The relationship between Anti-Mullerian Hormone (AMH) levels and pregnancy outcomes in patients undergoing assisted reproductive techniques (ART)

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A variety of predictors are available for ovarian stimulation cycles in assisted reproductive technology (ART) forecasting ovarian response and reproductive outcome in women including biomarkers such as anti-Mullerian hormone (AMH). The aim of our present study was to compare the relationship between AMH levels and pregnancy outcomes in patients undergoing intra-cytoplasmic sperm injection (ICSI). Overall, fifty patients (n=50), aged 20-45 years were recruited for the present prospective study. Three AMH levels were presented with high often poly cystic ovarian syndrome (PCOS) amongst 52.4% patients, 40.5% in normal and 7.1% in low to normal, correspondingly. There was statistically significant relationship between AMH and day of embryo transfer ($p < 0.05$). The Pearson analysis between AMH, age, E2 and FSH displayed no statistically significant relationship between E2 and AMH ($p < 0.05$) and negative correlation between FSH and age ($p > 0.05$). The area under the receiver operating characteristic curve for E_2 was 0.725 and for AMH levels as predictors of CPR was 0.497 indicating E_2 as better predictor than AMH. The number of oocytes, mature oocytes and fertilized oocytes all presented a weak positive relationship to AMH. Our results confirm the clinical significance of AMH to accurately predict ovarian reserve as a marker and its limitations to use as predictor for a positive pregnancy outcome. Additional prospective studies should be conducted to validate the predictive capability of AMH levels for the outcome of clinical pregnancy.

1 **Title**

2 **The Relationship Between Anti-Mullerian Hormone (AMH) Levels and**
3 **Pregnancy Outcomes in Patients Undergoing Assisted Reproductive**
4 **Techniques (ART)**

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Abstract

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29 technology (ART) forecasting ovarian response and reproductive outcome in women including
30 biomarkers such as anti-Mullerian hormone (AMH). The aim of our present study was to compare
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32 cytoplasmic sperm injection (ICSI). Overall, fifty patients (n=50), aged 20-45 years were recruited
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38 and age ($p > 0.05$). The area under the receiver operating characteristic curve for E_2 was 0.725 and
39 for AMH levels as predictors of CPR was 0.497 indicating E_2 as better predictor than AMH. The
40 number of oocytes, mature oocytes and fertilized oocytes all presented a weak positive relationship
41 to AMH. Our results confirm the clinical significance of AMH to accurately predict ovarian
42 reserve as a marker and its limitations to use as predictor for a positive pregnancy outcome.
43 Additional prospective studies should be conducted to validate the predictive capability of AMH
44 levels for the outcome of clinical pregnancy.

45

46 ***Keywords***: Anti-Mullerian hormone, Fertilization *in vitro*, Pregnancy, Enzyme-linked
47 immunosorbent assay, Oocytes, Clinical pregnancy rate

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Introduction

56 Couples in modern societies postpone childbearing amidst busy schedules and career positionings;

57 trying to conceive at a more advanced age contributing to a rise in the occurrence of infertility

58 (Caroppo et al., 2006). Most women are unaware that fertility starts to decline after the early

59 thirties in some individuals. With increasing female age, fecundity in natural and stimulated

60 ovarian cycles declines, as observed in population-based studies (Grynnerup et al., 2012) as well

61 as in IVF studies (Scheffer et al., 2018). For this reason, there is a growing number of women of

62 advanced age seeking treatment for infertility (Oskayli et al., 2019).

63 The hormonal control of ovarian function is influenced by administering exogenous follicle

64 stimulating hormone (FSH) (Richards, 2018). Prediction of ovarian responses prior to stimulation

65 is not only useful for patient counselling, but also important in tailoring the optimal dosage of

66 gonadotrophin for individual patients. The recruitment and development of multiple follicles in

67 response to gonadotrophin stimulation are essential for the successful treatment of infertility by

68 assisted reproductive techniques (ART) (Dewailly et al. 2014; Yang, Wu, and Zhang 2020).

69 Besides, poor ovarian response has been suggested to be associated with high cycle cancellation

70 rates (Saldeen, Källen, and Sundström 2007). Chang et al (1998) found that patients with antral

71 follicle number $\leq$ 3 had a significantly higher rate of cycle cancellation and higher human

72 menopausal gonadotropin (HMG) dosage as compared with those patients with antral follicle

73 number 4-10 or $\geq$ 10. Nevertheless, the AFC is presently believed to be the finest specific predictor

74 of ovarian response to stimulation in ART, and it can be used in clinical practice for pretreatment

75 counselling targets.

