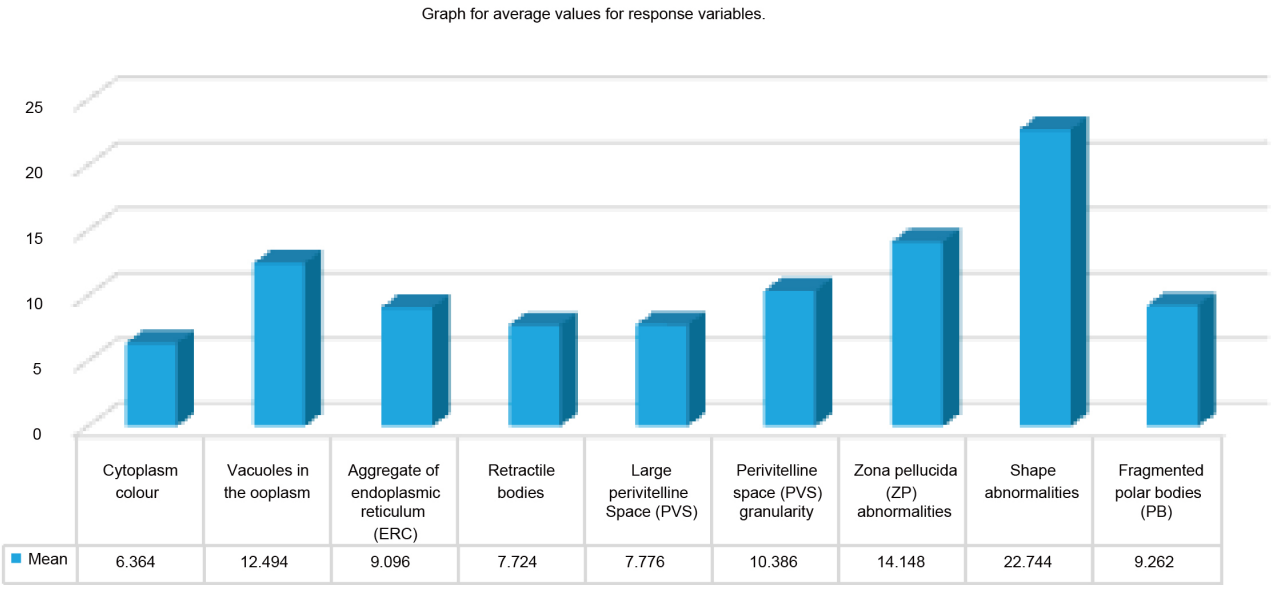

Figure 3. A bar chart showing specific dysmorphism in oocytes retrieved from a study participant.

IVF cycles to minimize repeated-measures bias. Eight (8) cycles were cancelled due to poor ovarian response. Varying predicted human oocyte maturity grading based on morphological characteristics in terms of the cumulus-oocyte-complex was observed among patients (Table 1). Summarized patients' social demographics, cycle characteristics, and controlled ovarian stimulation (COS) outcomes according to AMH levels of the participants, age, duration of stimulation, antral follicles, total dose of gonadotropin, follicles ≥ 18 mm on hCG day, number of total oocytes retrieved, and cancelled cycles are recorded (Table 2, Table 3).

Although the ages of women were found to be an independent predictor of ovarian reserve, there were no significant differences between groups concerning body mass index (BMI). Observed variables shown in Table 3 included patient age; serum AMH and antral follicle count (AFC) at the start of the cycle, number of stimulation days; number of follicles ≥ 18 mm on hCG day; number of oocytes retrieved and of metaphase II oocytes. The remaining 418 retrieval cycles were divided into five subgroups according to AMH categories: group 1 ≤ 0.30 (n = 20), group 2 > 0.30 ≤ 2.19 (n = 132), group 3 > 2.19 ≤ 4.00 (n = 158), group 4 > 4.00 ≤ 6.79 (n = 73), and group 5 ≥ 6.79 (n = 35) as indicated in (Table 4). Patients were divided into five subgroups according to AMH categories about oocyte dysmorphisms: group 1(10). Group 2(12), group 3 (24), group 4 (124), and group 5 (792) are shown in Table 5. Table 6 and Table 7 show Spearman's rank correlation coefficient between Age, AMH, and the number of oocytes retrieved, quality of metaphase II oocytes, with *p-values*.

Table 1. Predicted human oocyte maturity grading based on morphological characteristics in terms of the cumulus-oocyte-complex. Human oocyte maturity *in vivo* determines the outcome of blastocyst development *in vitro*.

