Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 10 (4): 75-86, 2015
ISSN 1819-3587 / DOI: 10.3923/tmr.2015.75.86

Effect of Basal Gonadotropins, Prolactin and Anthropometry as Predictive Markers of Ovarian Response in Patients Seeking Assisted Reproduction

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ABSTRACT

Assisted reproduction is expensive, time-consuming and stressful for patients. The accurate determination of ovarian reserve is important for this procedure. Basal hormonal levels may help to predict ovarian reserve after stimulation. The aim of this study was to assess basal hormonal markers; Luteinizing Hormones (LH), Follicle stimulating (FSH), prolactin and anthropometric indices as predictive markers of ovarian response in patients seeking assisted reproduction. A total of 104 subjects were recruited at the Lister Hospital Fertility Centre in Accra-Ghana for this study. Anthropometric parameters: Body Mass Index (BMI) and Waist-to-Hip Ratio (WHR) were measured. Lifestyle features; exercise and smoking patterns were assessed from a questionnaire. Blood samples were drawn on second day of their menstrual cycle in the month prior to the *in vitro* fertilization (IVF) procedure and basal luteinizing hormones, FSH and prolactin assayed by ELISA method. Subjects who yielded four or less oocytes were classified as poor responders whereas those who yielded more than 4 oocytes were termed as normal responders. Basal FSH and age (12.9±0.51 IU L^{-1} and 40±0.54 years) were significantly ($p<0.01$) higher in poor responders than normal responders 9.7±0.61 IU L^{-1} and 36±0.42 years, respectively. Increasing age, (42.44±0.47 years), high basal FSH (13.41±0.91 IU L^{-1}) and high FSH/LH ratio (2.74±0.64) significantly but negatively correlated with retrieved oocyte (7.28±2.03) and ovarian capacity (8.44±1.16) when compared to low age (28.08±0.65 years), normal FSH (7.64±1.21 IU L^{-1}) and FSH/LH (1.23±0.16) with retrieved oocyte (20.31±3.30) and ovarian capacity (20.92±2.63), respectively. Subjects engaged in moderate to high forms of exercise recorded a normal response. Number of retrieved oocytes and ovarian capacity were negatively correlated to WHR and BMI. Basal FSH and FSH/LH ratio better predicted response of subjects after ovarian stimulation. Obesity negatively impacted on ovarian response.

Key words: Antral follicle count, ovarian reserve, ovarian response

INTRODUCTION

The decline in female fecundity with age is reported to be associated with decreased oocytes quality and quantity in older women (Faber *et al*., 1997). In women of over 35 years of age, diminished oocyte quality is associated with decreased chances of a pregnancy and an increased risk of spontaneous abortions (Scott *et al*., 1995).

Obesity has been reported as a risk factor for lower fertility because of its social and possibly biological effect on reproduction. Studies have shown an association between obesity and reduced fertility (Pasquali *et al.*, 2003) and lower success rates for obese patients undergoing IVF treatment. In a recent study, body weight was found to predict the number of children that a person had and that obese and underweight women were less likely to have children than their normal-weight counterparts (Jokela *et al.*, 2008). Overweight and obesity in men is associated with decreased testosterone and Sex Hormone Binding Globulin (SHBG) levels, increased estradiol levels and, in severely obese men, changes in gonadotropin secretion resulting in decreased fertility (Chavarro *et al.*, 2010).

Several endocrinological conditions such as hyperprolactinaemia, polycystic ovarian syndrome and adrenal hyperandrogenaemia, high basal levels of plasma progesterone and FSH have also been implicated in the decline in female fecundity (Sherman *et al*., 1976; Lee *et al*., 1988) and the reason why some women are unable to achieve a pregnancy despite regular sexual intercourse with a normal male. Polycystic ovary syndrome is one of the most common causes of female infertility anovulation, (Hull, 1987) early pregnancy loss (Homburg *et al*., 1988) and later pregnancy complications (Boomsma *et al*., 2006). Women with this syndrome have hyperandrogenism, (Stein and Leventhal, 1935) and elevated levels of circulating luteinizing hormone (Rebar *et al*., 1976).