76 In assisted reproduction, serum levels for several hormones are used to assess the ovarian reserve

77 and to monitor the development of the follicles that have been stimulated by gonadotrophins(Alson

78 et al., 2018). Traditional techniques used to predict ovarian stimulation have incorporated serum

79 levels of hormones such as FSH, LH and estrogen (E_2) along with ultrasonographic guides such

80 as ovarian volume and number of early antral follicles as a reliable predictor of the outcome of in

81 vitro fertilization (IVF) (Kunt et al., 2011). Over the last years, Anti-Mullerian hormone (AMH)

82 has been projected as a novel marker for predicting ovarian response to gonadotrophin stimulation

83 (Alson et al., 2018, Zargar et al., 2018). AMH is a dimeric glycoprotein strongly produced by the

84 granulosa cells of the pre-antral (primary and secondary) and small antral follicles (AF's) in the

85 ovary and shown to be age dependent (Sahmay et al., 2014). Measurement of anti-Müllerian

86 hormone in serum is much more precise measure of the ovarian reserve than the other hormones

87 that have previously been available to us(Anderson et al., 2012).

88 The aim of this prospective study was to investigate the relationship between Anti-Müllerian

89 hormone levels and pregnancy outcomes in patients undergoing *in-vitro* fertilization or intra-

90 cytoplasmic sperm injection (ICSI).

91

92 **Materials and Methods**

93 **Patients**: Fifty women(n=50), aged 20-45 years were recruited from Centre of Assisted

94 Reproduction and Endocrinology (C.A.R.E) Clinic in Westville, Durban, South Africa who were

95 undergoing IVF treatment. This study was approved by Ethical Committee of the Durban

96 University of Technology (Project reference 128/16) and Research Committee, C.A.R.E. Clinic,

97 Durban, South Africa. After approving the study by the research ethics committees, written

98 informed consents were obtained from all the patients.

99 **GnRH antagonist Protocol:** A gonadotrophin-releasing hormone (GnRH) antagonist protocol

100 with recombinant FSH (GONAL-f, Merck Serono, Darmstadt, Germany) was used as

101 downregulatory (Park et al., 2015). The second approach was followed by administering 0.25

102 mg/day Cetrotide (Merck Serono). When at least 3 or more follicles reach a diameter equal or

103 above $17 - 18$ mm, the endometrial thickness reached at least 7 mm by ultrasound and E2 levels

104 were about 1500-1800 pmol/L then Human chorionic gonadotropin (hCG) was administered. All

105 patients received $5000 - 10\,000$ IU hCG (Ovitrelle®, Merck Serono). Oocyte retrieval was

106 performed 36 hours after the administration of the hCG. Conventional IVF or ICSI was performed

107 according to previously described protocols.

108 **Sample collection**: Blood samples were collected every 3-4 days on commencement of the

109 treatment. The blood samples were centrifuged at 3000 rpm for 10 minutes using a Biofuge

110 centrifuge (Biofuge Primo – Heraeus) to obtain the blood serum. AMH and FSH levels were

111   recorded, upon the first visit. Estrogen(E_2) and LH levels were monitored throughout the program

112   until a peak E_2 and LH level were reached.

113   **Hormone Assays**: Gen II ELISA (Beckman Coulter Inc., USA, catalog number A79765/A79766,

114   unmodified version). (Beckman Coulter, USA) kit was used to estimate hormone levels (FSH-Cat.

115   No. 33520 Access hFSH reagent, 100 determinations, 2 x 50 tests); E2 (Cat. No. B84493 Access

116   Sensitive Estradiol Reagent, 100 determinations) and AMH (Cat. No. B13127 Access AMH

117   Reagent, 100 determinations, 2 x 50 tests) from the blood serum according to manufacturer's

118   instructions. Insemination and intra cytoplasmic sperm injection (ICSI), oocyte retrieval, culture,

119   fertilization, embryo culture, and transfer were carried out as previously described by Gardener et

120   al. (Gardner et al., 2001).

121   Inclusion Criteria: The population of the study included female patients ranging between the ages

122   of 20–45

123   **Exclusion Criteria:** Patients undergoing cancer therapy and patients on immune suppressant

124   drugs were excluded from study.

125   **Statistical analysis:** The data were analysed using IBM SPSS software (Chicago, IL, USA).

