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Impact of Anti-Mullerian Hormone on Fertilization and Embryo Development in Some Patients Accessing IVF Treatment in a Private Fertility Center in Ghana

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Abstract

AMH has universally been accepted to correlate well with ovarian response to gonadotropin stimulation, but its effect on IVF outcomes remains undefined. Despite being a good marker of COS, AMH has not been able to predict fertilization rate, embryo development competence, and pregnancy outcomes in IVF programs. We conducted a prospective cohort study at an IVF center in Ghana. A total of 5238 oocytes were retrieved at ovum pick up (OPU) from 418 women, and a total of 4276 mature oocytes (metaphase II) were obtained. Following oocyte evaluation, 962 oocytes were found to be defective and therefore were discarded. Cycle and patient characteristics were expressed as mean ± standard deviation for continuous variables; percentages were used for categorical variables. Mean ± standard deviation was used to evaluate the relationship between serum AMH levels and continuous variables. Results were expressed in percentages. Bar charts, pie diagrams, and linear graphs were plotted to assess the influence of AMH levels on the number of metaphase II oocytes obtained, oocyte dysmorphism, fertilization rates, embryo development (on days 2, 3, and 5), and blastocyst formation (on day 5). This study presents preliminary ovarian biomarker baseline clinical data for ART practitioners on the application of AMH in IVF treatment for Ghanaian women. The clinical application depends on individual centers examining their data, correlating AMH levels and ultimate ovarian response in the form of meta-

phase II oocytes, fertilization rates, and embryo development. It is recommended that future research or further studies be conducted on a large sample size to define the role of AMH in IVF outcomes.

Keywords

Anti-Mullerian Hormone, AMH, IVF/ICSI, Fertilization, Embryo Development, Fertility Centers in Ghana

1. Introduction

AMH is now known to predict ovarian response and serves as a robust tool for starting controlled ovarian stimulation (COS) in women undergoing assisted reproductive technology (ART) treatments [1]-[5]. In vitro fertilization (IVF) can now be successfully carried out with numerous adaptations in individual laboratories, but specific detailed standard operating protocols (SOPs) are still required to optimize the microenvironment for successful cell culture and development. Both in vivo and in vitro, gametes and preimplantation embryos are produced in great excess, with only a tiny proportion surviving to implant and produce offspring. Human gametes are certainly error-prone, and the majority are never destined to begin a new life [5]. Some female gametes may undergo fertilization but subsequently fail to support further development due to deficiencies in the process of oogenesis [6]. Once gametes are selected, their successful interaction is probably one of the most difficult steps on the way to the formation of a new life. At this stage, the two genomes have not yet mixed, and numerous developmental errors can still occur, with failures in oocyte activation, sperm decondensation, or in the patterns of signals that are necessary for the transition to early stages of embryo development [5] [6].

A fertilized oocyte is a totipotent cell that initially divides into a few equally totipotent cells, but for a brief time, these cells can give rise to one (a normal pregnancy), none (a blighted ovum or an embryonic vesicular mole), or even several (monozygotic twinning) individuals [5]. Although fertilization is necessary for the life of a being, it is not the only critical event, as preimplantation embryo development can be interrupted at any stage by lethal processes or simple mistakes in the developmental program [7]. A series of elegantly programmed events begins at gametogenesis and continues through to parturition, involving a myriad of synchronized, interdependent mechanisms, choreographed such that each must function at the right time during embryogenesis [5].

Combinations of both physiological and chromosomal factors result in a continuous reduction, or "selection" of conception products throughout the stages that lead to the potential implantation of an embryo in the uterus [8]. Preimplantation embryogenesis might be described as a type of Darwinian filter where only the fittest embryos survive, and the survival of these is initially determined during

gametogenesis [5]. It may be argued that the task of elaborating and defining the concept of a "new individual" belongs to philosophers and moralists but for some, the beginning of human life coincides with the formation of a diploid body in which the male and female chromosomes are brought together [5] [9] and for others, true human life only occurs after implantation of the embryo in the uterine mucosa [5].

Some scientists believe that a new individual is formed only after the differentiation of the neural tube [10], whilst others believe that life begins when a fetus can live outside the uterus [10]. In its most extreme form, some philosophers consider the acquisition of self-awareness of the newborn to define a new life [10]. Most scientists would probably agree that life is a continuous cyclical process, with the gametes merely bridging the gap between adult stages. Science, one of the bases of human intellect and curiosity, is generally impartial and often embraces international and religious boundaries; ethicists, philosophers, and theologians cannot proceed without taking into account the new information and realities that are continuously generated in the fields of biology and embryology [5]. Advances in the expanding range and sensitivity of molecular biology techniques, in particular genomics, epigenomics, and proteomics, continue to further our understanding of reproductive biology, at the same time adding further levels of complexity to this remarkable process of creating a new life [11]. In the past decades, the field of human IVF has undergone significant transformation in many different ways (scientific knowledge gained from the use of sophisticated technology is one of them), and the management of patients and treatment cycles has also been influenced by commercial pressures as well as legislative issues [12].