Low levels of inhibin B and Anti-Mullerian Hormone (AMH) in advancing age have similar effects (Seifer *et al*., 2002; Silberstein *et al*., 2006). In IVF treatment, patients with low basal FSH levels had higher pregnancy rates per attempt than those with moderate or high FSH levels. Also an increased FSH level on cycle day 3 is associated with reduced ovarian response to gonadotropin stimulation and a decreased IVF outcome (Barnhart and Osheroff, 1998; Fasouliotis *et al*., 2000).

In order to obtain several embryos for replacement in IVF and to improve chances of success: (Waterstone *et al*., 1991; Callahan *et al*., 1994) require the stimulation, growth and maturation of several follicles. Follicle development is induced by daily administration of FSH, or Gonadotropin-Releasing Hormone (GnRH) analogues for the suppression of the pituitary which significantly decrease the incidence of premature luteinizing hormone surge (Felberbaum *et al*., 1996) and improves the chances of IVF success.

Early follicular phase level of basal FSH (Creus *et al*., 2000; Eldar-Geva *et al*., 2005), inhibin B (Corson *et al*., 1999), Antral Follicle Count (AFC) (Chang *et al*., 1998; Hsieh *et al*., 2001) and ovarian volume (Hendriks *et al*., 2005) have all been shown to correlate with ovarian response in IVF patients. However, some studies concluded that basal FSH is not useful in the prediction of IVF outcome (Eldar-Geva *et al*., 2005). Also basal LH and estradiol values have not improved the predictive value beyond that provided by FSH (Venetis *et al*., 2007; Fleming, 2008). Therefore women requiring assisted conception may need to know the chances of them achieving a pregnancy and this require several clinical tests for a diagnostic conclusion. The development of several screening tests has made it possible to provide a reliable assessment of the ovarian reserve and the prediction of gonadotropin stimulation response (Nahum *et al*., 2001). The result of an IVF treatment depends on ovarian response to hormonal stimulation. Poor ovarian response is associated with high cancellation rate, reduced number of oocytes and low pregnancy rate (Pellicer *et al*., 1987).

This study is aimed at assessing plasma basal hormonal levels of LH, FSH and prolactin and anthropometric indices as predictive markers of IVF success.

MATERIALS AND METHODS

A total of 104 subjects, age between 21-50 years seeking IVF treatment were selected for this study at Lister Hospital and Fertility Centre in Accra-Ghana. With infertility rate of 11.8%, (Geelhoed *et al*., 2002) and a precision of ±0.07, the needed sample size was calculated to be 82. Subjects undergoing donor IVF programs were excluded. The control subjects of 50, age range between 22-43 years consisted of women who had delivered without assisted conception. Subjects who were selected for this study, all did so willingly. A written informed consent form was completed by all the participants who were recruited into the study after the study was explained in a language they understood. All procedures were approved by the Committee on Human Research Publication and Ethics of School of Medical Sciences, KNUST (CHRPE/Student/113/09).

Sample collection: About 3-5 mL of blood was drawn from overnight fasted subjects and dispensed into serum separator tubes. Blood samples were taken on the second day of their menses in the month prior to the oocyte stimulation regimen. Samples were spun in a centrifuge at 8000 rpm for 5 min to yield serum. The serum was used to assay for the basal levels of LH, FSH and prolactin on the same day using the statfax 2200 semi-automated analyzer from Awareness Technologies (Palm City, Florida, USA).

Anthropometry: Anthropometric measurements included weight and height. Subjects stood upright on the weighing scale (Hospibrand ZT-120, England) without shoes and their weight taken. Heights were measured (to the nearest 1.0 cm), with the subject standing in an erect position against a vertical scale of a stadiometer (Fischer Scientific) and an L-square placed on the head and the head positioned so, that the top of the external auditory meatus was in level with the inferior margin of the bony orbit. The Body Mass Index (BMI) was then calculated. The waist circumference measurements were taken from the middle point between the iliac crest and the last rib, as recommended by the World Health Organization. Hip circumference was measured as the maximal circumference over the buttocks.