126   Pearson's correlation was used to determine the direction, strength, and significance of the

127   correlation between X and Y variables between the different semen parameters. A parametric

128   multiple linear regression analysis was used to evaluate the relationship between AMH and other

129   available endocrine markers. ROC curves were used to assess predictive value for E_2 and AMH

130   and evaluating cut off values to optimise sensitivity and specificity. A *p* value of < 0.05 was

131   considered statistically significant.

132   **Institutional Review Board approval**: This study was approved by Ethical Committee of the

133   Durban University of Technology (Project reference 128/16) and Research Committee, C.A.R.E.

134   Clinic, Durban, South Africa and was performed in accordance with the Helsinki Declaration of

135   1975 (as revised in 1983).

136   **Results**

137   The prospective study included fifty patients who met the inclusion criteria. From the initial sample

138   size of fifty, forty-two presented with data that could be analysed whilst 8 patients had oocytes

139   that where abnormal and did not result in transfer. The data from these 8 patients were not included
140   in the study due to poor embryo development (Table 1).

141   Table 1. AMH distribution in blood sample**s**

142   Amongst the 42 patients analysed, 4.76% were between 20-24 years, 9.52% were between 25-29
143   years, 40.47% were between 30-34 years, 35.7% were between 35-39 years and 9.52% were
144   between 40-44 years, respectively. As demonstrated by this study the clinical pregnancy rate for
145   patients 20 - 24 years was 100%, 25 -29 years was 50%, 30 – 34 years was 17.6%, 35 – 39 years
146   was 26.6% and 40 - 44 years was 25% (Fig. 1).

147   Table 2 shows number of oocytes retrieved, number of oocytes matured, and number of oocytes
148   fertilized into respective categories. Not all eggs obtained were at the metaphase 2 stages and had
149   to be matured in the incubator overnight and injected the following day. The results shown were
150   to some extent anticipated as AMH has been used an indicator of oocyte reserve in previous
151   studies(Yarde et al., 2013, Yao et al., 2015) whereas the resulting fertilized or transferred embryo's
152   may be due to a chance process based on many various factors such as   quality of oocyte and
153   sperm.

154   The Chi-square test for Independence was performed to check whether there was an association
155   between the number of oocytes fertilized and the AMH category (Table 2). A Chi-squared value
156   of 18.5, degrees of freedom $= 12$, with a $p = 0.10$ was found. There was no statistically significant
157   relationship between numbers of oocytes fertilized versus AMH category ($p > 0.05$).

158   Out of 22 patients, 43 embryos were transferred. Embryos were transferred depending on embryo
159   development and the number of embryos obtained. Most patients in the high and normal categories
160   resulted in a day 5 transfer, the Chi-squared test for independence of AMH and number of embryos
161   transferred gave a Chi-squared value of 6.384 with df $= 4$ and p-value $= 0.172$(Table 3), thus
162   statistically no significant association between AMH and number of embryos transferred was
163   observed. Whilst, Chi-square test for independence between the variables AMH and day of embryo
164   transfer (Table 4) gave a Chi-square value of 14.117, 6 degrees of freedom and $p = 0.028$ indicating
165   statistically significant relationship between AMH and day of embryo transfer ($p < 0.05$).

166   Pregnancy outcome and AMH category are as shown in Table 5. Out of twenty two cases in high
167   category, 6 resulted in a positive pregnancy; 6 resulted in a positive outcome (6/12 = 50.0%)

168   (Normal); while out of the 3 cases where the AMH was "Low to Normal", there were no

169   pregnancies reported. The Chi-squared test for independence of AMH category and pregnancy

170   outcome gave a Chi-Squared value of 0.502, 2 degrees of freedom and $p = 0.778$. There was no

171   statistically significant relationship between the pregnancy outcome and the AMH category ($p >$

172   0.05).

