

# Prevalence Elevated Day3fsh/LH Ratio, Prolactin Variation and Central Obesity With Menstrual Irregularities Among Primary Infertile Women in Babylon Province

Noran Jameel Ibraheem

College of science for women, university of Babylon

E.mail:noranjameel@gmail.com

## Abstract

**Background:** Infertility in women is a complex problem should be considered carefully by the physicians in order to find effective intervention and solution to avoid the expenditure of the money and time. **Subjects and Methods:** In this study, 180 women with primary infertility aged 20-40 years were investigated who attended to infertility unit in maternity and pediatrics teaching hospital in Hilla city, province of Babylon, sixty fertile women with similar age were enrolled as the controls. Pituitary gonadotropic hormone were analyzed and FSH/LH ratio was calculated, anthropometric measures included body mass index (BMI), waist circumference (WC), and waist-hip ratio (WHR), menstrual cycle characteristics were self-reported. **Results:** Hormonal abnormalities involved follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) level and elevated FSH/LH ratio were more frequent in infertile women, the majority of infertile women had overweight in comparison with that fertile group, menstrual disorders mainly oligomenorrhea, second amenorrhea and polymenorrhea were reported in the majority of the infertile women. There was statistically non-significant difference ( $p > 0.05$ ) among those values of thyroid hormone among case group as comparison with those in control group. **Conclusion:** Present study revealed that women infertility maybe result from problems in ovarian reserve, disorder of menstruation and prolactin level abnormality as well as central obesity. **Key words:** premature ovarian failure (POF), FSH:LH ratio, pattern obesity, menstrual pattern.

## الخلاصة

الخلفية: يعد العقم من المشاكل المعقدة عند النساء وينبغي على الأطباء النظر إليها بعناية لايجاد الحل الفعال لها لتجنب انفاق الوقت والمال. المرضى وطرائق العمل: تم فحص 180 امرأة مصابة بعقم ابتدائي تراوحت اعمارهن بين 20-40 سنة حضروا الى وحدة العقم في مستشفى الولادة وطب الاولاد التعليمي في محافظة بابل-مدينة الحلة، كما شمل الفحص 60 امرأة معروفة الخصوبة وبعمر مماثل كمجموعة ضابطة. تم قياس الهرمونات القنطرية الموجهة للغدد التناسلية وتم احتساب نسبة الهرمون المحفز للحويصلات الى الهرمون اللبوتيني في اليوم الثالث من الطمث. كما تم قياس مؤشر كتلة الجسم ومحيط الخصر ومحيط الورك ونسبة محيط الخصر الى الورك والسؤال عن نمط الدورة الشهرية. النتائج: تضمنت النتائج زيادة في عدم انتظام مستوى الهرمون المحفز للجريب والهرمون اللبوتيني والبرولاكتين وارتفاع نسبة الهرمون المحفز للجريبات الى الهرمون اللبوتيني بين النساء العقيمات، كما رافقت غالبية النساء العقيمات زيادة في وزن الجسم مقارنة مع النساء في المجموعة الضابطة، كما سجلت النساء المصابات بالعقم اكثر نسبة في عدم انتظام الدورة الشهرية لديها، في حين لم تحصل فروق معنوية في قيم هرمونات الدرقية بين مجموعة النساء المصابات بالعقم مقارنة مع تلك الموجودة في المجموعة الضابطة. الاستنتاج: يمكن ان ينشا العقم من حدوث مشاكل في احتياطي المبيض مع اضطراب في الدورة الشهرية وفي مستوى البرولاكتين مع تزامن وجود السمنة المركزية في الجسم.

الكلمات المفتاحية: فشل احتياطي المبيض المبكر، نمط السمنة، نسبة الهرمون المحفز للجريبات: الهرمون اللبوتيني، نمط الدورة الشهرية.

## Introduction

Infertility is a global health issue affecting approximately 8-10% of couples (Roupa *et al.*, 2009), infertility is a growing concern, with arising number of couples having difficulty becoming pregnant (Chandra *et al.*, 2005). In most Iraqi traditions, fertility is seen as blessing of the gods, therefore anyone who is infertile is considered to be cursed and women tend to bear the brunt of the social stigma because their role in marriage is to give birth to children to their extremely important events in every human's life and are strongly associated with the ultimate goals of completeness, happiness and family integration (Roupa *et al.*, 2009).

