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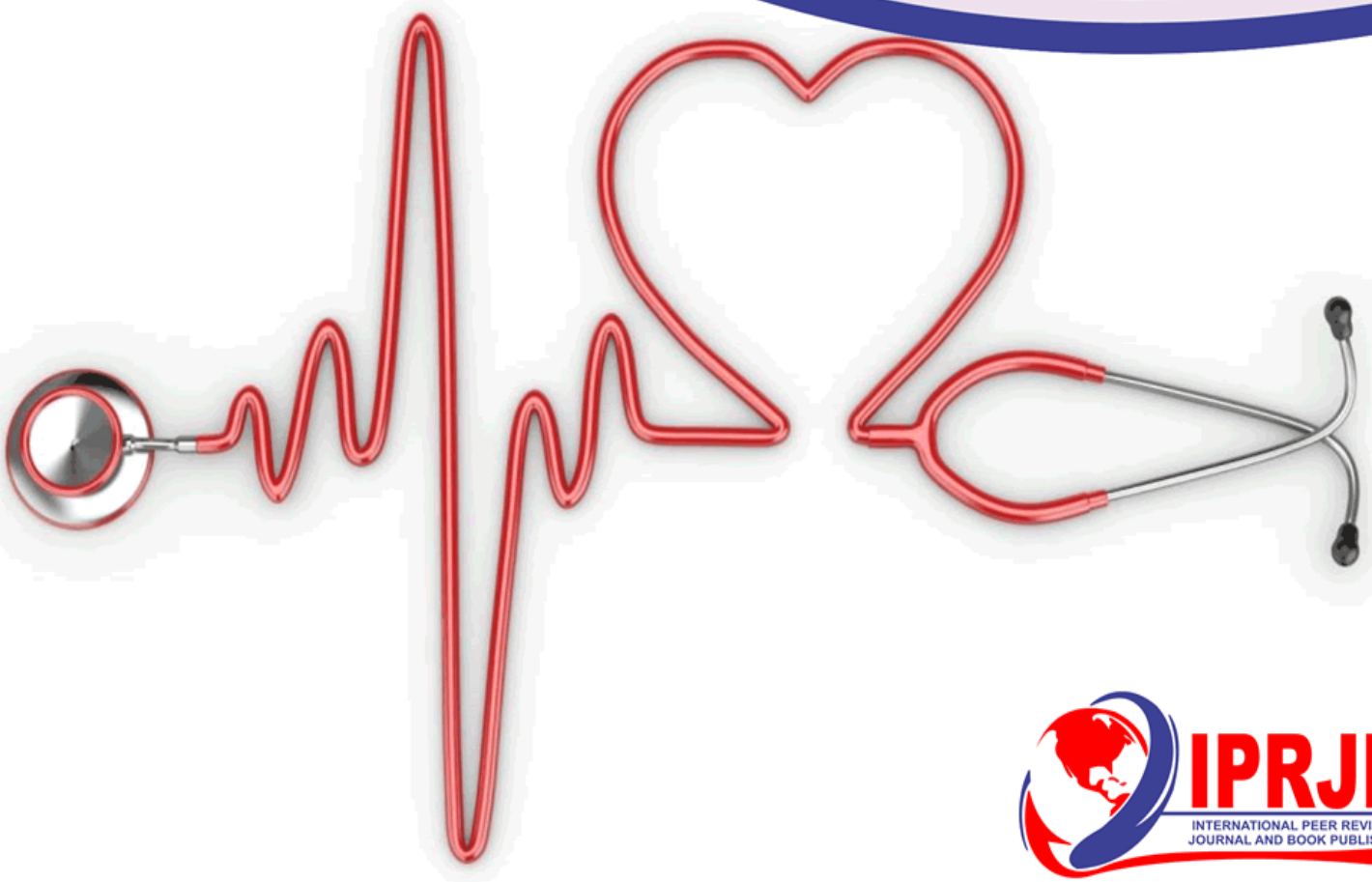

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SERUM AMH, INHIBIN B, FSH AND ESTRADIOL PROFILE IN WOMEN SEEKING CONCEPTION THROUGH *IN VITRO* FERTILIZATION IN NIGERIA

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Abstract

Purpose: This study was designed to evaluate the predictive ability of AMH, inhibin B, Follicle stimulating hormone and estradiol as biomarkers of ovarian reserve in assisted reproductive technology.