Estimation of fertility hormones: The NoviWell™ assay kits (HySkill Diagnostics, Bahlingen, Germany) were used. The kits were developed based on 'The Enzyme-linked immunosorbent' assay sandwich method for prolactin, (PRL) LH and FSH. The principle underlying the assay is the simultaneous binding of the hormone to two monoclonal antibodies; an immobilized one on a microplate and the other, a soluble one conjugated with Horse Radish Peroxidase (HRP). Briefly, 50 μL aliquots of standards and samples were dispensed into their respective wells in ready-to-use microtitre plates pre-coated with the corresponding hormones' anti-hormone IgG antibodies. After the addition of 100 μL of anti-hormone-HRP conjugate to each well, the plates were incubated for 60 min at room temperature in the dark. The contents of the wells were then decanted and the wells washed three times with 300 μL of distilled water. The enzyme reaction was started by the addition of 100 μL chromogen (tetramethylbenzidine/hydrogen peroxide system) or substrate into each well. The microtitre plates were then incubated for 15 min at room temperature. The reaction was terminated by the addition of 100 μL of 0.15 M Sulphuric acid (H_2SO_4). The end-point colour developed was directly proportional to the concentration of hormone. Absorbance was measured at 450 nm and the concentrations calculated from a standard curve generated using standards of known concentrations in a Stat Fax 2200 Plus Microplate Reader (Awareness Technology

Incorporated, Palm City, Florida, USA). Within-assays coefficient of variations were 6.1% for FSH and PRL and 5.4% for LH, The analytic sensitivities of the assays were 1.0 mIU mL^{-1} for FSH, LH and 1.0 ng mL^{-1} for PRL, as indicated by the manufacturer. The manufacturer's instructions for the performance of the assays were rigorously followed. Cut-off values for b-FSH and LH levels, measured on cycle days 2 or 3, were considered as basal FSH ≤10 mU mL^{-1} and basal LH ≤12 mU mL^{-1}.

Ovarian stimulation: In this study, one of two different oocyte stimulation protocols were followed depending on the age and basal FSH levels of the subjects measured on day 2 of menses. Subjects with high basal FSH levels, the 'long follicular protocol' was used as follows; On the 2nd or 3rd day of menses, they were started on D-Ser(But)6]-LH-RH(1-9) ethylamide (burserelin, Hoe 766), 50 ng day^{-1} injection subcutaneously for about 2-3 weeks and trans-vaginal scans done in between. The scans were done to ensure that the endometrium was thin enough and that there were no cysts formed. If cysts were formed, the protocol was discontinued or the cyst drained and regular menses allowed to flow. However, if there were no cysts, 450 IU of recombinant human FSH (r-hFSH or 139 urinary human FSH (u-hFSH HP, 150 IU day^{-1} was administered subcutaneously, follitropin alpha (Gonal) F was given in addition to the *burselin* injection for about 10 days. Trans-vaginal scans were done on the 8th and 10th day post FSH stimulation. Human chorionic gonadotropin (HCG, 10 000 IU), was administered subcutaneously once there was more than one follicle 18 mm in diameter and two others ≥16 mm. Oocyte retrieval was performed 36-38 h after HCG injection. The 'short protocol' was performed on subjects with normal basal FSH levels or below the age of 35 years. In this protocol, triptorelin (decapeptyl) injection was administered on the 21st day of menstrual cycle. On day 2 of the next cycle, 175-300 IU of FSH or Gonal F injection was administered for 10 days. Trans-vaginal scans were performed on the 8th and 10th day post stimulation. The 10,000 IU of hCG injection was given on the 11th day and egg collection done 36 h after the hCG injection.

All follicles 2-10 mm in size were also considered antral follicles during the scan on the 8th and 10th day post stimulation, counted and labeled as the ovarian capacity or Antral Follicle Count (AFC) for the purpose of the study.

In both protocols luteal phase progesterone support, (cyclogest) pessaries were administered after egg collection and continued till up to about 3 months if pregnancy occurs. This was done to prepare and maintain the endometrium ready for implantation of the embryo. Luteal phase endometrial support helps to maintain endometrial integrity to prevent spontaneous miscarriages once implantation occurs.