Robert (2002) provides a thorough evaluation that can identify many problems related to hormone imbalances that are associated with infertility as thyroid function hormone such as triiodothyronine( $T_3$ ), tetraiodothyronine( $T_4$ ), thyroid stimulating hormone(TSH) and follicle stimulating hormone(FSH) and leutinizing hormone (LH) and prolactine hormone(PRL). Ganong (1999) states the reproductive system of women shows regular cyclic changes that may be regarded as periodic preparations for fertilization and pregnancy; this cycle is the menstrual cycle was dependent on the proper functioning of the chain made up of hypothalamus-pituitary-ovary and uterus, the reproductive activities of gonadal tissue are controlled by the gonadotropins hormones( FSH and LH) from the interior pituitary gland(Elain and Marieb, 2000), LH and FSH both are subjects to feedback loops regulation by ovarian hormone, LH and FSH regulate the function of the ovaries(Zilfa *et al.*, 1999), therefore; LH along with FSH are ordered as part of the work up of infertility and useful in the investigation of menstrual irregularities(MC Donough, 2003).

Other literature(Carina and Lobo, 1999) refer to, that follicle stimulating hormone(FSH), luteinizing hormone (LH), prolactin hormone(PRL) and thyroid hormone are required for normal development of ova and need to be investigated in case of chronic anovulation, oligomenorhea and secondary amenorrhea(cessation of menstruation in female who was previously menstruating), Kim *et al.*, (1997) refer to that both FSH and LH may contribute information relevant to ovarian reserve as the elevation in FSH level at early follicular phase represent a standard clinical marker of reduced ovarian reserve and diminished responsiveness of the ovary to ovulation induction(Toner *et al.*, 1991). in which a condition of Premature ovarian failure (POF) normal ovarian function discontinues in women younger than 40 years old, for most women the cause remain unknown but hereditary factors and autoimmunity may play a role in the etiology( Unuane *et al.*, 2011) where developing follicles produce estrogen which signals the hypothalamus to increase or reduce the amount of FSH and LH, if ovaries are not working properly, LH and FSH are still released as long as pituitary gland healthy, but levels not drop again, causing high levels of one or both hormone( Jensen *et al.*, 2011).

Poor ovarian reserve diagnosis is easily made by determining FSH (showing elevated levels) and the best prediction is obtained by using the combination of FSH and LH (as opposed to the FSH:LH ration) therefore elevated FSH:LH ratio with a raised or normal FSH through early follicular phase is helpful in the diagnosis of reduce ovarian reserve(Kim *et al.*, 1997).

Study abroad suggests that hormonal balance covering the hypothalamic-pituitary-gonadal axis is affected by an individual's obesity affecting fertility (Murizah and Robert, 2008).

Filer (2009) confirms since obesity and overweight is the most common conditions its impact on reproduction is significant, incidence of obesity has been increasing secondary to an ignorance society, sedentary lifestyle, and high calorie foods, menstrual irregularity given the rising prevalence of overweight and obesity as defined by height and weight (BMI) or as defined by waist-hip ratio(WHR).

Several studies have been suggested that centrally distributed body fat may be more strongly associated with menstrual abnormalities and adverse hormonal profile than measures of peripheral body fat or overall adiposity such as BMI (Douche, 2002; Hollmann *et al.*, 1997). This study aims to describe the clinical as well as hormonal profile of infertile women and their menstrual pattern, and evaluated the FSH:LH ratio in infertile women (case group) in comparison with FSH:LH ratio in the fertile women (control group) on days 2-3 of menstrual cycle, and calculated the BMI and WHR to examine how this distribution of these characteristics may contribute in reduce of fertility in order to address these factors while making management protocol for infertile women community.

## Subjects and Methods

This study was conducted on 240 women, age group (20-40)years ,180 of them infertile women on their visit to( infertility unite) at maternity and pediatrics teaching hospital in Hilla city, province of Babylon (from February 2012to March 2013) .While control group included 60 matched fertile women.

Initial assessment was carried out by asking the women about duration of infertility, age, and last menstrual period, usual duration of cycle. asking also included for stress, weight change, diet or exercise habits, and for symptoms that raise the suspicious of thyroid disorders, included: weight change, palpitations, dry skin, irritability, eye problem and symptoms related to the presence of goiter. Clinical examination about galactorrhoea also included (Unuane *et al.*,2011).

Aregular menstrual cycle between 21 to 35 days was called normal, prolonged cycle of more than 6 months was termed oligomenorrhoea while a cycle longer than 6 months called secondary amenorrhea, cycle shorter than 21 days was called polymenorrhoea (whitley,1999 ).

Obesity was calculated depending on WHO report (2000) according to body mass index, BMI was measured the subjects were categorized as follow : desirable weight 18.5-24.9,over weight 25-29.9 and obese when BMI was  $> 30 \text{ kg/m}^2$  , waist circumference (WC) was measured using non stretch measuring tape at the narrowest point between the lower costal border and the iliac crest. Hip circumference was measured at the level of the greater posterior protuberance of the buttocks, WC was categorized as  $\leq 70, 70-79.9, 80-87.9,$  and  $\geq 88\text{cm}$ , WHR was calculated by dividing waist by hip circumference and classified as  $\leq 0.72, 0.72-0.78,$  and  $\geq 0.79$ , central obesity was defined as  $\text{WC} \geq 88\text{cm}$  or  $\text{WHR} \geq 0.79$  . both LH and FSH were measured preferably on day 3 of the cycle, normal LH levels was 1.5-8mIU/ml, if the level of FSH was under 6 it was excellent, 6-9 good ,10-12 fair, as per kit supplier's instruction.