Pearson correlation

174   Pearson correlation coefficients were calculated to determine if any statistical significance exists

175   between AMH on a quantitative scale and age, E_2 and FSH (Table 6). The Pearson Correlation co-

176   efficient of 0.151 indicates that a very weak positive relationship existence between E_2 and AMH,

177   which is not statistically significant ($p = 0.341$). Furthermore, Pearson correlation coefficient

178   between the AMH and age had a coefficient of -0.028 thus showing no statistical significance $p =$

179   0.859 ($p > 0.05$). The Pearson Correlation between AMH and FSH produced a coefficient of -

180   0.185 thus indicating no statistical significance $p = 0.240$ ($p > 0.05$). Pearson correlation coefficient

181   showed no significant association between AMH and number of oocytes ($p = 0.191$), number of

182   mature oocytes ($p = 0.300$) and number of oocytes fertilized ($p = 0.146$). The number of oocytes,

183   mature oocytes and oocytes fertilized all presented a no statistically significant correlation with

184   AMH (0.206, 0.164, and 0.228, respectively).

Logistic regression analysis

186   A logistic regression model was used to determine the possible predictor variables for the

187   pregnancy outcome. The model was fitted to the data with the result of the pregnancy namely,

188   "Positive" or "Negative" as the binary dependent variable and age, E_2 LH, Basal FSH, Basal AMH

189   and number of oocytes fertilized as independent variables (Table 7). As shown in Table 7, LH has

190    $p = 0.042$ ($p < 0.05$) and E_2 has $p = 0.065$, which is statistically not significant at a 5% level. The

191   SPSS output for the model is given in Table 1(Supplementary data S1) signifying that overall, 73.8

192   % of the cases were correctly classified, while 5/12 =0.417 or 41.7 % of the positives were

193   correctly classified, and 86.7% of the negative cases were correctly classified.

Area under the curve

195   The ROC curves of the serum AMH concentrations and E_2 for the prediction of the clinical

196   pregnancy are depicted in Fig. 2. The areas under the curves (AUC) for E_2 were 0.725 and for

197   AMH (AUC = 0.497). E_2 is therefore a better single predictor of pregnancy outcome when

198   compared to AMH. It has been shown that E_2 can better predict the number of oocytes obtained.

199   **Discussion**

200   In the current study, we investigated the relationship between AMH levels and pregnancy

201   outcomes in patients undergoing intra-cytoplasmic sperm injection.

202   *Correlation between basal AMH and E_2*

203   Pearson analysis between E_2 and AMH presented a Pearson Correlation co-efficient of 0.151

204   with $p = 0.341$ ($p < 0.05$) which indicates that a weak significant relationship exists between E_2

205   and AMH. Most previous studies (Ramalho de Carvalho et al., 2012, Ubaldi et al., 2005) have

206   shown a relationship between a raised basal E_2 level and a reduced ovarian response using different

207   values to express elevated estrogen levels which replicated the findings in this study therefore

208   showing that a low AMH can result in low estrogen levels. Also, it can be concluded that a poor

209   AMH value results in a poor ovarian reserve indicating follicles produced will not be correlated to

210   a raised estrogen level, therefore indicating poor follicle growth, thus reducing the number of

211   oocytes produced. However, it was determined that meagre response to stimulus in IVF, indicative

212   of a lower ovarian reserve, is associated with declined baseline serum AMH concentrations (Van

213   Rooij et al., 2004). Consequently, when women have regular ovarian reserve and decent retort,

214   disappointment of IVF must look for additional infertility reasons, e.g., male specific issue i.e. Y

215   chromosome microdeletion. Furthermore, this conclusion is reinforced by the data of woman

216   undergoing IVF which indicated that male factor infertility resulted in an unsuccessful cycle.

217   Although E2 levels in these cases were above those of controls, they are still within the range of

218   25-100 pg/ml (Sahmay et al., 2014), suggesting that E2 single-handedly is not capable of

219   predicting the female reproductive potential.

220   *Correlation between basal AMH and Age*

221   Pearson correlation between AMH and age (Table 6) presented a co-efficient of -0.028 thus

222   displaying a weak, negative association with a $p = 0.859$ ($p > 0.05$). A stronger relationship between

223   these two variables was expected as it is known that as age increases, AMH should decrease. This

224   contrary association corresponds as reported by Van Rooij et al., (2004), where serum AMH levels

225   decline with age in normal women with proven fertility. Also, it is suggested that serum AMH is

226   identified as the improved endocrine marker to measure the reproductive capability in advanced

227   age.