Methodology: Thirty (30) female partners of infertile couples seeking assisted reproductive technology through in vitro fertilization (IVF) and twenty seven (27) fertile women who are oocyte donors were recruited from Life Life Hospital, Lily Hospitals and Shepherd Fertility Hospital in Southern Nigeria from April to November 2017 and evaluated for AMH, inhibin B, estradiol and FSH using Enzyme-linked Immunosorbent assay technique. Subjects were divided into four groups (A,B,C,D) on the basis of age. A: Oocyte donors < 23 years; B: Oocyte donors ≥ 23years; C: Female partners of infertile couples <35years; D: Female partners of infertile couples ≥35years. Blood sample was collected from each participant on the day 3 of their menstrual cycle.

Findings: Mean AMH serum levels for female partners of infertile couples and oocyte donor were 4.26±1.7ng/ml and 5.13±1.12ng/ml respectively (p=0.04); mean FSH serum levels for female partners of infertile couples and oocyte donor were 8.64 ±3.40mIU/ml and 6.37±1.48mIU/ml, respectively (p=0.02); mean inhibin B serum levels for female partners of infertile couples and oocyte donors were 60.57±13.99 pg/ml and 77.52±12.33pg/ml respectively (p=0.00); mean estradiol serum levels for female partners of infertile couples and oocyte donors were 69.38±18.83pg/ml and 44.06±8.70pg/ml respectively (p=0.00).

Unique contribution to theory, practice and policy: AMH has a stronger predictive power for ovarian reserve function than FSH only in women who are 35 years and above.

Keywords: *Ovarian Reserve, Anti-Mullerian Hormone, Follicle Stimulating Hormone, Inhibin B, Estradiol.*

1.0 INTRODUCTION

Ovarian reserve is directly connected with reproductive capacity and it is largely determined by female age. Assisted reproduction has focused more on ovarian reserve function with the aim of improving minimal risk of ovarian hyperstimulation syndrome.[1] A range of prognostic biochemical markers of ovarian reserve include follicle stimulating hormone (FSH), estradiol (E_2), inhibin B and Anti-Mullerian hormone (AMH).[2,3] Follicular growth is modulated by AMH, which inhibits recruitment of follicles from primordial pool by modifying the FSH sensitivity of the follicles.[4,5] AMH reflects the non-FSH dependent follicular growth.[6,7] AMH have been shown to be a highly reliable marker for measuring ovarian reserve because they are not affected by gonadotropin, and exhibit minimal variability within or among menstrual cycles.[8,9,10] However, Inhibin B, on the other hand, is released by the granulosa cells of the follicle[3] and is involved in the regulatory functions in developing follicle.[11]

2.0 MATERIALS AND METHODS

2.1 Subjects

This study was carried out among thirty (30) female partners of infertile couples and twenty seven (27) women who are egg oocyte donors serving as controls in two selected clinics; Lily Hospitals, Warri and Shepherd Specialist Hospital, Warri, Southern Nigeria. Subjects were divided into four groups on the basis of age. Group A: Oocyte donors below 23 years of age (between the ages of 18 and 22); Group B : Oocyte donors 23years of age and above (between the ages of 23 and 25); Group C: Female partners of infertile couples below 35years of age (between the ages of 29 and 34); Group D: Female partners of infertile couples 35years of age and above (between the ages of 35 and 44). Only female partners of infertile couples (most of who have presented with 1^o infertility) with regular menstrual cycle and about to commence IVF treatment were recruited as test subject and single ladies who are oocyte donors were recruited as controls for this study.

2.2 Collection of Blood Samples

5ml of whole blood was collected into plane tubes on day 3 of the menstrual cycle of all the participants. Sample was allowed to clot and retract and then spun at 4000rpm for 4mins and serum harvested and stored at -20^{o}C until the day of analysis. Biomarkers of ovarian reserve determined in this study include Anti-Mullerian Hormone (AMH), Inhibin B, Follicle Stimulating Hormone (FSH) and Estradiol.

2.3 Exclusion Criteria

Exclusion was based on infertile women who met the criteria of recruitment but had additional health challenges such as hypertension, diabetic, metabolic syndromes and other chronic infections as retrieved from their case file. Women who are surrogates (carrying pregnancy for other women without using their own oocytes) were also excluded from this study.

2.4 Ethical Approval

Ethical approval was sought from the Central Ethics Committee of the Nnamdi Azikiwe University. Reference number: NAU/CEC/STU/EXT/003

2.5 Research Grant

Grant for this research was received from Nigeria's TETFund institution based research fund; reference number: TETFUND/DESS/NAU/AWKA/RP/Vol VIII.