Oocyte retrieval: Matured oocytes were collected 36 h after the hCG injection was administered. Ultrasound Directed Follicle Aspiration (UDFA) technique was used with a 7.5 MHz transducer vaginal probe. The retrieved oocytes were analyzed for quality and quantity and placed in the incubator at 37°C. Subjects who yielded 4 or less oocytes were classified as poor responders whereas those who yielded more than 4 oocytes were termed as normal responders. The retrieved oocytes were analyzed for maturity. Conventional *in vitro* fertilization (IVF) or Intra-Cytoplasmic Sperm Injection (ICSI) was performed with the corresponding partner's sperms. Embryo culture was performed in a 6% CO_2 incubator at 37°C. Embryo transfers were performed on either day 2 or day 3 post oocyte retrieval.

Data analysis: Results were expressed as Mean±SEM. Data were analyzed by one-way ANOVA followed by the Bonferroni test for multiple comparison using Graph Pad Prism version 4 (Graph Pad Software, San Diego California). Unpaired Student t-tests were used to assess for significance. Statistical significance was set at p-values ≤0.05 for the various parameters in the study. A linear regression and multivariate regression analyses, was done to find predictors of ovarian capacity from the various parameters.

RESULTS

Figure 1 expresses the gonadotropin levels between the subjects and the controls. Basal FSH was significantly higher in the subjects than the control. There were no significant changes in basal LH and PRL between the subjects and the controls.

In Table 1, subjects were grouped into 20-30, 31-40 and 41-50 years on the bases that fecundity is an age factor. The mean age of the 41-50, group (42.44±0.47) was obviously significantly higher than the subject mean age (36.25±047). Also subjects in the 41-50 age range had significantly higher ($p<0.01$) duration of infertility, waist circumference and WHR. However, FSH, ovarian capacity and oocytes retrieved were significantly reduced ($p<0.001$). There were no significant changes in the BMI, height, weight, LH and prolactin levels across the age groups.

When subjects were classified on the basis of their BMI and WHR, the overweight and obese BMI: 25-29.9 and >30, respectively had significantly reduced ovarian capacity and