Morphological characteristics	*Grade 1 Mature or preovulatory*	*Grade 2 Approximately mature*	*Grade 3 Immature*	*Grade 4 Post mature*	*Grade 5 Atretic*
Cumulus mass	Very expanded	Expanded	Dense and compact	Very expanded, often having clumps	Rarely present
Corona radiata	Very radiant, revealing a distinct zona pellucida	Slightly compact	If present: Very adherent, compact layer of corona cells	Radiant, yet often clumped and irregular or incomplete; zona pellucida visible	If present: Clumped and very irregular; zona pellucida very visible
Ooplasm	Clear		If visible: Revealing the presence of the germinal vesicle	Either slightly granular or dark	Dark and frequently misshapen
Detached membrane granulosa cells	Expanded and well-aggregated	Expanded and well-aggregated	Compact and non-aggregated	Small and relatively non-aggregated	Very small clumps of cells

The grading takes into account the cumulus compactness, corona density, and cytoplasm granularity of the oocyte if visible [20].

Table 2. Cycle outcomes according to AMH.

Groups	*Initiated cycles*	*Cancelled cycles*	*Actual cycles after cancellations*	*Number of oocytes retrieved*	*Mature (metaphase II) oocytes were obtained*	*Immature oocytes*	*Number of oocytes*
Group 1	22	6	16	140	130	10	9
Group 2	138	2	136	310	298	12	5
Group 3	158	0	158	914	890	24	0
Group 4	73	0	73	1858	1734	124	0
Group 5	35	0	35	2016	1224	796	0
Total	426	8	418	5238	3220	218	14

Table 3. Social demographic characteristics and ovarian stimulation outcomes according to AMH levels of the participants.

Parameter	*Group* 1 *AMH* < 0.30	*Group* 2 *AMH* > 0.30 ≤ 2.19	*Group* 3 *AMH* > 2.19 ≤ 4.0	*Group* 4 *AMH* > 4.0≤ 6.79	*Group* 5 *AMH* ≥ 6.79
Started cycles (426 total)	n = 22	n = 138	n = 158	n = 73	n = 35
Age (years)	43.96 ± 3.88	38.64 ± 3.10	33.20 ± 3.02	28.01 ± 2.22	23.04 ± 2.02
BMI (kg/m^2)	24.10 ± 3.84	23.24 ± 3.72	23.75 ± 2.42	22.95 ± 2.84	22.96 ± 2.78
Serum FSH	≥12	11-12	6-8	5-7	4 -5
AFC	5 ± 2.44	6 ± 3.22	8 ± 3.10	9 ± 4.20	10 ± 2.22
Stimulation days	12 ± 4	12 ± 3	12 ± 2	12 ± 2	12 ± 2
FSH administered (IU)	6300 ± 558.20	2700 ± 707.02	3600 ± 684.26	1800 ± 698.46	1600 ± 569. 84
Follicle ≥ 18 mm on hCG day	3 ± 2.28	5 ±3.62	6 ± 3.90	8 ± 3.61	8 ± 4.20
Number of oocytes retrieved	140	310	914	1858	2016
Number of metaphase II oocytes	130	298	890	1734	1224

Data are presented as mean values (± standard deviation); AMH: Anti-Müllerian hormone (ng/mL); FSH: Follicle Stimulating hormone; AFC: Antral Follicle Count; BMI: Body Mass Index; hCG: Human Chorionic Gonadotropin.

Table 4. Anti-Müllerian hormone levels and number of Oocytes obtained in the study groups.

Serum level of AMH	*Variable*	*Group* 1 < 0.30 (*n* = 20)	*Group* 2 > 0.30 ≤ 2.19 (*n* = 132)	*Group* 3 > 2.19 ≤ 4.0 (*n* = 158)	*Group* 4 > 4.00 ≤ 6.79 (*n* = 73)	*Group* 5 ≥ 6.79 (*n* = 35)	*Total*
	Number of oocytes retrieved	140	310	914	1858	2016	5238
	Number of metaphase (Met II) mature oocytes	130	298	890	1734	1224	4276
	Number of immature oocytes retrieved	10	12	24	124	792	962

Table 5. Percentage of Oocyte dysmorphisms affected by serum level of anti-Müllerian hormone.

Predictor variable	*Oocyte abnormalities/dysmorphism*						
AMH levels	*Response variable* (*nature of oocyte abnormality*)	*Group* 1	*Group* 2	*Group* 3	*Group* 4	*Group* 5	*Mean* (*SD*)
	Cytoplasm colour	0 (0.00)	1 (8.33)	2 (8.33)	8 (6.45)	69 (8.71)	80 (6.36)
	Vacuoles in the ooplasm	2 (20.00)	1 (8.33)	2 (8.33)	14 (11.29)	115 (14.52)	134 (12.49)
	Aggregate of endoplasmic reticulum (ERC)	1 (10.00)	1 (8.33)	3 (12.50)	8 (6.45)	65 (8.20)	78 (9.10)
	Retractile bodies	1(10.00)	1 (8.33)	2 (8.33)	7 (5.65)	50 (6.31)	61 (7.72)
	Large perivitelline Space (PVS)	1 (10.00)	1 (8.33)	2 (8.33)	7 (5.65)	52 (6.57)	63 (7.78)
	Perivitelline space (PVS) granularity	1 (10.00)	2 (16.67)	3 (12.50)	8 (6.45)	50 (6.31)	64 (10.39)

Continued

Zona pellucida (ZP) abnormalities	1 (10.00)	3 (25.00)	3 (12.50)	8 (6.45)	133 (16.79)	148 (14.15)
Shape abnormalities	2 (20.00)	1 (8.33)	4 (16.67)	60 (48.39)	161 (20.33)	228 (22.74)
Fragmented polar bodies (PB)	1 (10.00)	1 (8.33)	3 (12.50)	4 (3.23)	97 (12.25)	106 (9.26)
Total	10	12	24	124	792	962

SD = Standard Deviation.