2.6 Stimulation Protocol

Ovarian stimulation protocol used was either the long GnRH agonist protocol [12] the short GnRH antagonist protocol,[13] or the minimal stimulation protocols[14] as described by researchers in reproductive medicine.

2.7 Oocyte Retrieval

Oocyte retrieval was done by the reproductive physician, using ultrasound-controlled fine-needle aspiration[15].

2.8 Methods of Analysis

Follicle Stimulating Hormone was assayed by quantitative sandwich immunoassay using ELISA kits manufactured by CALIBIOTECH Inc., 1935 Corell Ct., El Cajon, CA 92020.[16] Estradiol was assayed by competitive binding immunoassay using ELISA kits manufactured by CALIBIOTECH Inc., 1935 Corell Ct., El Cajon, CA 92020.[17] Inhibin B was assayed by competitive binding immunoassay using ELISA kits manufactured by RayBiotech Inc., 3607 Parkway Lane, Suite 100, Nircross, GA 30092.[18] Anti-Mullerian Hormone was assayed by Sandwich-ELISA using kits manufactured by Elabscience, 14780 Memorial Drive, Suite 216, Houston, Texas 77079.[19] All assays were done at Lahor Research and Diagnostic Laboratory, Benin City. The laboratory is accredited by the Medical Laboratory Science Council of Nigeria (MLSCN) as well as Ministry of Health for both routine and research assays.

2.9 Statistical Analysis

The test statistics used include the independent Student's t-test to compare mean value, ANOVA with LSD post hoc to compare means between groups and Pearson's Correlation Analysis for the determination of association between variables.

3.0 FINDINGS AND DISCUSSIONS

Results from 30 female partners of infertile couples seeking IVF treatment and 27 oocyte donors were analyzed. The mean age for female partners of infertile couples and oocyte donors were 37 ± 5 and 22 ± 2 years respectively; the mean AMH serum levels for female partners of infertile couples and *oocyte donor* were 4.26 ± 1.7 ng/ml and 5.13 ± 1.12 ng/ml respectively ($p = 0.04$); the mean FSH serum levels for female partners of infertile couples and oocyte *donor* were 8.64 ± 3.40 mIU/ml and 6.37 ± 1.48 mIU/ml, respectively ($p = 0.02$); the mean inhibin B serum levels for female partners of infertile couples and oocyte donors were 60.57 ± 13.99 pg/ml and 77.52 ± 12.33 pg/ml respectively ($p = 0.00$); the mean estradiol serum levels for female partners of infertile couples and oocyte donors were 69.38 ± 18.83 pg/ml and 44.06 ± 8.70 pg/ml respectively ($p = 0.00$) [See table I].

TABLE I: Mean ± SD blood levels of FSH, AMH, Inhibin B and Estradiol in female partners of infertile couples seeking IVF treatment (test) and oocyte donors

	Oocyte donor n = 27	Test n = 30	t-value	p-value
FSH (mIU/ml)	6.37 ± 1.48	8.64 ± 3.4	- 3.210	0.02*
AMH (ng/ml)	5.13 ± 1.12	4.26 ± 1.17	- 2.984	0.04*
INHIBIN B (pg/ml)	77.52 ± 12.33	60.57 ± 13.99	4.826	0.00*
ESTRADIOL (pg/ml)	44.06 ± 8.7	69.38 ± 18.83	- 6.399	0.00*

Key

* Significant.

IVF = *in vitro* fertilization

AMH = Anti-Mullerian Hormone

FSH = Follicle Stimulating Hormone

On ANOVA, there was significant difference between the mean value of FSH, AMH, inhibin B and estradiol among oocyte donors and female partners of infertile couples (p=0.001, p=0.005, p=0.000 and p=0.000 respectively). On post HOC analysis of FSH, there was significant difference between the mean values of FSH, AMH, Inhibin B and Estradiol of oocyte donors under 23years and female partners of infertile couples who are 35years and above (p=0.000, p=0.002, p=0.000, p=0.000 respectively). There was also significant difference between the mean values of FSH, AMH, Inhibin B and Estradiol of oocyte donors who are 23years and above and female partners of infertile couples who are 35years and above (p=0.004, p=0.004, p=0.000, p=0.000 respectively). Also, there was significant difference between the mean values of FSH, AMH and Inhibin B of female partners of infertile couples who are below 35 years and those who are 35years and above (p=0.015, p=0.028, p=0.018 respectively) [see table II].