The rapid expansion in both the number of cycles and range of treatments offered has introduced a need for more rigorous control and discipline in the IVF laboratory routine, and IVF laboratory personnel must have a good basic understanding of the science that underpins the attempts to create the potential beginning of a new life [13]. IVF is practiced globally, including in Ghana, and the number of babies born is estimated to be in the order of at least 10 million. A vast and comprehensive collection of published literature covers clinical and scientific procedures and protocols, as well as information gained from modern molecular biology techniques in some advanced countries, but there are limited or no published data in sub-Saharan African countries like Ghana [12]. Previous studies found that, besides ovarian response to COS, the serum level of AMH can also be used as a predictor of the severity of ovarian hyperstimulation syndrome (OHSS) and endometriosis [14]. AMH measurements also offer relatively high specificity and sensitivity as a diagnostic marker for polycystic ovarian syndrome [15]-[17]. The number of oocytes retrieved and the quality of oocytes and embryos obtained significantly impact pregnancy outcomes. Although it is universally accepted that AMH correlates well with ovarian response to gonadotropin stimulation, its effect on IVF outcomes remains undefined. Despite being a good marker of COS, AMH has not been able to predict fertilization rate, embryo development competence,

and pregnancy outcomes in IVF programs [14] [18]-[20]. A recent survey in Ghana by Mawusi *et al*. showed that research on the relevance of AMH within the Ghanaian setting remains limited [21]. There is limited literature concerning the predictive value of AMH in Ghanaian women undergoing IVF treatment and its effect on fertilization and embryo development. Available evidence mostly relates to reported outcomes from patients in the developed world, including Europe and the United States [12]. The present study aimed to assess the effect of AMH on fertilization and embryo development in some Ghanaian women. Our findings from an investigative study of an underserved part of the world provide data for comparison with studies elsewhere.

2. Materials and Methods

2.1. Ethical Consideration

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 University 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.

2.2. Study Design and Site

This prospective cohort study was carried out at the Airport Women's Hospital (AWH) and Fertility Centre in Ghana between February 2018 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.

2.3. Study Population

The study included 426 women assessing IVF treatment at AWH in Accra, Ghana. The inclusion criteria comprised Ghanaian women of good health, with regular menstrual cycles, between 20 and 45 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.

2.4. 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 21. Controlled Ovarian Stimulation (COS) 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 trans-vaginal 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.

2.5. Insemination with Intracytoplasmic Sperm Injection (ICSI) Methodology

Intracytoplasmic sperm injection (ICSI) was used to inject sperm directly into the oocytes. The purpose was to achieve higher fertilization rates and was usually used for couples with severe male factor infertility and women with poor ovarian stimulation outcomes. Micromanipulation was performed on an inverted microscope (x200 magnification, Leica, SparMed and Integra 3, Copenhagen, Denmark) with Hoffman modulation contrast (Modulation Optics Incorporated, Greenvale, New York, United States of America), an electronically controlled heated stage, and hydraulic micromanipulations (Luigs and Neumann, Ratingen, Germany). The technique of ICSI, as described in detail by Ebner [22], was used. The denuded oocytes were placed into the droplets of Quinn's Advantage medium with HEPES-buffered media (Origio, Copenhagen, Denmark), one oocyte per droplet, a maximum of four oocytes at a time. The injection and holding pipettes were first primed with the wash droplet in the ICSI dish by aspirating some medium in and out of the pipettes to reduce the risk of the sperm sticking to the inside of the injecting pipette. The sperm were selected and immobilized individually by using the injection pipette to hit the membrane of the sperm tail. The sperm was brought to the tip of the injecting pipette, and the needle was pushed slowly through the zona pellucida (ZP) into the oocyte, exclusively with the polar body (PB) at the six (6) or twelve (12) o'clock position. To avoid the expulsion of the sperm into the perivitelline space, the oolemma was broken by the gentle suction of a small amount of cytoplasm into the injection pipette. The aspirated cytoplasm and the sperm in the injecting needle were reinjected deep (50% - 75%) into the oocyte diameter. Into the cytoplasm to ensure that the sperm was not dragged out with the injection pipette. The pipette was slowly retracted from the oocyte, and the oocyte was released or removed from the holding pipette. Following injection of all oocytes, they were rinsed or washed through several drops of Fert medium and then placed into a new equilibrated SAGE 1-step culture media (Cooper Surgical,

Copenhagen, Denmark) and incubated overnight in a 6% carbon dioxide (CO_2) medium. Metaphase I oocytes that had matured to metaphase II based on an extruded polar body were also injected. The oocytes were assessed for fertilization by the presence of two PN and two polar bodies on the following day (16 - 20 hours post-injection/insemination).

2.6. Fertilization Assessment

The evaluation of pronuclei 16 - 18 hours post sperm injection provided a significant amount of useful information about the potential of the resulting embryo [23]-[25]. Scott and Smith were the first to correlate zygote morphology with rates of implantation and pregnancy based on a combination of pronuclear size with the number and distribution of nucleoli [26].

2.7. Embryo Morphology Evaluation

Embryo morphology was assessed 24 hours post-ICSI and on the mornings of days 2, 3, and 5 using an inverted Leica microscope under 400× magnification. The embryos were then categorized as "good", "fair", or "poor" in terms of quality, based on the number and grade of blastomeres. Cleavage stage morphology was assessed based on the following parameters: number of blastomeres, percent fragmentation, variation in blastomere symmetry, presence of multinucleation and defects in the zona pellucida and cytoplasm [27] [28] "Good" quality embryos were cleavage stage embryos showing the following characteristics: 4 cells on day 2 or 8 - 10 cells on day 3, <25% were considered to be of "fair" quality. Poor cleavage stage embryos showed severe fragmentation (>25%) and asymmetrical cells. These embryos were slow-growing and contained a very low number of cells [29]. In blastocyst formation assessment on day 5, the embryos were graded using the formation of the inner cell mass (ICM) and the Trophectoderm (TE). Inner cell mass grading: "Good": numerous cells in the epithelium, cells appear healthy and are tightly packed. "Fair": large cells that are loosely packed, and "Poor": few cells, inner cell mass not distinguishable, and the presence of degenerative cells. Trophectoderm grading: "Good": many cells forming an epithelium, "Fair": a few large cells forming an epithelium, and "Poor": a few cells that appear poor or uneven, and the presence of a degenerative appearance [30]-[32].