Table 6. Spearman's rho correlation coefficients between age, AMH, number of oocytes retrieved, and metaphase II oocytes.

Variables		*Age*	*AMH*	*Number of oocytes retrieved*	*Metaphase II oocytes*
1. Age	N	426	423	419	426
	Correlation Coefficient	1.000	−0.630**	−0.765**	−0.808**
	P value	-	0.000	0.000	0.00
2. AMH	N	423	423	416	423
	Correlation Coefficient	−0.630**	1.000	0.767**	0.806**
	p value	0.000	-	0.000	0.000
3. Number of oocytes retrieved	N	419	416	419	419
	Correlation Coefficient	−0.765**	0.767**	1.000	0.923**
	p value	0.000	0.000	-	0.000
4. Metaphase II oocytes	N	426	423	419	426
	Correlation Coefficient	−0.808**	0.806**	0.923**	1.000
	p value	0.000	0.00	0.000	-

**Correlation is significant at the 0.01 level (2 2-tailed).

Table 7. Spearman's rank correlation coefficient between Age, AMH, and the number of oocytes retrieved, quality of metaphase II oocytes, with *p* values.

Variable		*Age*	*AMH*	*No. of oocytes retrieved*	*Metaphase II oocytes*
Age	*r*		−0.473	−0.756	−0.726
	p value		0.000	0.000	<0.001
AMH	*R*	−0.473		0.615	0.633
	p value	<0.001		0.001	<0.001

There is a strong negative correlation between AMH and the age of women.

5. Discussion

In this study, we assessed the association between AMH levels and oocyte quality (metaphase II oocytes retrieved). The quantity and quality of oocytes obtained after egg retrieval have been regarded as variables that influence fertilization, embryo development, and implantation of derived embryos [14] [21]. The relation

between AMH levels and the number of metaphase II oocytes obtained in each group was recorded in **Table 4**. A significant correlation between AMH levels and ovarian response was observed in the number of oocytes retrieved during COS. Our results show that age and AMH levels are the superior parameters predicting ovarian response. However, even in women of comparable age and AMH levels, there was a wide variation in the individual ovarian reserve [22]-[25]. AMH is a very promising and possibly the best actual candidate to evaluate individual ovarian response to gonadotropin stimulation and to detect poor responders with levels of AMH ≤ 0.30 ng/mL.

Considering that both extreme forms of ovarian response (low and hyper-response) may be associated with diminished oocyte quality [14]-[16], it is hypothesized that lower or higher levels of AMH might harm the number of oocytes and embryo development. Subgroup analysis of the five AMH categories showed that group 1 (22) patients, 6 cycle cancellations (27.27%), 130 MII oocytes, group 2 (138 patients), 2 cycle cancellations (1.45%), 298 MII oocytes, group 3 (158 patients), no cycle cancellations, 890 MII oocytes, group 4 (73 patients), no cycle cancellation, 1734 MII oocytes and group 5 (35 patients), 1224 MII oocytes with no cycle cancellations. AMH levels ≤ 0.30 ng/mL (patients with poor prognosis) were observed in 16 patients (3.83%) in group 1. Patients within this AMH level range accounted for 27.27% of cancelled or failed cycles among the study groups.

Though the total number of oocytes retrieved in group 5 was higher (2016) than those in group 4 (1858), the total number of mature (metaphase II) oocytes obtained in group 5 was less (1224) as compared to those in group 4 (1734). This could be attributed to a hyper-response to COS in group 5 that is associated with diminished oocyte quality [26] [27]. Initial data obtained in chapter 2 of this study confirmed the reduced number of metaphase II oocytes obtained in group 5: women with AMH values greater than median value of 3.5 ng/mL, mean value of 4.85 ng/mL, a standard deviation of 3.34 ng/mL and AMH levels ≥ 6.75 ng/mL (group 5). These patients were considered "high responders" with PCOS-like features without meeting the full diagnostic criteria for exclusion. In assisted reproduction, women with high AMH, especially those with PCOS, are more likely to be "high responders" to gonadotropin stimulation. While this can increase egg yield, it also raises the risk of ovarian hyperstimulation syndrome (OHSS), a potentially serious complication [28] [29]. OHSS is a notable iatrogenic complication in IVF treatment with the retrieval of usually more than fifteen (15) oocytes with a high number of immature oocytes and smaller numbers of mature (metaphase II oocytes) following a standard COS protocol [15]. IVF patients with PCOS constitute 20% of clients undergoing COS, and less than one-fifth of them present symptoms of OHSS [30]. Increased levels of serum AMH in PCOS patients have been suggested to be a result of excess induction of androgens in small antral follicles [30]-[32]. It has been previously described that when cumulus cells are denuded for ICSI, more than 60% of all oocytes show at least one abnormal mor-