Correlation between Basal AMH and FSH

229   Basal FSH is one of the primary endocrine markers presented into ART program. The Pearson

230   correlation amid AMH and FSH (Table 6) had a coefficient of -0.185 thus displaying a weak,

231   negative relationship and with a *p*= 0.240 (*p*> 0.05). This study specifies a negative correlation,

232   i.e., the higher the FSH the higher the chances the patient can present with a poor ovarian reserve

233   and early menopause. It is routine practice to frequently measure the basal FSH level and to start

234   IVF treatment only when the FSH level is lower than threshold value in a cycle. This was strategic

235   on the awareness that these women will retort well to ovarian stimulation while the basal FSH

236   level is lesser at the beginning of the cycle. The outcomes of this study revealed that woman who

237   were poor respondents or had a reduced ovarian reserve had a poor outcome and frequent testing

238   is of no worth. Women who had a history of high FSH level must undergo treatment without

239   further delay. By postponing treatment for these patients can be detrimental as they get older and

240   fast approaching menopause (Uzumcu and Zama, 2016).

Correlation between Age and FSH and Number of oocytes

242   The Pearson Correlation between FSH and age (Table 6) displayed no statistical significance, *p* =

243   0.583 (*p* > 0.05). For most of Pearson Correlation analysis, no significant relationships were found

244   with most of the *p*-values, being greater than 0.05. This may be due to the small sample size used

245   in this study of 42 patients. AMH being compared to age and number of oocytes showed a slightly

246   negative correlation which is expected as it is shown in previous studies that AMH and number of

247   oocytes decrease with maternal age (van Rooij et al., 2004) and Gobikrushant et al. (2018). This

248   inverse relationship is in agreement by Van Rooij et al.   (2004), who reported that serum

249   AMH levels deteriorate with age in normal women with proven fertility. Additionally, serum AMH

250   indicates the simplest endocrine marker to measure the age-related decline of reproductive

251   competence. AMH levels, in our group who were high respondents were over 3.0 ng/ml, normal

252   respondent over 1.0 ng/ml and low respondents found to be below 0.9 ng/ml. Oocytes were still

253   recovered even with low AMH levels. Neither fertilization rate nor embryo quality can be assessed

254   using basal AMH levels. This contrasts with the findings reported by Vaegter et al. (2017), where

255 embryos had superior morphology and cleavage performance in patients with AMH levels > 2.7

256 ng/ml as compared with patients with values below this threshold.

257 *Correlation between AMH and the number of oocytes, number of mature oocytes and number of*

258 *oocytes fertilized*

259 Our second objective of the study was to examine if AMH levels affected oocyte quality. In this

260 study, the Pearson Correlation test (Table 6) showed no significant relationship between AMH and

261 number of oocytes ($p = 0.191$), several mature oocytes ($p = 0.300$) and number of oocytes fertilized

262 (p = 0.146). The number of oocytes, mature oocytes and oocytes fertilized all showed a weak

263 positive relationship to AMH (0.206, 0.164, and 0.228, respectively). These findings are in

264 agreement with that reported by La Marca and Sunkara (2014); La Marca et al., (2010) and

265 Dehghani-Firouzabadi et al. (2008), where mean amount of oocytes was lower in poor responding

266 patients than in normal patients attending IVF programs. This therefore led to the inference that

267 ovarian response can be regarded as a reflection of the ovarian reserve. The Chi-square test for

268 Independence was done to determine whether there is an association between the number of

269 oocytes collected and the AMH category (Table 8). A Chi-squared value of 21.246, degrees of

270 freedom = 8, with a $p = 0.007$ was observed. There was a significant relationship between the

271 numbers of oocytes collected versus AMH category ($p < 0.05$). The Chi-square test for

272 Independence was performed to see whether there is an association between the number of oocytes

273 fertilized and the AMH category (Table 9). A Chi-squared value of 18.5, degrees of freedom = 12,

274 with a $p = 0.10$ was found. There was thus no statistically significant relationship between the

275 numbers of embryo's fertilized versus AMH category ($p > 0.05$).This is anticipated as AMH has

276 been used an indicator of oocyte reserve in previous studies whereas the resulting fertilized or

277 transferred embryo's may be due to a chance process based on many various factors such as the

278 quality of the oocyte and sperm (Yarde et al., 2013). Ebner et al. (2006), demonstrated that AMH

279 serum levels were related with oocyte quality in stimulated cycles. The quality of the embryos was

280 not assessed using baseline AMH which agrees with our findings. However, the fertilization rate

281 was not correlated with the serum AMH which varied with the results of the present study.