2.8. Blastocyst Evaluation

Viable blastocyst-stage embryos were scored strictly according to the guidelines published by David Gardner, William Schoolcraft, and others (Ferrick *et al*., 2019; Niederberger *et al*., 2018). The scoring system was used to assess the developmental capacity of blastocysts based on their morphological appearance and thereby enabled selection for transfer or embryo freezing (cryopreservation). Scoring of blastocysts was performed on an inverted microscope (Leica, SparMed, Copenhagen, Denmark) with a monitor and a camera attached while maintaining physiological pH and temperature. The implantation potential of a given blastocyst was

assessed based on three (3) parameters: quality of inner cell mass (ICM), quality of trophoblast or trophectoderm (TE), based upon cell number and cohesion, and blastocoelic capacity or blastocoel expansion. Blastocysts were given an alphanumeric score from I to VI, based on their degree of expansion and hatching status, and two-letter scores for inner cell mass (ICM) and trophectoderm were all performed on the inverted microscope.

3. Statistical Analysis

A total of 5238 oocytes were retrieved at ovum pick up (OPU) from 418 women, and the total number of mature, metaphase II oocytes obtained was 4276. Cycle and patient characteristics were expressed as mean ± standard deviation for continuous variables; percentages were used for categorical variables. Mean ± standard deviation was used to evaluate the relationship between serum AMH levels and continuous variables. Results were expressed in percentages. Bar charts were plotted to assess the influence of AMH levels on fertilization rates and embryo development (on day 2, 3, and blastocyst formation (on day 5). Pearson's chi-square (χ^2) test of association was used to evaluate the relationship between AMH, fertilization rate, and embryo development for each subgroup. The t-test of difference in proportions was also used to compare the proportions of fertilization and embryo development. The results were expressed as proportions, 95% confidence intervals (CI) for the difference in population proportions, and p-values. Significance was assigned to events with a p-value < 0.05. Data analysis was carried out on Minitab Statistical Software (version 22.2.0) and SPSS (version 31). All p-values < 0.05 were considered statistically significant. Data was plotted using bar charts.

4. Results

Fertilization Rates

The fertilization rate in percentages for each AMH category value are as follows: group 1 = 123/130 (94.61%), group 2 = 292/298 (97.99%), group 3 = 888/890 (99.77%) group 4 = 1730/1734 (99.77%) and group 5 = 1220/1224 (99.69%). The mean fertilization rate of MII oocytes in the study groups is 4253/4276 (99.46%). The highest fertilization rate was observed in groups 3 and 4 (99.77% and 99.77%, respectively), and the lowest fertilization rate was in group 1 (94.61%), compared to the mean fertilization rate of 99.46% (Table 1).

The relationship between AMH and embryo development was assessed on day 2, day 3, and day 5 (Table 2). Table 3 gives a summary of the statistical analysis of AMH, fertilization rate, and embryo development. The bar chart in Figure 1 represents cases of fertilization rate in each group, while Figure 2 demonstrates the effect of AMH on embryo development (mean) in the study groups. There was no association between AMH and fertilization ($\chi^2 = 99.98$, $p > 0.111$) (Table 3).

All other relationships between AMH and embryo development are listed in the summary Table 3.

Table 1. Cross-tabulation of percentage fertilization rates in AMH group categories.

AMH	Variable	Group 1	Group 2	Group 3	Group 4	Group 5	Total
	no. of oocytes retrieved	140	310	914	1858	2016	5238
	no. of met II oocytes obtained	130	298	890	1734	1224	4276
	no. of immature oocytes retrieved	10	12	24	124	792	962
	fertilization rates	123[a]	292[b]	888[c]	1730[d]	1220[e]	4253
	not fertilized	7[a]	6[b]	2[c]	4[d]	4[e]	23

There was no association between AMH and fertilization in subgroups (χ^2 (310.980, 5) p > 0.0001). The *t*-test of difference in proportions is not significant (p > 0.05) in fertilization among the various AMH subgroups.

Table 2. Relationship between serum level of Anti-Müllerian hormone (AMH) and embryo development on day 2, day 3, and day 5.

Predictor variable	Study groups	Fertilization rates (%)	Response variable	Day 2 (%)	Day 3 (%)	Day 5 (%)	Mean (SD)
			Embryo quality				
Serum level of AMH	Group 1 <0.30 (n = 20)	94.62 [123/130]	Good	34.96 [43/123]	32.56 [14/43]	28.57 [4/14]	32.03
			Fair	24.39 [30/123]	93.33 [28/30]	96.42 [27/28]	71.38
			Poor	40.65 [50/123]	92.00 [46/50]	97.83 [45/46]	76.83
	Group 2 >0.30 ≤2.19 (n = 132)	97.77 [292/298]	Good	50.68 [148/292]	47.97 [71/148]	42.25 [30/71]	46.97
			Fair	28.08 [82/292]	95.12 [78/82]	97.43 [76/78]	73.54
			Poor	21.23 [62/292]	83.33 [135/162]	100 135/135]	68.12
	Group 3 >2.19 ≤4.0 (n = 158)	99.76 [888/890]	Good	67.79 [602/888]	58.14 [350/602]	44.57 [156/350]	56.77
			Fair	13.51 [120/888]	95.83 [115/120]	98.26 [113/115]	84.85
			Poor	18.69 [166/888]	90.36 [150/166]	98.78 [405/410]	69.16
	Group 4 >4.00 ≤6.79 (n = 73)	99.77 [1730/1734]	Good	93.64 [1620/1730]	91.60 [1484/1620]	64.69 [960/1484]	83.31
			Fair	93.64 [60/1730]	90.00 [54/60]	98.15 [53/54]	93.93
			Poor	2.89 [50/1730]	96.00 [48/50]	97.92 [47/48]	65.40
	Group 5 ≥6.79 (n = 35)	99.67 [1220/1224]	Good	65.90 [804/1224]	63.18 [508/804]	51.18 [260/508]	60.08
			Fair	23.61 [288/1220]	95.83 [276/288]	98.19 [271/276]	72.54
			Poor	10.49 [128/1220]	96.75 [124/128]	99.19 [123/124]	68.81

Table 3. Summary of statistical analysis of AMH, fertilization rate, and embryo development.