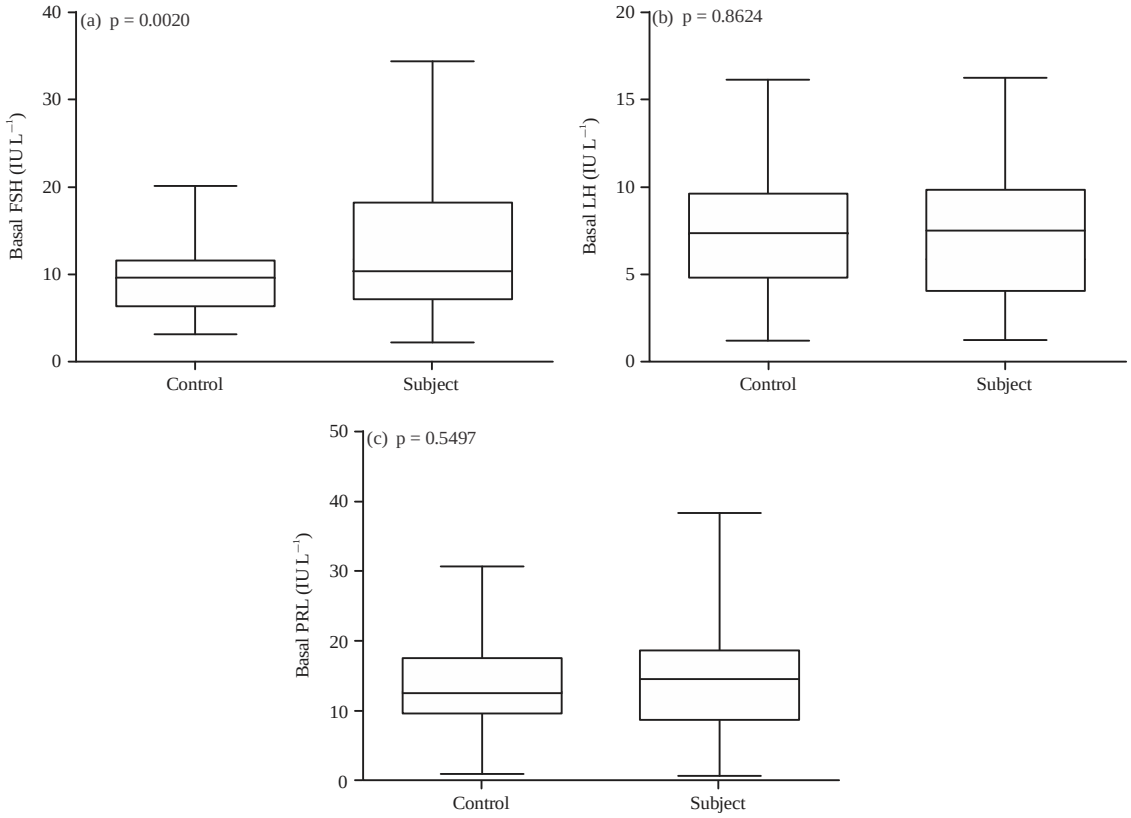

Fig. 1(a-c): A Basal gonadotropin level in subjects and the control, (a) Basal FSH levels, (b) Basal LH levels and (c) Basal PRL levels

Table 1: Characteristics of whole study population

	Age stratification			
Characteristics	21-30	31-40	41-50	All
Number (n)	13	73	18	104
Age (years)	28.08±0.65	36.18±0.35	42.44±0.47***	36.25±0.47
Duration of infertility (months)	52.23±11.37	74.59±1.71	117.30±13.22^{ns}	79.19±4.61
WC (CM)	80.01±2.50	87.50±0.95	85.00±2.08^{ns}	85.00±0.90
HC (CM)	102.50±3.00	110.20±1.10	102.50±2.10**	107.00±0.98
WHR	0.79±0.01	0.79±0.0	0.82±0.01*	0.79±0.0
Weight (kg)	69.00±1.6	71.00±0.98	73.00±1.7^{ns}	71.00±0.78
Height (m)	1.60±0.02	1.60±0.01	1.60±0.01^{ns}	1.60±0.01
BMI (kg m^{-2})	25.00±0.76	27.00±0.40	28.00±0.70^{ns}	27.00±0.33
LH (IU L^{-1})	6.76±0.87	7.42±0.43	6.99±0.93^{ns}	7.26±0.36
FSH (IU L^{-1})	7.64±1.21	12.21±1.24	13.41±0.91*	12.48±0.71
Prolactin (ng mL^{-1})	13.97±1.48	13.55±0.81	13.98±1.96^{ns}	13.68±0.68
FSH/LH	1.23±0.16	2.23±0.20	2.74±0.64^{ns}	2.19±0.18
Ovarian capacity/follicle count	20.92±2.63	13.62±0.96	8.44±1.16***	13.63±0.83
Retrieved oocytes	20.31±3.30	11.99±1.04	7.28±2.03***	12.21±0.96

Data are presented as Mean±SEM or number (n) by count, BMI: Body mass index, WHR: Waist-to-hip ration, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, *p<0.05, **p<0.001, ***p<0.0001, One way ANOVA was performed

Table 2: Effects of obesity on measured outcomes of ovarian stimulation

	BMI				WHR			
Weight	Grade	% (n)	Ovarian capacity	Retrieved oocytes	Grade	% (n)	Ovarian capacity	Retrieved oocytes
Normal	18.5-24.9	26.0 (27)	20.0±1.9	16.0±2.0	<0.80	51.9 (54)	17.0±1.2	15.0±1.4
Overweight	25.0-29.9	61.5 (64)	13.0±0.9*	12.0±1.2*	0.80-0.85	40.4 (42)	11.0±1.1	10.0±1.4
Obese	>30	12.5 (13)	5.8±0.7**	5.1±1.1**	>0.85	7.7 (8)	9.5±1.8	7.0±1.6

Data are presented as percentages (number by count) or Mean±SEM, *p<0.05, **p<0.001