282 *AMH category and positive pregnancies*

283 Embryo quality has been suggested to be of paramount importance to predict the occurrence of

284 pregnancy after IVF. In a regression model E_2 has a $p = 0.017$ ($p < 0.05$) and LH has a $p = 0.035$

285 (*p* <0.05). Both variables are significant and age and basal AMH play a role in the pregnancy
286 outcome and the model is thus adjusted for these two variables

287 In this study, AMH value for predicting pregnancy outcomes does not exist because oocyte quality
288 is not accounted for by ovarian reserve markers. As demonstrated in this study the clinical
289 pregnancy rate for patients 20 - 24 years was 100%, 25 - 29 was 50%, 30 – 34 years was 18%, 35
290 – 39 years was 27% and 40 - 44 years was 25% (Figure 1). Patients presenting with a low AMH
291 did not vary from those women presenting with higher AMH concentrations in same age group. A
292 positive pregnancy outcome was logged across all age groups regardless of the AMH level. These
293 results advocate that low ovarian reserve is not correlated with low oocyte quality in patients and
294 the prediction remains the similar despite low AMH concentrations. Kini et al. (2010) stated the
295 role of AMH in foreseeing cumulative pregnancy outcome during IVF treatment. It was recognized
296 that serum AMH concentration on day 6 of stimulation was suggestively higher in participants
297 who resulted in an ongoing pregnancy in IVF compared to those who did not. Serum AMH is a
298 suitable indicator of ovarian hyper-response. In a metanalysis study conducted by Yao et al. (2015)
299 to evaluate role of serum AMH role in forecasting the pregnancy outcome in IVF/ICS, it was
300 concluded that there is positive correlation between serum AMH and pregnancy. Nevertheless,
301 association between serum AMH and non-pregnancy cannot be ruled out either.

302 **Conclusion**

303 In conclusion, the outcomes of our investigations specify that AMH has established to be a
304 valuable marker for ovarian reserve and might benefit woman who plan for pregnancy. AMH
305 hormone seems to be the best endocrine marker, however, the valuable role of AMH and its role
306 in ovarian function should be looked at in relation to the other markers to assess the decline of the
307 ovarian pool. While appropriate reference values are being generated per age category and until
308 the consequences of having a low or high AMH for one's age are being established, AMH should
309 only be determined in the context of clinical studies. At present, the most important clinical role
310 of AMH at this stage is to serve as a red flag for reduced ovarian reserve in women of reproductive
311 age who must undergo further diagnostics. As per the study conducted, we can infer that AMH
312 can accurately predict ovarian reserve but cannot predict the oocyte quality or a positive pregnancy
313 outcome. The more oocytes obtained, increases a patient's chance of more viable embryos and
314 therefore, improving chances of a healthy pregnancy and ultimately a live birth. Further research

315 on the implication of varying levels of AMH within the follicular fluid may be representative as

316 an indicator of "quality" in addition to the number of growing follicles.

317 **Study Limitation**

318 A noteworthy restraint of the current study was the lack of antral follicle count (AFC) at time of

319 oocyte collection.

320 **Conflict of Interest**

321 Authors declare no conflict of interest

322 **Authors Contribution**

323 [SU] and [JKA] contributed to the study conception and design. [SU] performed all experiments,

324 [SU], [KSBN] and [JKA] performed material preparation, data collection and analysis. The first

325 draft of the manuscript was written by [KSBN] and all authors commented on previous versions

326 of the manuscript. All authors read and approved the final manuscript.

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Number of patients associated with pregnancies in relevant age groups