Comparison groups	Test used	Proportion (%)	95% CI for Difference	χ^2/t-value	*p*-value
AMH vs fertilization rate	Chi-square	Low AMH: 94% High AMH: 99%	90% - 99%	$\chi^2 = 99.98$	0.111
AMH vs embryo development	Chi-square	Low AMH: 62% High AMH: 79%	10.2% - 23.0%	$\chi^2 = 7.12$	0.008
AMH vs fertilization rate	t-test of proportions	Difference: 14%	6.1% - 21.9%	t = 3.02	0.300
AMH vs embryo development	t-test of proportions	Difference: 17%	8.5% - 25.5%	t = 3.47	0.001

Proportions are expressed as percentages of successful fertilization and embryo development per subgroup. Confidence intervals (95% CI) reflect the range of the difference in proportions between groups. Significance was determined at $p < 0.05$. Software used: Minitab (v22.2.0) and SPSS (v31.0).

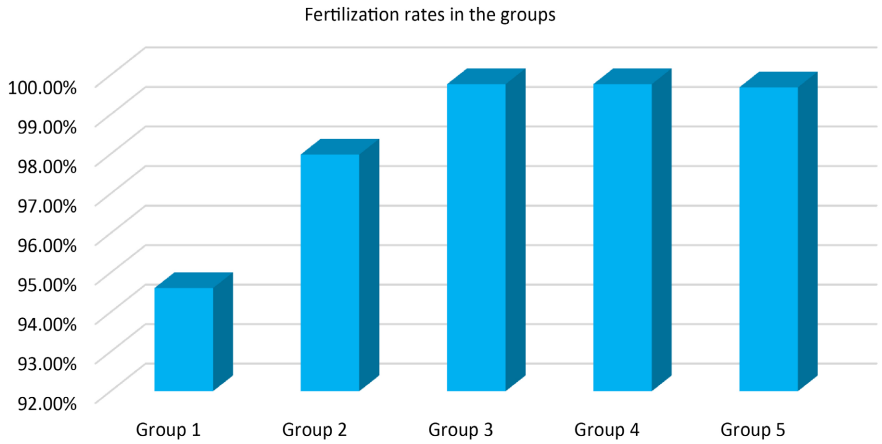


Figure 1. A bar chart showing fertilization rates in the study groups.

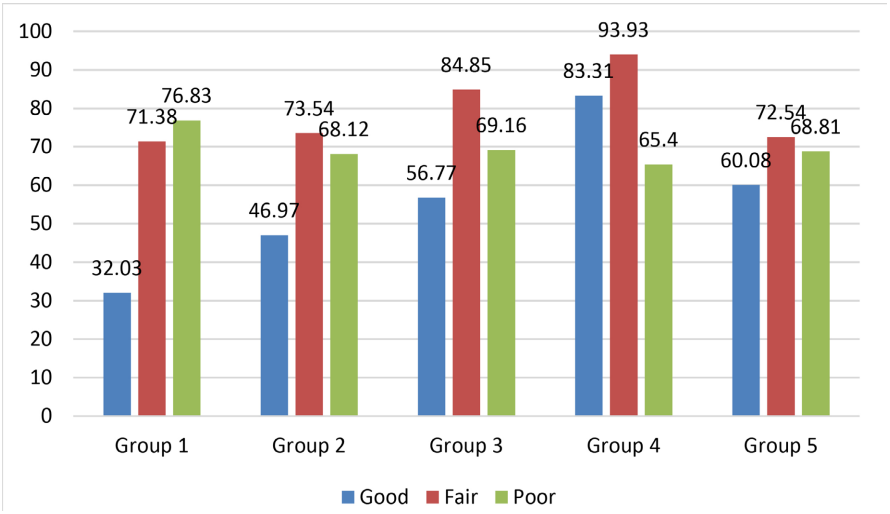


Figure 2. A bar chart showing the effect of AMH on embryo development (mean) in the study groups.

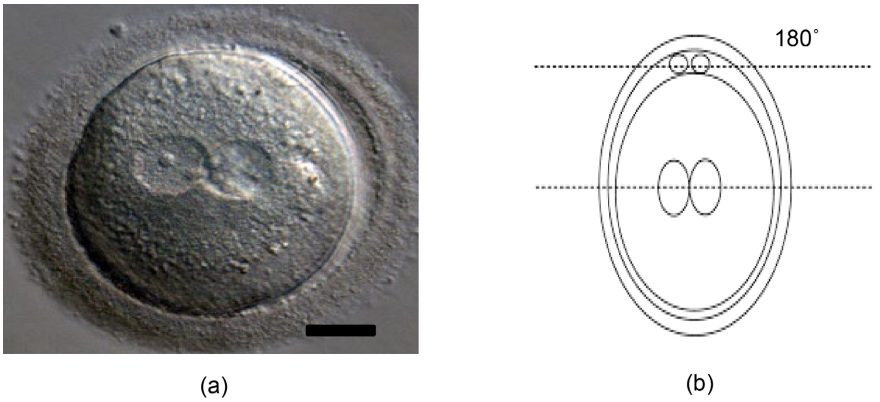


Figure 3. An inverted microscope micrograph showing (a) a fertilized oocyte with two pronuclei and nucleoli within each pronucleus (×2000) and (b) a schematic diagram of the dorsal-ventral axis in the pronuclear oocyte.