TABLE II: Mean ±SD blood levels of FSH, AMH, Inhibin B and Estradiol in female partners of infertile couples seeking IVF treatment (test) and oocyte donors with regard to age groups.

Groups	n	FSH mIU/ml	AMH ng/ml	INHIBIN B pg/ml	ESTRADIOL pg/ml
Grp 1 (Donor)	19	6.32 ± 1.62	5.05 ±1.1	74.70 ± 12.21	43.09 ± 7.72
Grp 2 (Donor)	8	6.45 ± 1.16	5.30 ± 0.80	84.18 ± 10.46	46.35 ± 10.92
Grp 3 (Test)	11	7.24 ± 3.06	4.86 ± 1.03	68.20 ± 14.49	66.88 ± 24.10
Grp 4 (Test)	19	9.62 ± 3.18	3.95 ± 1.15	56.84 ± 11.70	70.23 ± 15.15
F value		6.37	4.72	11.63	13.40
p value		0.001*	0.005*	0.000*	0.000*
1 vs 2		0.919	0.586	0.073	0.607
1 vs 3		0.350	0.644	0.160	0.000*
1 vs 4		0.000*	0.002*	0.000*	0.000*
2 vs 3		0.502	0.385	0.007*	0.005*
2 vs 4		0.004*	0.004*	0.000*	0.000*
3 vs 4		0.015*	0.028*	0.018*	0.557

Key

*Significant

Grp 1 – Oocyte donors between 18 and 22 years

Grp 2 – Oocyte donors between 23 and 25 years

Grp 3 - Female partners of infertile couples seeking IVF treatment between 29 and 34 years

Grp 4 - Female partners of infertile couples seeking IVF treatment between 35 and 44 years
IVF = *in vitro* fertilization
AMH = Anti-Mullerian Hormone
FSH = Follicle Stimulating Hormone

On chi square analysis of FSH and AMH values using a 2 by 2 contingency table, there was significant difference among female partners of infertile couples who are 35 years and above (p=0.014) [see table III]. Also, on chi square analysis of FSH and inhibin B values using a 2 by 2 contingency table, there was significant difference among female partners of infertile couples who are 35 years and above (p=0.004) [see table IV].

Table III: Comparability of FSH and AMH values in assessing ovarian reserve functions using their reference intervals for Female Partners of Infertile Couples who are between 35 and 44 years.

		AMH			
		Below RI	Within RI	Above RI	
FSH	Below RI	0	11	0	
	Within RI	0	5	0	
	Above RI	3	0	0	
TOTAL		3	16	0	19

$X^2 = 5.989$, $p = 0.014$

Key
RI = Reference Interval
FSH = 2.0 – 10.0 mIU/ml (Reference interval)
AMH = 2.5 – 6.9ng/ml (Reference interval)

Table IV: Comparability of FSH and inhibin B values in assessing ovarian reserve functions using their reference intervals for female partners of infertile couples between 35 and 44 years.

		Inhibin B			
FSH		Below RI	Within RI	Above RI	
	Below RI	0	0	0	
	Within RI	0	11	0	
	Above RI	4	4	0	
TOTAL		4	15	0	19

$X^2 = 8.466$, $p = 0.004$

Key
RI = Reference Interval
FSH = 2.0 – 10.0 mIU/ml (Reference interval)
Inhibin B = 45 – 139pg/ml (Inhibin reference interval)

There was no significant correlation between FSH and AMH values among oocyte donors, but there was significant negative correlation between values of FSH and AMH among female partners of infertile couples when analyzed together (p=0.000) as well analyzed as when analyzed separately as subgroups (p=0.018 for these who are 35 and above; p=0.030 for those who are below 35 years) [see table V]. Among all recruited participants, there was significant correlation between Age and blood levels of FSH, AMH, inhibin B, estradiol (p= 0.000, p=0.000, p=0.000, p=0.000 respectively) [see table VI]. Also among all recruited participants, there was significant correlation

between number of oocytes retrieved and blood levels of FSH, AMH, inhibin B, estradiol (p= 0.004, p=0.000, p=0.000, p=0.000 respectively) [see table VII].