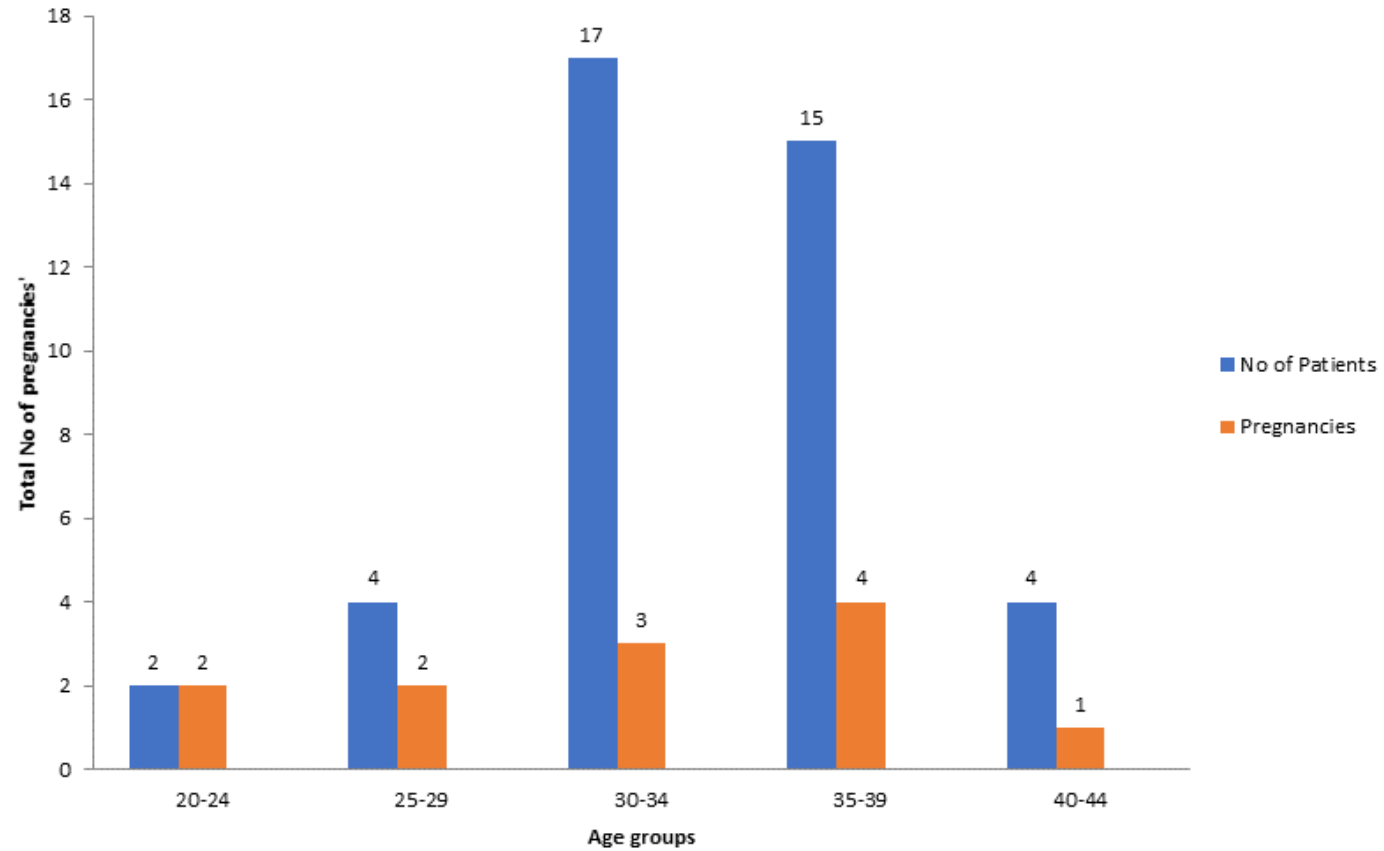
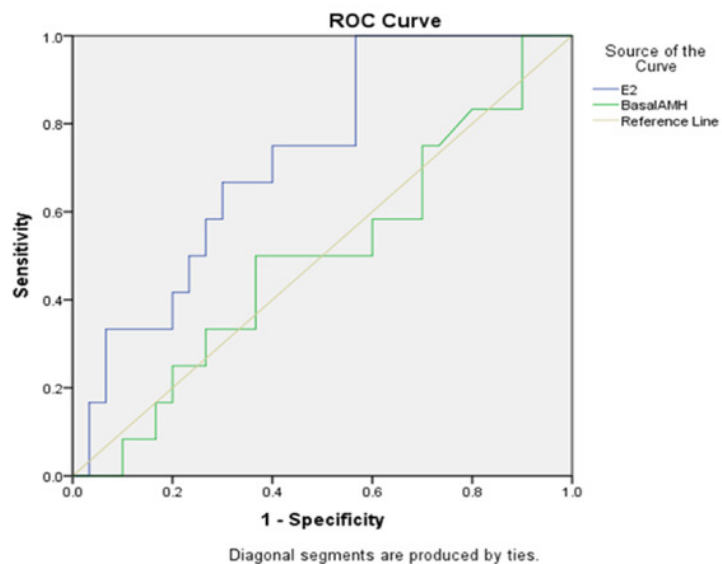



Figure 2

Sensitivity and specificity of E_2 and AMH in predicting pregnancy.