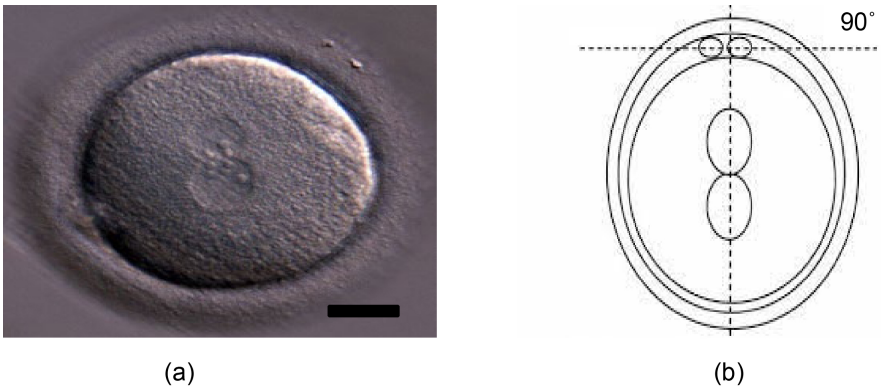


Figure 4. An inverted microscope micrograph showing (a) a fertilized oocyte with two pronuclei. Nucleoli within each pronucleus are lined up at adjacent borders (b). A schematic diagram of the anterior-posterior axis in the pronuclear oocyte (×2000).

5. Discussion

This study assessed the impact of AMH levels on fertilization rates and embryo development among women who received IVF treatment. Subgroup analysis showed that neither fertilization rate nor embryo quality could be estimated using serum AMH levels. There were similar rates of normal fertilization with a mean fertilization rate of 99.46%, indicating that AMH levels are not associated with the process of fertilization [33] [34]. **Figure 3** and **Figure 4** show the morphology of a fertilized oocyte with two distinct pronuclei.

Embryos were categorized as "good", "fair", or "poor" quality, based on the number and grade of blastomeres. Cleavage stage morphology was assessed based on the following parameters: number of blastomeres, per cent fragmentation, variation in blastomere symmetry, presence of multinucleation, and defects in the zona pellucida and cytoplasm [34].

Figures 5(a)-(c) show the morphology of "good" day 2 and 4 - cell stage cleavage embryos with the following characteristics: two equal blastomeres on one plane of focus and a further two alternate oppositions on another plane with sym-

metrical blastomeres of equal size, with no cytoplasmic fragmentation. Figures 6(a)-(c) show the morphology of "fair" day 2 and 4-cell stage cleavage embryos with cytoplasmic fragmentation (<25%) and granular grades in left blastomeres. Figures 7(a)-(c) show the morphology of "poor" day 2 cleavage stage embryos with irregular blastomeres with severe cytoplasmic fragmentation (>85%) and asymmetrical cells. Figures 8(a)-(c) and Figures 9(a)-(c) show the morphology of "good" day 3 embryos with regular blastomeres. Figures 10(a)-(c) and Figures 11(a)-(c) show "good" day 3 and 8 - 10 cell stage cleavage embryos with regular blastomeres with near-perfect morphology. Figures 10(a)-(c) show the morphology of "fair" 6-cell stage embryos with minor cytoplasmic fragmentations in the lower blastomeres on day 3. Figures 11(a)-(c) show the morphology of "poor" 8-cell stage day 3 embryos with severe fragmentation scattered throughout the surface surroundings and intermingled with blastomeres. These embryos were slow-growing and contained a very low number of cells.

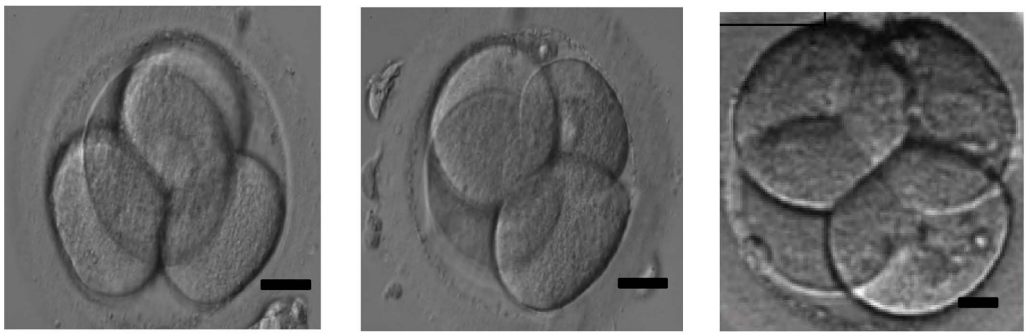


Figure 5. An inverted microscope micrograph showing the morphology of "good" day 2 embryos. (a) Four-cell stage embryo with slightly irregular blastomere; (b) Four–cell stage embryo showing two blastomeres on one plane of focus and a further two in alternate opposition on another and (c) Four-cell stage embryo with symmetrical blastomeres and of equal size, two lies in one plane of focus and two others, with opposing polarity lie in another with slight granular grades (×2000).