phological feature [33]-[35]. It is unclear whether the correlation between AMH and the presence of oocyte abnormalities observed was due to decreased secretion by granulosa cells in poor-quality oocytes [36] or as a result of a possible detrimental effect of low levels of AMH on oocyte quantity and quality [14] [17] [21]. The list of dysmorphic markers associated with reduced quality and development of oocytes was observed and recorded. Oocyte dysmorphisms such as refractile bodies in ooplasm may affect fertilization and embryo quality [37], while the presence of vacuoles in ooplasm, according to the literature, has been found to reduce fertilization rate in an ICSI study [37]. Whether or not disturbances occur may mainly depend on the size of the vacuole; it is yet to be established. The presence of smooth endoplasmic reticulum (sER) clusters may be indicative of a disturbance that may affect embryo development since the clinical pregnancy rate was much lower in oocytes with sER clusters compared to those without sER clusters [6]. In addition, a baby born from a cluster-positive oocyte that was fertilized had Beckwith-Wiedemann syndrome, a disease associated with disturbed expression from an imprinted gene [6] [38] is a long debate on the predictive value of the size and morphology of the first polar body (PB).

A study by Ebner et al. reported that oocytes with normal-sized, intact first-polar bodies produced embryos with a higher success rate of conception cycles. Oocytes with fragmented PBs had a significantly lower blastocyst formation rate [6] [36] [38]. The fragmentation rate is likely to increase over time after egg retrieval; this may explain the negative results found in two other studies where the timing of ICSI and PB morphology analysis differed [39]. There was no link between PB shape and the chromosomal makeup of PBs in oocytes from older patients [11] [40]. Mouse models suggest that very large PBs indicate loss of cell cycle control and disrupted spindle function [40]-[42]. Further research is needed to determine if this also applies to humans. The rapid degeneration of the first PB and potential differences in maturation kinetics influenced by maternal age and stimulation protocols make it challenging to evaluate the usefulness of PB scoring for oocyte selection.

Clear, colorless cytoplasm is considered a marker of good quality in oocytes, while extensive cytoplasmic granularity, particularly central granulation, was negatively correlated with a conception cycle and was associated with increased aneuploidy in embryos [43]. The presence of dark cytoplasm in oocytes decreased by 83% the likelihood of obtaining a good quality embryo and thus may be considered negatively correlated with oocyte quality [43]-[45]. Increased cytoplasmic viscosity is also considered a marker of low oocyte quality but is difficult to quantify objectively [3] [46] [47]. Funnel persistence as a result of high viscosity can only be analyzed in ICSI cycles after the microinjection of sperm and can thus not be used for oocyte selection before fertilization. Oocytes with larger than larger-than-normal perivitelline space were associated 1.8 times higher chance of having good-quality embryos compared to those with perivitelline dysmorphisms [2]. Perivitelline granularity was not predictive of fertilization and cleavage rate, but ra-

ther a physiological maturation-dependent phenomenon related to stimulation protocol [3] [38] [47]. Zona pellucida (ZP) is one of the cellular constituents of the oocyte that is synthesized during oocyte growth and maturation. The relative order of fibers might reflect a continuously favorable environment during a period of oocyte development when it acquires nuclear and cytoplasmic maturational competence. Several studies have focused on measuring zona thickness, but differences between groups were always fairly low and therefore not very useful for selecting oocytes [48]-[50]. The study showed a mean (SD) (14.15) of zona pellucida abnormalities in the oocytes obtained. In a retrospective analysis of the mean retardance of light by the three layers of the zona pellucida in a line scan across the zona from oocytes transferred after ICSI showed that the mean magnitude of light retardance was nearly 30% higher ($p < 0.001$) in the inner layer of the zona pellucida of oocytes contributing to a conception cycle compared to a non-conception cycle [49]. The magnitude of light retardance by the zona pellucida inner layer appears, therefore, to present a unique non-invasive marker for oocyte developmental potential and/or cycles with high probability of success. A study employing the same techniques fully confirmed the finding by showing a correlation between the mean retardance of the human oocyte spindle and the zona developmental potential of the oocytes [40] [42]. Indeed, AMH might play an important role in primordial follicle selection and cyclic growing follicle recruitment [51]. Moreover, AMH might regulate the selection of the dominant follicle through the inhibitory effects of AMH on the initial recruitment of primary follicles from the resting primordial follicle pool and through the regulation of FSH sensitivity in the human ovary [52].