AMH distribution in blood samples

6   Table 1. AMH distribution in blood samples

Category	AMH Blood Level Concentration	Frequency	% Patients
High (often PCOS)	≥ 3.0 ng/ml	22	52.4
Normal	≥ 1.0 ng/ml	17	40.5
Low Normal Range	$\leq 0.3 - 0.9$ ng/ml	3	7.1

Correlation between AMH and number of oocytes collected, matured, and fertilised during stimulation

4   Table 2. Correlation between AMH and number of oocytes collected, matured, and fertilised
5   during stimulation

		Total no. of oocytes collected	Total no. of oocytes matured	Total no. of oocytes fertilized	% oocytes collected	% oocytes matured	% oocytes fertilized
AMH category	High	81	62	69	65.4%	60%	61.6%
	Normal	38	36	38	30.6%	35%	33.9%
	Low to Normal	5	5	5	4.0%	5%	4.5%
Total		124	103	112	100%	100%	100%

Chi Square analysis of number of embryos transferred and AMH

4   Table 3. Chi Square analysis of number of embryos transferred and AMH

	Value	Degrees of Freedom (df)	Asymptotic Significance (2-sided)
Pearson Chi Square	6.384[a]	4	.172
Likelihood Ratio	7.001	4	.136
N of Valid Cases	42		

6   *a.   7 cells (77.8%) have expected count less than 5. The minimum expected count is 0.08*

Chi Square analysis of AMH and day of embryo transfer

5 Table 4. Chi Square analysis of AMH and day of embryo transfer.

	Value	Degrees of Freedom (df)	Asymptotic Significance (2-sided)
Pearson Chi Square	14.117[a]	6	0.028
Likelihood Ratio	6.432	6	0.377
N of Valid Cases	42		

6   *a. 10 cells (83.3%) have expected count less than 5. The minimum expected count is 0.05*

Pregnancy outcome and AMH category

6   Table 5. Pregnancy outcome and AMH category

AMH Category	Pregnancy Result		
	Negative	Positive	Total
High	16	6	22
Normal	11	6	17
Low Normal	3	0	3
Total	30	12	42

Pearson correlation between basal AMH and E_2, Age and FSH and oocytes

5   Table 6. Pearson correlation between basal AMH and E_2, Age and FSH and oocytes

		E_2	Age	FSH	Number of oocytes	Number of mature oocytes	Number of oocytes fertilized
AMH	Pearson correlation coefficient	0.151	-0.028	-0.185	0.206	0.164	0.228
	Significance value (2-tailed)	0.341	0.859	0.240	0.191	0.300	0.146
	No. in the sample	42	42	42	42	42	42
Age	Pearson Correlation			-0.087	-0.271		
	Significance value (2-tailed)			0.583	0.082		
	No. in the sample			42	42		

Logistic regression analysis of the variables for the prediction of pregnancy

3   **Table 7.** Logistic regression analysis of the variables for the prediction of pregnancy

	B	Standard error (S.E.)	Wald	Degree of Freedom (df)	p-value	OR = Exp (B)
E_2	.001	.000	3.396	1	.065	1.001
LH	-.556	.273	4.144	1	.042	.574
Basal AMH	-.335	.239	1.967	1	.161	.715
Age	-.146	.091	2.593	1	.107	.864
Non-Fertilized	.150	.368	.166	1	.683	1.162
Basal FSH	-.102	.136	.559	1	.455	.903
Constant	4.451	3.452	1.663	1	.197	85.744

6   *E_2, Estrogen; LH, luteinizing hormone; AMH, anti-Mullerian hormone; FSH, follicle stimulating*
7   *hormone. LH has p = 0.042 (p < 0.05) and E_2 has p = 0.065, which is significant at a 10 % level.*
8   *In this logistic regression model, the remaining variables are not significant (p-values>0.10).*

Chi Square analysis of Number of oocytes fertilized and AMH

5   Table 8: Chi Square analysis of Number of oocytes collected and AMH.

	Value	Degrees of Freedom (df)	Asymptotic Significance (2-sided)
Pearson Chi Square	21.246[a]	8	.007
Likelihood Ratio	21.317	8	.006
N of Valid Cases	42		

7   *[a]13 cells (86.7%) have expected count less than 5. The minimum expected count is 0.12*

Chi Square analysis of Number of oocytes collected and AMH

5   Table 9. Chi Square analysis of Number of oocytes fertilized and AMH.

	Value	Degrees of Freedom (df)	Asymptotic Significance (2-sided)
Pearson Chi Square	18.504[a]	12	.101
Likelihood Ratio	17.736	12	.124
N of Valid Cases	42		

7   [a]*18 cells (85.7%) have expected count less than 5. The minimum expected count is 0.02*