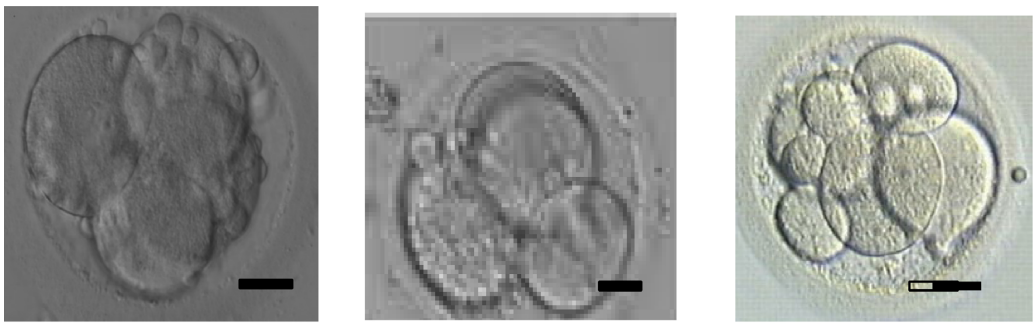
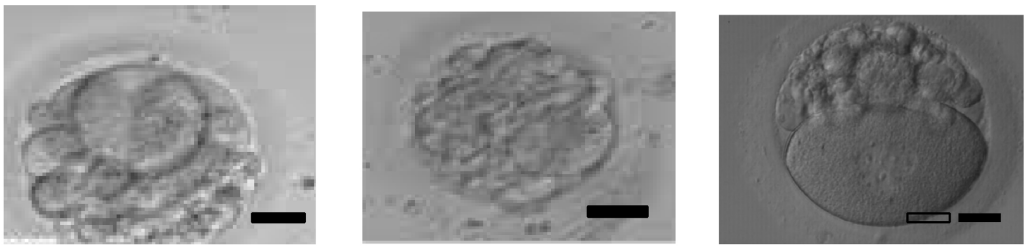
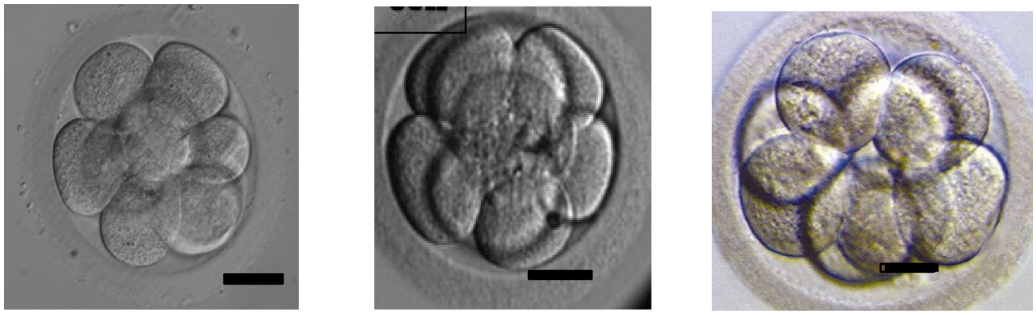


Figure 6. An inverted microscope micrograph showing the morphology of "fair" day 2 embryos. (a) Four-cell stage embryo with cytoplasmic fragmentation in blastomeres (b). Four-cell stage embryo with cytoplasmic fragmentation at the left lower blastomere and (c) Four-cell stage embryo with cytoplasmic fragmentation and granular grades in left blastomeres (×2000).



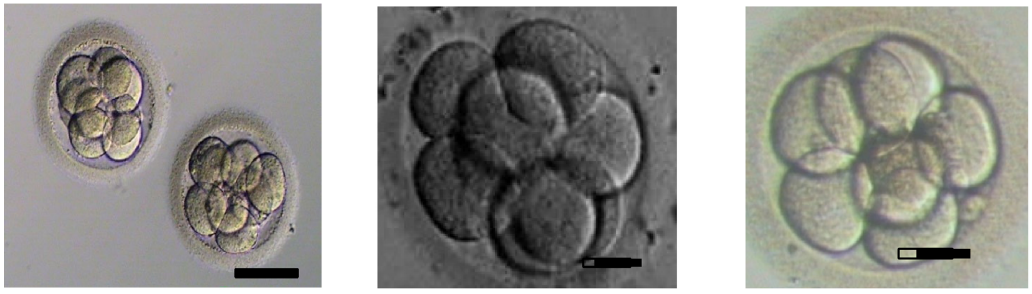
(a) (b) (c)

Figure 7. An inverted microscope micrograph showing the morphology of "poor" day 2 embryos. (a) Three-cell stage embryo showing irregular blastomeres with significant cytoplasmic fragmentation (b), Four-cell stage embryo showing irregular blastomeres with significant cytoplasmic fragmentation, and (c) Two-cell stage embryo with significant cytoplasmic fragmentation at the upper blastomere (×2000).



(a) (b) (c)

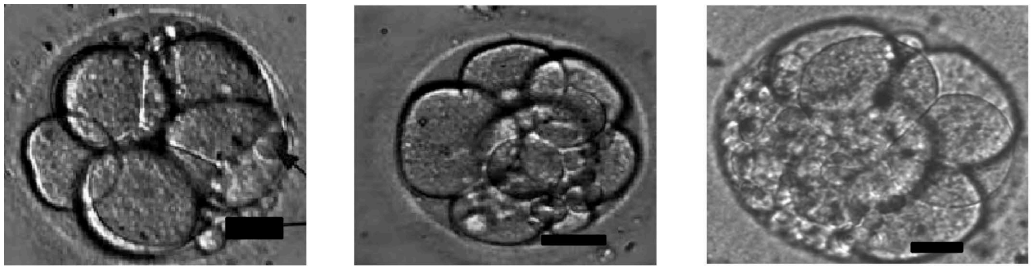
Figure 8. An inverted microscope micrographs showing the morphology of "good" day 3 embryos. (a) Eight-cell stage embryo with regular blastomeres (b), an Eight-cell stage embryo showing minor but normal variation in blastomere size, and (c) an Eight-cell stage embryo with a near-perfect morphology. The zona is relatively compact and exhibits almost uniform thickness (×2000).