In summary, the results confirm the relevance of AMH measurement as an important screening test for reduced ovarian reserve in women. Furthermore, a level of AMH ≤ 0.3 ng/mL correctly predicts a very poor response with two oocytes in some cases and a poor outcome even in groups of high age-related heterogeneity. If AMH levels are ≤ 0.30 ng/mL, an antral follicle count should be added to exclude false positive results and increase specificity, especially in a routine clinical setting with heterogeneous groups of patients. Chronological age fairly well predicts ovarian response and the upcoming perimenopausal and menopausal transition. Important studies have shown that AMH is a predictor for the occurrence of perimenopausal transition (cycle length irregularity) within 3 - 5 years [15] at levels of 0.92 ng/mL.

Our study affirms that by early detection of women with a reduced ovarian response, using a cut-off level < 0.30 ng/mL, treatment options are still available (high-dose stimulation in IVF, poly-ovulation) with favorable pregnancy rates, not statistically different from those of women with 'normal' ovarian reserve. Therefore, women who want to get pregnant should be advised to get AMH levels determined if they do not achieve pregnancy after six cycles of regular intercourse. AMH is a suitable screening test and may replace FSH, which gains relevance only in the late reproductive phase. AMH plays a major role in assisted.

Reproductive technology allows not only the quantification of the ovarian reserve but also the prediction of eventual ovarian response to IVF stimulation.

Although AMH is good for predicting oocyte quantity, the age of women remains the strongest predictor of oocyte quality. To evaluate or complement the prediction of oocyte quality, different or complementary markers, both biochemical and clinical, such as AFC, day 3 FSH, and Estradiol, mitochondrial biomarkers, serum progesterone in the late follicular phase, and Preimplantation Genetic Diagnosis Testing for Aneuploidy (PGT-A) have shown promise to better predict oocyte quality. Cumulus cell gene expression and other markers like HAS2, PTGS2, and GREM1 have also shown promise, mainly in research but not in routine or clinical use yet.

6. Limitation of the Study

The association between AMH and the quality of human oocytes observed in our fertility center in this study should be interpreted with caution due to several limitations. The single-center design restricts the diversity of clinical practices, laboratory protocols, and patient demographics, reducing the generalizability of the findings. Results may not apply to other fertility centers within Ghana or internationally, as they are influenced by the specific genetic, environmental, lifestyle, and socioeconomic characteristics of the studied population. Additionally, cultural and healthcare access differences may further limit extrapolation. Larger, multicenter studies across varied populations are needed to confirm and extend these observations to broader contexts.

7. Conclusions

Our study indicates that anti-müllerian hormone is a dependable measure for predicting oocyte quality in Ghanaian women undergoing IVF treatment, but it is not a reliable indicator of oocyte nuclear maturation. Our findings show that low AMH levels alone are not appropriate for withholding fertility treatment, and further research is necessary to understand the link between AMH and oocyte nuclear maturation.

Author Contributions

D.M., M.M.M. conceived and designed the study; M.M.M. collected the data with supervision from D.M. M.M.M. and D.M. conducted the data analysis; D.M. and G.K.N. Authored the article with significant contributions from M.M.M. All authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Ethical approval for this study (number: CHRPE/AP/276/17) was provided by the Committee on Human Research, Publication, and Ethics (CHRPE) of the School of Medicine and Dentistry, College of Health Sciences, Kwame Nkrumah Univer-

sity of Science and Technology (KNUST). We obtained written consent from all participants before the data collection.

Informed Consent Statement

Not applicable. Data Availability Statement: The dataset for this study will be made available upon reasonable request from the corresponding author.

Acknowledgements

We are extremely grateful to all the staff of Airport Women's Hospital for their support and cooperation during the recruitment and data collection. We are also grateful to all the women who consented to participate in this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Iussig, B. (2015) Morphological Selection of Oocytes. *Current Trends in Clinical Embriology*, **2**, 103-114. https://doi.org/10.11138/cce/2015.2.3.103

[2] Magli, M.C., Jones, G.M., Lundin, K. and van den Abbeel, E. (2012) Atlas of Human Embryology: From Oocytes to Preimplantation Embryos *Human Reproduction*, **27**, 1-91. https://doi.org/10.1093/humrep/des229

[3] Rienzi, L., Balaban, B., Ebner, T. and Mandelbaum, J. (2012) The Oocyte. *Human Reproduction*, **27**, i2-i21. https://doi.org/10.1093/humrep/des200

[4] Hennet, M.L. and Combelles, C.M.H. (2012) The Antral Follicle: A Microenvironment for Oocyte Differentiation. *The International Journal of Developmental Biology*, **56**, 819-831. https://doi.org/10.1387/ijdb.120133cc

[5] Dumesic, D.A., Meldrum, D.R., Katz-Jaffe, M.G., Krisher, R.L. and Schoolcraft, W.B. (2015) Oocyte Environment: Follicular Fluid and Cumulus Cells Are Critical for Oocyte Health. *Fertility and Sterility*, **103**, 303-316. https://doi.org/10.1016/j.fertnstert.2014.11.015

[6] Balaban, B. and Urman, B. (2006) Effect of Oocyte Morphology on Embryo Development and Implantation. *Reproductive BioMedicine Online*, **12**, 608-615. https://doi.org/10.1016/s1472-6483(10)61187-x

[7] Zaninovic, N., Gosden, R. and Veeck Gosden, L. (2005) Visualization of Meiotic Spindles and Chromosomes in Living Human Oocytes Using Conventional Hoffman Modulation Microscopy. *Fertility and Sterility*, **84**, S368-S369. https://doi.org/10.1016/j.fertnstert.2005.07.964

[8] Neri, Q.V., Pereira, N., Cozzubbo, T., Rosenwaks, Z. and Palermo, G.D. (2017) Intracytoplasmic Sperm Injection: Technical Aspects. In: Palermo, G.D., Ed., *Textbook of Assisted Reproductive Techniques*, 5th Edition, CRC Press, 183-197.