Table V: Correlation of blood levels of FSH, AMH, Inhibin B and Estradiol among all female partners of infertile couples seeking IVF treatment of infertility

	n	R	p-value
FSH vs AMH	30	-0.619	0.000*
FSH vs Inhibin B	30	-0.240	0.205
FSH vs Estradiol	30	-0.044	0.816
AMH vs Inhibin B	30	0.299	0.109
AMH vs Estradiol	30	-0.209	0.268
Inhibin B vs Estradiol	30	-0.369	0.045*
	Group D		
FSH vs AMH	19	-0.537	0.018*
FSH vs Inhibin B	19	-0.046	0.853
FSH vs Estradiol	19	0.036	0.884
AMH vs Inhibin B	19	0.090	0.714
AMH vs Estradiol	19	-0.180	0.461
Inhibin B vs Estradiol	19	0.330	0.168
	Group C		
FSH vs AMH	11	-0.651	0.030*
FSH vs Inhibin B	11	-0.382	0.246
FSH vs Estradiol	11	-0.138	0.686
AMH vs Inhibin B	11	0.319	0.339
AMH vs Estradiol	11	-0.209	0.537
Inhibin B vs Estradiol	11	0.651	0.030*

* is significant

Key

FSH = Follicle stimulating hormone

AMH = Anti-Mullerian hormone

IVF = *in vitro* fertilization

Table VI: Correlation of blood levels of FSH, AMH, Inhibin B, Estradiol and Age

	n	R	p-value
AGE vs FSH	57	0.560	0.000*
AGE vs AMH	57	-0.445	0.001*
AGE vs Inhibin B	57	-0.609	0.000*
AGE vs Estradiol	57	0.592	0.000*
	Group A		
AGE vs FSH	19	0.224	0.357
AGE vs AMH	19	0.132	0.591
AGE vs Inhibin B	19	0.174	0.476
AGE vs Estradiol	19	-0.137	0.576
	Group B		
AGE vs FSH	8	-0.105	0.804
AGE vs AMH	8	-0.092	0.829
AGE vs Inhibin B	8	0.010	0.981
AGE vs Estradiol	8	-0.003	0.994
	Group C		
AGE vs FSH	11	0.250	0.459
AGE vs AMH	11	-0.067	0.846
AGE vs Inhibin B	11	-0.553	0.078
AGE vs Estradiol	11	-0.490	0.126
	Group D		
AGE vs FSH	19	0.513	0.025*
AGE vs AMH	19	-0.348	0.145
AGE vs Inhibin B	19	-0.462	0.046*
AGE vs Estradiol	19	0.061	0.804

* is significant

Key

FSH = Follicle stimulating hormone

AMH = Anti-Mullerian hormone

IVF = *in vitro* fertilization

Table VII: Correlation blood levels of FSH, AMH, Inhibin B, Estradiol and Oocyte retrieved among all participants

	n	R	p-value
OOCYTES vs FSH	57	-0.370	0.004*
OOCYTES vs AMH	57	0.581	0.000*
OOCYTES vs Inhibin B	57	0.587	0.000*
OOCYTES vs Estradiol	57	-0.531	0.000*
	Group A		
OOCYTES vs FSH	19	0.284	0.239
OOCYTES vs AMH	19	0.483	0.035*
OOCYTES vs Inhibin B	19	0.567	0.011*
OOCYTES vs Estradiol	19	-.380	0.109
	Group B		
OOCYTES vs FSH	8	0.011	0.979
OOCYTES vs AMH	8	0.529	0.177
OOCYTES vs Inhibin B	8	-0.508	0.199
OOCYTES vs Estradiol	8	-.585	0.128
	Group C		
OOCYTES vs FSH	11	-0.803	0.003*
OOCYTES vs AMH	11	0.662	0.026*
OOCYTES vs Inhibin B	11	0.627	0.039*
OOCYTES vs Estradiol	11	0.336	0.313
	Group D		
OOCYTES vs FSH	19	-0.338	0.100
OOCYTES vs AMH	19	0.643	0.003*
OOCYTES vs Inhibin B	19	0.187	0.444
OOCYTES vs Estradiol	19	0.156	0.523

* is significant

Key

FSH = Follicle stimulating hormone

AMH = Anti-Mullerian hormone

IVF = *in vitro* fertilization

4.0 SUMMARY AND CONCLUSIONS

4.1 Discussions

This study used serum follicle stimulating hormone (FSH), Anti-Mullerian hormone (AMH), Inhibin B and Estradiol to evaluate the ovarian reserve function of female partners of infertile couples seeking IVF treatment. The expected value for follicular phase FSH is 2.0 – 10.0mIU/ml, the expected value for AMH is 2.5 – 6.9ng/ml; the expected value for estradiol in follicular phase is 30 – 100pg/ml, the expected value for inhibin B is 45 – 139pg/ml

This study revealed that FSH was higher and AMH lower in female partners of infertile couples seeking IVF treatment (older women) when compared with oocyte donors (younger women). This implies that FSH level becomes higher in the early follicular phase as a woman ages with concomitant reduction in circulating AMH levels as a woman ages.