(a) (b) (c)

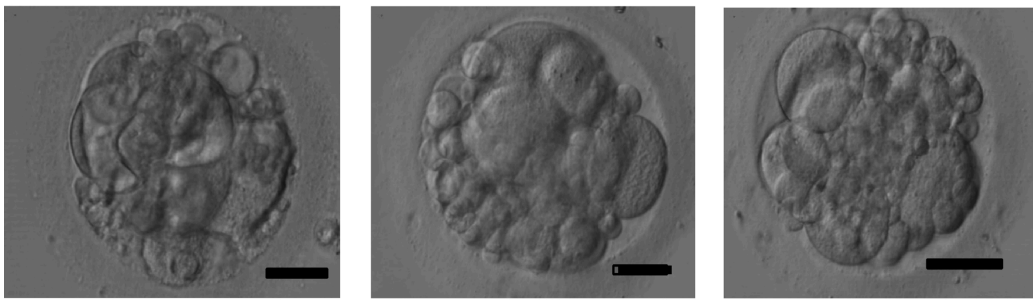
Figure 9. An inverted microscope micrographs showing the morphology of "good" day 3 embryos. (a) These eight-cell stage embryos were transferred to a 37-year-old woman. One singleton pregnancy was achieved (b). This 1 eight-cell embryo was transferred to a 42-year-old woman. One singleton pregnancy was achieved. (c) seven-cell stage embryo with a near-perfect morphology (×2000).

In blastocyst formation assessment on day 5, the embryos were graded using the formation of the inner cell mass (ICM) and the Trophectoderm (TE). Inner cell mass grading: "Good" blastocysts showed numerous cells in the epithelium, cells appeared healthy and were tightly packed, as shown in **Figures 12(a)-(c)**.



(a) (b) (c)

Figure 10. An inverted microscope micrographs showing the morphology of "fair" day 3 embryos. (a) Six-cell stage embryo with minor cytoplasmic fragmentation in the lower blastomeres (b), Six-cell stage embryo with significant cytoplasmic fragmentation and (c) Six-cell stage embryo with significant cytoplasmic fragmentation and granules in blastomeres (×2000).



(a) (b) (c)

Figure 11. An inverted microscope micrographs showing the morphology of "poor" day 3 embryos. Severe fragmentation scattered throughout the surface surroundings and intermingled with the blastomeres of an eight-cell stage embryo; (b) Multinucleated blastomere with numerous large and small cytoplasmic fragments, and (c) Complete cytoplasmic fragmentation of an eight-cell stage embryo (×2000).

(a) (b) (c)

Figure 12. An inverted microscope micrographs showing the morphology of a "good" day 5 blastocyst. (a) Day 5 full blastocyst displaying grade 1 expansion; no thinning of the zona pellucida. The inner cell mass (ICM; 8-o'clock) is made up of highly compacted cells with many cells in the trophectoderm, (b) Day-5 fully expanded blastocyst with compacted ICM at almost 9 o'clock position and a cohesive trophectoderm with many cells and (c) Day-5 blastocyst with a cohesive trophectoderm and a rounded inner cell mass at the 11- o'clock position (×2000).

"Fair" blastocysts showed: large cells that are loosely packed, as indicated in **Figures 13(a)-(c)**. "Poor" blastocysts showed: few cells, inner cell mass not distin-

guishable, and the presence of degenerative cells as indicated in Figures 14(a)-(c). For Trophectoderm grading of day 5 embryos, "Good" blastocysts in Figure 12 showed many cells forming an epithelium, "Fair" blastocysts in Figure 13 showed a few large cells forming an epithelium, and "Poor" blastocysts in Figure 14 showed a few cells that appeared poor, uneven, and the presence of degenerated cells.

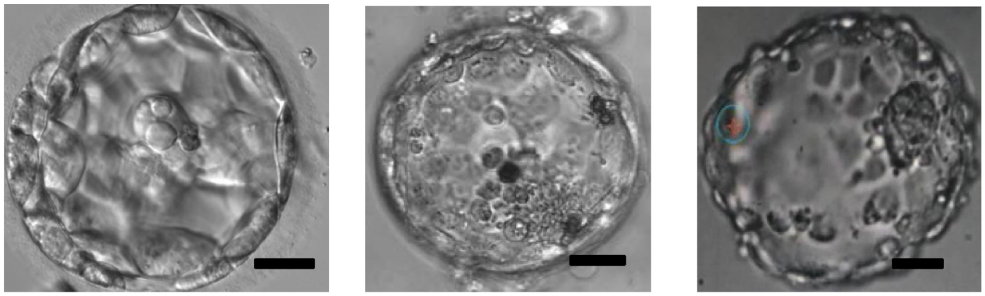


Figure 13. An inverted microscope micrographs showing the morphology of a "fair" day 5 blastocyst. (a) Day-5 blastocyst displaying small cells in the trophectoderm and inner cell mass; (b) blastocyst on day-5 with poor quality inner cell mass and trophectoderm [low cell numbers] and (c) Day-5 blastocyst displaying an irregular zona pellucida with low cell numbers in the trophectoderm and poor-quality inner cell mass at 2 o'clock position (×2000).