[9] Lasienë, K., Vitkus, A., Valanèiûtë, A. and Lasys, V. (2009) Morphological Criteria of Oocyte Quality. *Medicina*, **45**, Article 509. https://doi.org/10.3390/medicina45070067

[10] Holubcová, Z., Kyjovská, D., Martonová, M., Páralová, D., Klenková, T., Otevřel, P.,

et al. (2019) Egg Maturity Assessment Prior to ICSI Prevents Premature Fertilization of Late-Maturing Oocytes. *Journal of Assisted Reproduction and Genetics*, **36**, 445-452. https://doi.org/10.1007/s10815-018-1393-0

[11] Teplá, O., Topurko, Z., Mašata, J., Jirsová, S., Moosová, M., Fajmonová, E., *et al.* (2021) Relative Position of the Meiotic Spindle and Polar Body as a Marker of Oocyte Maturation Improves the Utilization and Pregnancy Rates. https://doi.org/10.21203/rs.3.rs-284509/v1

[12] Ubaldi, F. and Rienzi, L. (2008) Morphological Selection of Gametes. *Placenta*, **29**, 115-120. https://doi.org/10.1016/j.placenta.2008.08.009

[13] Demyda-Peyrás, S., Hidalgo, M., Dorado, J., Anter, J., Género, E. and Moreno Millán, M. (2013) Short Communication: *In Vitro* Oocyte Maturation and Fertilization Rates in the Spanish Lidia Bovine Breed. *Spanish Journal of Agricultural Research*, **11**, 356-361. https://doi.org/10.5424/sjar/2013112-3317

[14] Borges, E., Braga, D.P.A.F., Setti, A., de Cássia Figueira, R. and Iaconelli Júnior, A. (2017) The Predictive Value of Serum Concentrations of Anti-Müllerian Hormone for Oocyte Quality, Fertilization, and Implantation. *JBRA Assisted Reproduction*, **21**, Article No. 176. https://doi.org/10.5935/1518-0557.20170035

[15] Broer, S.L., Dolleman, M., Opmeer, B.C., Fauser, B.C., Mol, B.W. and Broekmans, F.J.M. (2011) AMH and AFC as Predictors of Excessive Response in Controlled Ovarian Hyperstimulation: A Meta-Analysis. *Human Reproduction Update*, **17**, 46-54. https://doi.org/10.1093/humupd/dmq034

[16] van Rooij, I.A.J., de Jong, E., Broekmans, F.J.M., Looman, C.W.N., Habbema, J.D.F. and te Velde, E.R. (2004) High Follicle-Stimulating Hormone Levels Should Not Necessarily Lead to the Exclusion of Subfertile Patients from Treatment. *Fertility and Sterility*, **81**, 1478-1485. https://doi.org/10.1016/j.fertnstert.2003.10.054

[17] Fong, S.L., Baart, E., Martini, E., Schipper, I., Visser, J., Themmen, A., *et al.* (2008) Anti-Müllerian Hormone: A Marker for Oocyte Quantity, Oocyte Quality and Embryo Quality? *Reproductive BioMedicine Online*, **16**, 664-670. https://doi.org/10.1016/s1472-6483(10)60480-4

[18] Smeenk, J.M.J., Verhaak, C.M., Vingerhoets, A.J.J.M., Sweep, C.G.J., Merkus, J.M.W.M., Willemsen, S.J., *et al.* (2006) Stress and Outcome Success in IVF: The Role of Self-Reports and Endocrine Variables. *Human Reproduction*, **20**, 991-996. https://doi.org/10.1093/humrep/deh739

[19] Sanchez, F., Romero, S., De Vos, M., Verheyen, G. and Smitz, J. (2015) Human Cumulus-Enclosed Germinal Vesicle Oocytes from Early Antral Follicles Reveal Heterogeneous Cellular and Molecular Features Associated with in Vitro Maturation Capacity. *Human Reproduction*, **30**, 1396-1409. https://doi.org/10.1093/humrep/dev083

[20] Lin, Y., Chang, S., Lan, K., Huang, H., Chang, C., Tsai, M., *et al.* (2003) Human Oocyte Maturity *in Vivo* Determines the Outcome of Blastocyst Development *in Vitro*. *Journal of Assisted Reproduction and Genetics*, **20**, 506-512. https://doi.org/10.1023/b:jarg.0000013651.37866.0c