Table 3: Basal gonadotropins and other parameters in predicting ovarian response

Variables	Total	Normal responders	Poor responders	p-value
Number	104	81	23	
Age (years)	37(23.00-48.00)	36 (23.00-48.00)	40 (28.00-43.00)	<0.01
Dur. of infertility (months)	72(7.000-240.0)	72 (7.000-180.0)	84 (12.00-240.0)	0.0761
Primary infertility (n)	84	76.2 (64)	23.8 (20)	
Secondary infertility (n)	20	85.0 (17)	15.0 (3)	
Anthropometry				
BMI	26.5 (20.1-44.4)	26.5 (20.1-44.4)	26.9 (23.5-36.1)	0.754
WHR	0.79 (0.69-0.93)	0.80 (0.69-0.93)	0.79 (0.70-0.87)	0.3829
Basal markers				
LH (IU L^{-1})	7.3 (1.2-16.3)	7.3 (1.2-16.3)	7.0 (1.2-14.9)	0.9469
FSH (IU L^{-1})	10.6 (2.0-34.4)	9.7 (2.0-34.4)	12.9 (6.4-33.9)	<0.05
Prolactin	13.5 (0.9-38.4)	13.3 (0.9-30.7)	13.9 (2.7-38.4)	0.5835
FSH/LH	1.62 (0.35-11.36)	1.5 (0.35-11.36)	2.39 (0.75-7.83)	<0.01
Pregnancy				
Positive β-hCG (% (n))	31.7 (33)	35.8 (29)	17.4 (4)	

Data are presented as median (range) or percentage (number by count), Mann: Whitney test was performed

retrieved oocytes. Also subjects with WHR significantly higher than 0.85 (normal) had significantly reduced ovarian capacity and retrieved oocytes (Table 2).

Eighty four subjects had primary infertility whilst 20 were diagnosed with secondary infertility. For those diagnosed with primary infertility 76.2% were normal responders whilst 23.8% poor responders. Out of the 20 subjects with secondary infertility 85% were normal responders and 15% poor responders. There were no significant changes in BMI and WHR in terms of normal or poor responders. Poor responders had significantly higher FSH and FSH/LH ratio ($p<0.01$) (Table 3).

High FSH was associated with older subjects. There was a significant reduction in AFC and retrieved oocytes ($p<0.001$ and $p<0.001$), respectively in subject with high FSH. FSH did not significantly change pregnancy rate (Table 4).

Table 4: Outcomes of subjects with high and normal FSH levels

Subjects	Normal FSH	High FSH	p-value
Number (n)	48.00	56.00	
Age (years)	35.10±0.79	37.23±0.52	<0.05
AFC	16.71±1.34	11.00±0.91	<0.001
Retrieved oocytes	16.17±1.60	8.82±0.95	<0.0001
Positive β-hCG (% (n))	17.00 (38.6%)	16.00 (30.8%)	

Data are presented as Mean±SEM

Table 5: Multivariate regression analysis of anthropometry and outcome of ovarian stimulation

	BMI		WHR		WC	
	r^2	p-value	r^2	p-value	r^2	p-value
No. of retrieved oocytes	0.1201	0.0003	0.0723	0.0058	0.0023	0.6296
No. of follicle count	0.2478	<0.0001	0.1342	0.0001	0.0303	0.0774

Statistically significant: p<0.05

DISCUSSION

The mean age of the women seeking IVF treatment at the Lister Hospital was 36.25±0.47 (Table 1). Seventy percent of the subjects were in the age range of 31-40 years. Similar mean ages have been reported (Van Noord-Zaadstra *et al*., 1991). It is usually within this age that a lot of women, particularly, those with primary infertility become desperate and therefore seek for assisted reproductive technique. The least number, 13 were aged range 21-30 years. This is also in agreement with the reports that fecundity decreases with age (Frank *et al*., 1994), hence very few women have conception difficulties at this early age range. Expectedly, the number of subjects were fewer 18 in the older age range 41-50 possibly because many patients in this age group have given up on the chances of making babies knowing very well the chances dwindle with age and therefore reluctant to take chances on a very expensive procedure.