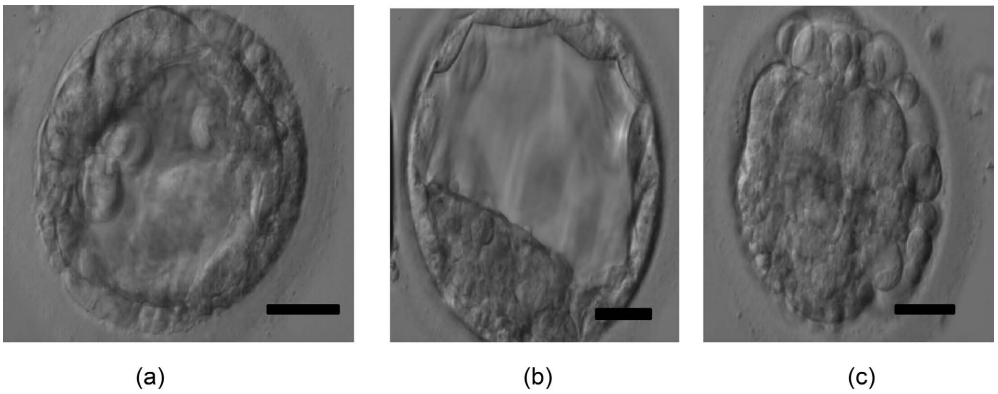


Figure 14. An inverted microscope micrographs showing the morphology of a "poor" day 5 blastocyst. (a) abnormal trophectoderm with degenerated inner cell mass; (b) cytoplasm filled with fluid and unhealthy appearing trophectoderm, and (c) degenerated trophectoderm associated with fragmented inner cell mass (×2000).

There was a sharp increase in the number of good-quality embryos obtained from group 2 to 4. The proportion of fair-quality embryos (from cleavage stage to blastocyst formation) was highest in group 4 [mean = 93.93] and lowest in group 1 [mean = 71.38]. There is evidence that embryo selection on days 2 and 3 based on morphological criteria may be unreliable, resulting in the transfer of embryos that are abnormal or arrested at later developmental stages [34]. The rate of blastocyst formation decreases significantly across all study groups with embryos with fragmentation on days 2 and 3. Several hypotheses have been suggested to explain

the detrimental effects of fragments on embryo development [30]. First, fragments might physically impede cell-cell interactions, interfering with compaction, cavitation, and blastocyst formation. In addition, ultrastructural observations of degeneration in blastomeres adjacent to fragments suggest that fragments might also release toxic substances and therefore damage nearby cells [35]. Alternatively, fragments might also reduce the volume of cytoplasm and deplete the embryos of essential organelles or polarized domains. In this study, the fragmented polar bodies (PB) [mean = 9.26]. Higher aneuploidy rates were reported for >35% fragmented embryos, whereas it was also speculated that in the case of moderate fragmentation, various temporal or spatial patterns of fragmentation have quite different effects on embryo development than the occurrence of fragments [35].

Finally, the proportion of poor-quality embryos (from cleavage stage to blastocyst formation) was highest in group 1 (mean = 76.83) and lowest in group 4 (65.40). Research has shown that when embryos are cultured in vitro, about 50% cease development during the first week [36]. The reasons for this high rate of embryo loss during early development are not fully understood. This might include chromosomal abnormalities, suboptimal culture conditions, or inadequate oocyte maturation [30] [37]. If present in the oocytes, these abnormalities and conditions could be one possible reason for the high rate of fair and poor-quality embryos on days 3 to 5 of the culturing period. The association between AMH levels and fertilization rate -particularly in ICSI (Intracytoplasmic Sperm Injection) cycles- remains underexplored in Ghana. However, preliminary findings from our center suggest relatively high fertilization rates, even among women with lower AMH levels, contrasting with global benchmarks that typically associate diminished AMH with reduced oocyte yield and lower fertilization potential. This discrepancy may be partly attributed to procedural factors specific to our center (in Ghana), such as stringent oocyte selection protocols, optimized culture media, early timing of insemination post-retrieval, and skilled embryologists performing ICSI. Additionally, patient demographics, including a younger average maternal age and lower prevalence of comorbidities compared to Western populations, may contribute to more favorable embryological outcomes despite suboptimal AMH levels. While international literature often presents AMH as a strong predictor of ovarian reserve rather than fertilization success per se, these localized outcomes underscore the importance of contextual factors in interpreting AMH's utility in assisted reproductive technologies within Ghana.

6. Limitations of the Study

A key limitation of our study (impact of AMH on fertilization and embryo development in Ghana) is the absence of pregnancy and live birth outcome data. While fertilization rates and embryo quality provide important intermediate indicators of ART success, they do not necessarily predict clinical pregnancy or live birth, which are the ultimate measures of reproductive outcome. Without this data, the study could not fully assess the predictive value of AMH across the entire ART

process. This limits the ability to draw meaningful conclusions about the hormone's clinical utility in guiding treatment decisions or counselling patients. Furthermore, the absence of pregnancy and live birth outcomes may overlook potential confounding factors such as implantation failure or early miscarriage, which could vary independently of AMH levels.

7. Conclusion

This study presents a viable base of data on AMH, a robust parameter for predicting oocyte quantity and quality after controlled ovarian stimulation (COS), fertilization, and embryo development in women undergoing IVF treatment in Ghana. It has also been established that, by early detection of women with a reduced ovarian response, using a cut-off level of ≤0.30 ng/mL, treatment options are still available (high-dose stimulation in IVF, polyovulation) with favorable pregnancy rates, not statistically different from those of women with 'normal' ovarian reserve. To some extent, this study has produced some preliminary ovarian biomarker baseline clinical data for ART practitioners on the application of AMH in IVF treatment for Ghanaian women. The clinical application depends on individual centers examining their data, correlating AMH levels and ultimate ovarian response in the form of metaphase II oocytes, fertilization rates, and embryo development. It is recommended that future research or further studies be conducted on a large sample size to define the role of AMH in IVF outcomes.

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Conflicts of Interest

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

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