[21] Lehmann, P., Vélez, M.P., Saumet, J., Lapensée, L., Jamal, W., Bissonnette, F., *et al.* (2014) Anti-Müllerian Hormone (AMH): A Reliable Biomarker of Oocyte Quality in IVF. *Journal of Assisted Reproduction and Genetics*, **31**, 493-498. https://doi.org/10.1007/s10815-014-0193-4

[22] Coccia, M.E. and Rizzello, F. (2008) *Ovarian Reserve. Annals of the New York Academy of Sciences*, **1127**, 27-30. https://doi.org/10.1196/annals.1434.011

[23] Gleicher, N., Weghofer, A. and Barad, D.H. (2011) Defining Ovarian Reserve to Bet-

ter Understand Ovarian Aging. *Reproductive Biology and Endocrinology*, **9**, Article No. 23. https://doi.org/10.1186/1477-7827-9-23

[24] Tal, R. and Seifer, D.B. (2017) Ovarian Reserve Testing: A User's Guide. *American Journal of Obstetrics and Gynecology*, **217**, 129-140. https://doi.org/10.1016/j.ajog.2017.02.027

[25] Tremellen, K.P., Kolo, M., Gilmore, A. and Lekamge, D.N. (2005) Anti-Müllerian Hormone as a Marker of Ovarian Reserve. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, **45**, 20-24. https://doi.org/10.1111/j.1479-828x.2005.00332.x

[26] Howie, R. and Kay, V. (2018) Controlled Ovarian Stimulation for *In- Vitro* Fertilization. *British Journal of Hospital Medicine*, **79**, 194-199. https://doi.org/10.12968/hmed.2018.79.4.194

[27] Pilsgaard, F., Grynnerup, A.G.-., Løssl, K., Bungum, L. and Pinborg, A. (2018) The Use of Anti-Müllerian Hormone for Controlled Ovarian Stimulation in Assisted Reproductive Technology, Fertility Assessment and -Counseling. *Acta Obstetricia et Gynecologica Scandinavica*, **97**, 1105-1113. https://doi.org/10.1111/aogs.13334

[28] Tshzmachyan, R. and Hambartsoumian, E. (2020) The Role of Letrozole (LE) in Controlled Ovarian Stimulation (COS) in Patients at High Risk to Develop Ovarian Hyper Stimulation Syndrome (OHSS). A Prospective Randomized Controlled Pilot Study. *Journal of Gynecology Obstetrics and Human Reproduction*, **49**, Article 101643. https://doi.org/10.1016/j.jogoh.2019.101643

[29] Uppangala, S., Fernandes, G., Salian, S.R., Kumar, P., Talevi, R., Kalthur, G., *et al.* (2020) Reduced Ovarian Response to Controlled Ovarian Stimulation Is Associated with Increased Oxidative Stress in the Follicular Environment. *Reproductive Biology*, **20**, 402-407. https://doi.org/10.1016/j.repbio.2020.04.005

[30] Wang, F., Niu, W., Kong, H., Guo, Y. and Sun, Y. (2017) The Role of AMH and Its Receptor SNP in the Pathogenesis of PCOS. *Molecular and Cellular Endocrinology*, **439**, 363-368. https://doi.org/10.1016/j.mce.2016.09.023

[31] Broer, S.L., Broekmans, F.J.M., Laven, J.S.E. and Fauser, B.C.J.M. (2014) Anti-Müllerian Hormone: Ovarian Reserve Testing and Its Potential Clinical Implications. *Human Reproduction Update*, **20**, 688-701. https://doi.org/10.1093/humupd/dmu020

[32] Homburg, R. and Crawford, G. (2014) The Role of AMH in Anovulation Associated with PCOS: A Hypothesis. *Human Reproduction*, **29**, 1117-1121. https://doi.org/10.1093/humrep/deu076

[33] Ebner, T. (2009) Extracytoplasmic Markers of Human Oocyte Quality. *Journal of Mammalian Ova Research*, **26**, 18-25. https://doi.org/10.1274/jmor.26.18

[34] Maggiulli, R., Ubaldi, F. and Rienzi, L.F. (2012) Oocyte Insemination and Culture. In: Ginsburg, E. and Racowsky, C., Eds., *In Vitro Fertilization*, Springer, 83-98. https://doi.org/10.1007/978-1-4419-9848-4_6

[35] Rienzi, L.F., Maggiulli, R. and Ubaldi, F.M. (2019) Oocyte Denuding. In: Nagy, Z., Varghese, A. and Agarwal, A. Eds., *In Vitro Fertilization*, Springer International Publishing, 133-145. https://doi.org/10.1007/978-3-319-43011-9_14

[36] Ebner, T., Sommergruber, M., Moser, M., Shebl, O., Schreier-Lechner, E. and Tews, G. (2006) Basal Level of Anti-Müllerian Hormone Is Associated with Oocyte Quality in Stimulated Cycles. *Human Reproduction*, **21**, 2022-2026. https://doi.org/10.1093/humrep/del127