Anthropometry: The subject population had a mean Waist Circumference (WC) of 85±0.9 cm. There was no significant difference in the WC across the age ranges (Table 1). The mean Hip Circumference (HC) (102.50±2.10 cm) was significantly lower in the 41-50 age groups. The waist and hip circumferences were not significantly different from that of the normal women population in Ghana; 82.50 and 100.00 cm, respectively (Owiredu *et al*., 2008). The BMI change was not significant across all the age ranges. WHR was significantly ($p<0.001$) negatively correlated with retrieved oocytes and ovarian capacity, similarly BMI was also significantly ($p<0.001$) negatively correlated with retrieved oocytes and ovarian follicular count (Table 5). High WHR is an index of central adiposity. Women with PCOS, central adiposity may have higher oestrone concentrations compared with women with a peripheral fat distribution (Pasquali *et al*., 1994) and high oestrone concentration is associated with reduction in fertility/ovarian response. Measured outcomes of ovarian stimulation, number of retrieved oocytes and ovarian capacity were significantly ($p<0.001$) negatively correlated to both obesity indices, WHR and BMI. Correlation coefficient of $r = -0.37$ and -0.50, respectively for ovarian capacity and retrieved oocytes correlation coefficient of $r = -0.28$ and -0.34, respectively. This implies, increases in BMI and WHR results in the reduction of retrieved oocyte and ovarian capacity. This is evident from the multivariate regression analysis (Table 5) that BMI and WHR are better predictors of the outcomes of ovarian stimulation than WC.

Obesity and fertility: Obesity is reported to reduce fertility in women both in natural cycles and infertility treatment cycles. Several investigators have shown a decrease in pregnancy and live birth rates in overweight and obese compared with normal weight women (Fedorcsak *et al*., 2004; Sneed *et al*., 2008), Indeed obese women requiring IVF treatment require increased gonadotropin

during ovarian stimulation (Wittemer *et al*., 2000; Metwally *et al*., 2008). Higher rates of miscarriage (Fedorcsak *et al*., 2004) and congenital anomalies (Stothard *et al*., 2009; Aune *et al*., 2014) have also been reported in obese women. These assertions have been supported by other findings. Clark *et al*. (1995) reported that even a small weight loss in an ovulatory obese infertile women, resulted in an improvement in ovulation.

This study confirms some of these findings, the ovarian capacity of the overweight and obese subjects (13.0±0.9, 5.8±0.7, respectively) were significantly lower than that of the normal weight (20.0±1.9) (Table 2). Similarly, significantly lower oocytes were retrieved from the overweight and obese subjects (12.0±1.2 and 5.1±1.1) respectively than that of the normal weight (16.0±2.0). Fedorcsak *et al*. (2000) similarly observed that, obese patients had fewer retrieved oocytes and that low oocyte numbers was associated with an increase in the risk of abortion than among lean patients. Obesity is an independent risk factor for early pregnancy loss which is, in partly, related to the lower number of oocytes (Fedorcsak *et al*., 2000). Obesity, particularly with PCOS may have severe hyperandrogenism and lower SHBG and therefore impaired ovarian function (Pasquali *et al*., 2003).

Gonadotropins: Metabolic defects of the endocrine system resulting in conditions such as hyperprolactinaemia, PCOS and adrenal hyperandrogenaemia, high basal levels of plasma progersterone and FSH, have been shown to have negative fertility effects in both male and female patients (Sherman *et al*., 1976; Seifer *et al*., 2002). In normogonadotrophic women, studies have shown that even low amounts of LH can induce normal follicle and oocyte development (Chappel and Howles, 1991). However, elevated follicular phase LH is associated with reduced fertility and increased risk of miscarriage (Regan *et al*., 1990). The success of IVF depends on adequate follicle recruitment either by the appropriately suppression or stimulation of the gonads, which have been successfully used in IVF treatment. Whilst LH and Prolactin levels were not significantly changed across the age ranges, FSH was significantly higher in the 41-50 age groups and this tallied with significantly reduced ovarian capacity and retrieved oocytes compared to the younger age group (Table 1). Similar results have also been observed by Hansen *et al*. (2005).

The number of large follicles developing after stimulation was higher in younger subjects than older subjects.