This study has also demonstrated significantly lower number of retrieved oocytes in women with high levels of FSH and significantly higher number of oocytes retrieved in women with high serum levels of AMH among oocyte donors and female partners of infertile couples seeking IVF treatment. This finding is in line with earlier reports of researchers that FSH levels in women increases with age, resulting in a decline in fertility potential. [20,21] This indicates that high day 3 serum FSH levels as well as low circulating levels of AMH can be good indicators of poor ovarian reserve. This agrees with the outcome a study carried out by Ashrafi et al [22] who observed that women with high day 3 FSH levels had fewer aspirated oocytes and larger number of cancelled IVF cycles than women with lower FSH levels. AMH is secreted without dependence on other circulating hormones; hence, AMH is expressed at a constant level without alteration during the phases of the menstrual cycle. This makes AMH a very direct measure of ovarian reserve. Studies have also demonstrated that serum AMH with normal levels (ranging from 2.5ng/ml to 6.9ng/ml) is highly predictive of good ovarian reserve, hence, the conclusion that AMH is a good biochemical marker for the assessment of ovarian reserve function. [23,24,25] This study has also revealed that there is no difference between FSH and AMH with regards to the power of prediction of ovarian reserve function among younger women implying that FSH and AMH are both good predictors of ovarian reserve function in women who are less than 35 years old. On However, the study revealed that AMH gives a stronger prediction of ovarian reserve function over FSH in women who are 35years and above affording the physician the opportunity to determine the stimulation protocol to use in the bid to getting good result of ovarian stimulation as well as positive pregnancy outcome for any form of assisted reproductive technologies.

This study revealed that the number of oocytes retrieved was high with high levels of serum inhibin B and vice versa as observed both in oocyte donors and in female partners of infertile couples. Inhibin B levels was also shown to decline with increase in age in women. It can be deduced from these findings that inhibin B can be a marker of good ovarian reserve because, similar to AMH, inhibin B is a granulosa cell specific hormone secreted by developing follicles; hence, high levels of circulating inhibin B can be an indicator of folliculogenesis. This finding is corroborated by previous studies that concluded that circulating levels of inhibin B are reduced when the pool of recruitable follicles become reduced with age, stating that circulating inhibin levels positively correlates with good ovarian reserve and vice versa.[26.27] However, Corson et al[27] observed and concluded from their research that inhibin B has no clinical value in the assessment of ovarian reserve. On chi square analysis of FSH and inhibin B among female partners of infertile couples using a 2 by 2 contingency table, inhibin B was shown to be a better predictor of ovarian reserve function than FSH. However, some researchers have concluded that inhibin B should not be considered as a marker for ovarian reserve due to its intracycle and intercycle variation.[29,30]

This study shows fewer numbers of retrieved oocytes with high level of day-3 serum estradiol as well as decline in estradiol in older women. Estradiol also shows negative correlation with circulating AMH levels. An early follicular phase estradiol level above normal early in the menstrual cycle suggests that follicular development is in an advanced stage that is inappropriate for day 3.[31] Evidence suggests that elevated early follicular estradiol levels are associated with poorer prognosis.[32] However, estradiol levels can be increased for two very different reasons: due to the occurrence of rapid folliculogenesis as in women with polycystic ovarian syndrome (PCOS), where estradiol is being secreted by a large number of antral follicles.[33] Estradiol has earlier been classified as an indirect marker of ovarian reserve. However, other studies have failed to prove its importance in predicting ovarian reserve as similar day 3 estradiol levels have been demonstrated in serum of both good and poor responders to ovarian stimulation in IVF cycles.[34]

4.2 Conclusions

The study concludes that AMH is only a stronger predictor for ovarian reserve function in women who are 35 years and above. Hence, the power of prediction of ovarian reserve function by FSH in women younger than 35 years is not different from that of AMH. Also, inhibin B increases alongside increase in AMH in younger women, but not in older women. The study also shows that there is no difference between estradiol in younger and older women seeking IVF treatment. The female partners of infertile couples responded well to stimulation as evidenced by the oocyte production, although the response drops with age. This is an indication that the female partners recruited in this study may not be the primary cause of infertility of the couples.

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