[37] Walls, M., Junk, S., Ryan, J.P. and Hart, R. (2012) IVF versus ICSI for the Fertilization

of *In-Vitro* Matured Human Oocytes. *Reproductive BioMedicine Online*, **25**, 603-607. https://doi.org/10.1016/j.rbmo.2012.08.001

[38] Balaban, B., Ata, B., Isiklar, A., Yakin, K. and Urman, B. (2008) Severe Cytoplasmic Abnormalities of the Oocyte Decrease Cryosurvival and Subsequent Embryonic Development of Cryopreserved Embryos. *Human Reproduction*, **23**, 1778-1785. https://doi.org/10.1093/humrep/den127

[39] Hossein, M.S., Jeong, Y.W., Kim, S., Kim, J.J., Park, S.W., Jeong, C.S., *et al.* (2008) Protocol for the Recovery of *in Vivo* Matured Canine Oocytes Based on Once Daily Measurement of Serum Progesterone. *Cloning and Stem Cells*, **10**, 403-408. https://doi.org/10.1089/clo.2008.0001

[40] De Santis, L., Cino, I., Rabellotti, E., Calzi, F., Persico, P., Borini, A., *et al.* (2005) Polar Body Morphology and Spindle Imaging as Predictors of Oocyte Quality. *Reproductive BioMedicine Online*, **11**, 36-42. https://doi.org/10.1016/s1472-6483(10)61296-5

[41] Kilani, S. and Chapman, M.G. (2011) The Use of Polarized Light Microscopy in IVF. *Expert Review of Obstetrics & Gynecology*, **6**, 241-246. https://doi.org/10.1586/eog.11.17

[42] Sun, M., Li, Z. and Gui, J. (2010) Dynamic Distribution of Spindlin in Nucleoli, Nucleoplasm and Spindle from Primary Oocytes to Mature Eggs and Its Critical Function for Oocyte-to-Embryo Transition in Gibel Carp. *Journal of Experimental Zoology Part A*: *Ecological Genetics and Physiology*, **313**, 461-473. https://doi.org/10.1002/jez.618

[43] Trebichalská, Z., Kyjovská, D., Kloudová, S., Otevřel, P., Hampl, A. and Holubcová, Z. (2021) Cytoplasmic Maturation in Human Oocytes: An Ultrastructural Study. *Biology of Reproduction*, **104**, 106-116. https://doi.org/10.1093/biolre/ioaa174

[44] Combelles, C.M.H. and Rawe, V.Y. (2013) Determinants of Oocyte Quality: Impact on *in Vitro* Fertilization Failures. In: *Oogenesis*, Springer London, 307-327. https://doi.org/10.1007/978-0-85729-826-3_21

[45] Alves, B.G., Alves, K.A., Lúcio, A.C., Martins, M.C., Silva, T.H., Alves, B.G., *et al.* (2014) Ovarian Activity and Oocyte Quality Associated with the Biochemical Profile of Serum and Follicular Fluid from Girolando Dairy Cows Postpartum. *Animal Reproduction Science*, **146**, 117-125. https://doi.org/10.1016/j.anireprosci.2014.02.019

[46] Balaban, B. (2017) Analysis of Fertilization. In: Taylor and Francis Group, Eds., *Handbook of In Vitro Fertilization*, CRC Press, 169-180.

[47] Balaban, B., Barut, T. and Urman, B. (2012) Assessment of Oocyte Quality. In: Nagy, Z., Varghese, A. and Agarwal, A., Eds., *Practical Manual of in Vitro Fertilization*, Springer, 105-119. https://doi.org/10.1007/978-1-4419-1780-5_13

[48] Bokhove, M. and Jovine, L. (2018) Structure of Zona Pellucida Module Proteins. In: *Current Topics in Developmental Biology*, Elsevier, 413-442. https://doi.org/10.1016/bs.ctdb.2018.02.007

[49] Familiari, G., Relucenti, M., Heyn, R., Micara, G. and Correr, S. (2006) Three-Dimensional Structure of the Zona Pellucida at Ovulation. *Microscopy Research and Technique*, **69**, 415-426. https://doi.org/10.1002/jemt.20301

[50] Litscher, E.S. and Wassarman, P.M. (2020) Zona Pellucida Genes and Proteins and Human Fertility. *Trends in Developmental Biology*, **13**, 21-33.

[51] Carlson, B.M. (2018) Human Embryology and Developmental Biology-Inkling Enhanced E-Book. Elsevier Health Sciences.

[52] Homburg, R., Hendriks, M.L., Konig, T.E., Anderson, R.A., Balen, A.H., Brincat, M., *et al.* (2012) Clomifene Citrate or Low-Dose FSH for the First-Line Treatment of Infertile Women with Anovulation Associated with Polycystic Ovary Syndrome: A Prospective Randomized Multinational Study. *Human Reproduction*, **27**, 468-473. https://doi.org/10.1093/humrep/der401