When the subjects were classified into poor or normal responders according to the quantity of retrieved oocytes, considering that subjects with 4 or less oocytes as poor responders and those with more than 4 oocytes as normal (Table 3). The study did not show any significant difference ($p = 0.9469$) in the level of basal LH and prolactin (7.3 IU L^{-1} and 7.0 ng mL^{-1}, respectively) (Fig. 1b and c) between poor responders and good responders. However, the FSH levels were significantly increased ($p<0.05$) in the poor responders (9.7 and 12.9 IU L^{-1}) (Fig. 1a). The FSH/LH ratio was significantly higher ($p<0.01$) in the poor responders (Table 3). Similar results have been reported by Shrim *et al*. (2006). Basal cycle high FSH/LH ratio was associated with poor follicular development and oocyte quality, however a high FSH/LH ratio was a better early biomarker of poor ovarian response (Morgante, 2014).

Follicle stimulating hormones: Basal FSH of the subjects were statistically significantly higher than the control ($p<0.001$) and negatively correlated with the number of retrieved oocytes and ovarian capacity (Fig. 1a) ($r = -0.37$ and -0.41, respectively). Early follicular phase basal FSH levels (Creus *et al*., 2000), in some studies, measuring Antral Follicle Count (AFC) (Chang *et al*., 1998) and ovarian volume (Frattarelli *et al*., 2000) were shown to be correlated with ovarian response in

Table 6: Multivariate regression analysis of basal gonadotropins and outcome of ovarian stimulation

	LH		FSH		FSH/LH		Prolactin	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
Retrieved oocytes	0.003614	0.5444	0.1391	<0.0001	0.0522	0.0197	0.002216	0.6351
No. of follicle count	0.001076	0.741	0.1664	<0.0001	0.0717	0.006	0.000257	0.8717

Statistically significant: $p<0.05$

IVF patients. FSH/LH ratio was significantly increased ($p<0.01$), FSH/LH ratio also showed a negative correlation to the number of oocytes and ovarian capacity with correlation coefficients r = -0.23 and -0.27, respectively. There were however no significant changes in LH and Prolactin.

This implies basal level of plasma FSH before stimulation was predictive of ovarian response. High FSH (12.9 IU L^{-1}) was associated with older subjects (37.23±0.52 years), low AFC (11.00±0.91), lower retrieved oocyte (8.82±0.95) (Table 4). Subjects with normal plasma FSH showed a significantly higher response ($p<0.01$), i.e., higher AFC (16.71±1.34) and retrieved oocyte (16.17±1.60) ($p<0.001$) and lower FSH was also associated with younger age. Indeed FSH stimulation outcome for IVF correlated significantly with the number the of oocytes retrieved (Yong *et al*., 2003). The multivariate regression analysis (Table 6) reveals that basal FSH level is better predictor of ovarian response, followed by FSH/LH ratio, whilst independent levels of prolactin and LH, poorly predicts ovarian response. However, chemical pregnancy rate (positive β-HCGn of 38.6 and 30.8%, respectively) were not significantly different between subjects with normal or higher FSH levels. Similar results showed that patients with low basal FSH levels had higher pregnancy rates per attempt than those with moderate levels and both of which were higher than those with high FSH levels. It was therefore concluded that basal LH and estradiol values did not improve the predictive value beyond that provided by FSH (Venetis *et al*., 2007).

CONCLUSION

Basal level of plasma FSH before stimulation is predictive of ovarian response. Normal level of FSH was associated with increased ovarian capacity and retrieved oocyte. High basal FSH and older subjects was associated with fewer retrieved and poor quality retrieved oocyte and poor ovarian capacity. Comparatively, basal FSH was a better predictor of ovarian response, followed by FSH/LH ratio, whilst independent levels of prolactin and LH, poorly predicts ovarian response. On the basis of achieving a pregnancy, having a normal basal FSH does not have any significant advantage over those with high basal FSH. It is therefore inferred that in the Ghanaian population, patients with higher than normal basal FSH should not be used as the sole basis to encourage patients to undergo a donated oocyte program. It is therefore important that such patients are advised of the significant impact that high basal FSH has on measured outcomes such as retrieved oocytes and ovarian capacity.

ACKNOWLEDGMENT

The authors are grateful to the staff and management of Lister hospital for their assistance and for making the facilities available for this work.

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