In general FSH was often used as a gauge of ovarian reserve (McClure and Thompson,1997), diminished ovarian reserve was considered at FSH levels of  $> 12\text{mIU/ml}$ .,the normal ratio of FSH:LH was usually close to 1:1 ,but if the ratio equivalent or more than 2.5 may indicate low ovarian reserve (Mukherjee *et al.*,1996).

Normal prolactin level was 3.8-23.2 ng/ml, more than 23.2 ng/ml was termed hyperprolactinemia, but less than 3.8 was termed hypoprolactinemia.The normal range for thyroid stimulating hormone (TSH) was taken as (0.25-5 mIU/ml),a high level with a low or normal  $T_4$  and  $T_3$  level indicated hypothyroidism while hyperthyroidism was indicated if the levels were vice versa as per kit supplier's instruction.

The hormone collected in the two- three days of the cycle, for women with secondary amenorrhea care was taken and that they had not taken any hormone or withdrawal bleeding for at least six weeks before the collective (whitley,1999), the hormonal assay was performed by MINIVIDAS, is an automated quantitative test for the determination of human hormone using the ELFA technique (enzyme linked fluorescent assay) method as per the instruction manual for kit (Biomerieux SA).

Data were reported as mean $\pm$  SD, with the levels of significance set at  $p < 0.05$  and student t-test followed by one way ANOVA ,(Mann Whitney U multiple comparison) test was used to assess significance for all data.

## The Results

Unadjusted comparison of the levels of various reproductive hormones between cases and controls is presented in table 1, the data obtained were collected from 240 women, who subdivided into two groups, case group consisted 180 were infertile women, fertile women were 60 women they were considered as control group.

The mean level of LH hormone for cases was  $5.1 \pm 6.9$  mIU/ml, while for control group was  $6.0 \pm 2.3$  mIU/ml ( $p=0.04$ ). the mean level of FSH hormone in infertile group was  $15.2 \pm 16.9$  mIU/ml but the control was  $7.6 \pm 1.9$  mIU/ml ( $p=0.006$ ). The mean of FSH/LH ratio for cases was found to be  $2.9 \pm 1.5$  compared to  $1.2 \pm 0.6$  in control group ( $p=0.008$ ). In addition, the infertile women had a mean level of

prolactin  $30.8 \pm 18.1$  ng/ml, which was greater than control group range  $20.9 \pm 7.3$  ng/ml ( $p=0.004$ ).

The remaining measured hormone, i.e., T3, T4, and thyroid-stimulating hormone (TSH), statistically were nonsignificantly different between cases and controls groups.

Although we showed the BMI within undesirable range in both study group (cases and control), but cases had significantly higher BMI than control group ( $28.9 \pm 4.1$  kg/m<sup>2</sup> vs.  $26.0 \pm 2.5$  kg/m<sup>2</sup>,  $p=0.02$ ) respectively.

As well as, infertile women showed elevated in their WHR as compared with control group ( $0.99 \pm 0.03$  vs  $0.76 \pm 0.06$ ,  $p=0.007$ ) respectively.

Table1: comparison of the characteristics of infertile women and control

Variables	subjects	NO.	Mean $\pm$ SD	Sig.p-value
LH mIU/ml	infertile	180	$5.1 \pm 2.3$	<b>0.04</b>
	control	60	$6.0 \pm 2.3$	
FSH mIU/ml	infertile	180	$15.2 \pm 7.9$	<b>0.006</b>
	control	60	$7.6 \pm 1.9$	
FSH/LH	infertile	180	$2.9 \pm 0.5$	<b>0.008</b>
	control	60	$1.2 \pm 0.6$	
T4 mIU/ml	infertile	180	$87.9 \pm 14.7$	<b>0.06</b>
	control	60	$85.1 \pm 12.1$	
T3 mIU/ml	infertile	180	$1.65 \pm 0.48$	<b>0.92</b>
	control	60	$1.65 \pm 0.47$	
TSH mIU/ml	Infertile	180	$2.4 \pm 0.2$	<b>0.07</b>
	Control	60	$1.9 \pm 0.2$	
PRL ng/ml	infertile	180	$30.8 \pm 10.1$	<b>0.004</b>
	Control	60	$20.9 \pm 7.3$	
BMI(kg/m <sup>2</sup> )	Infertile	180	$28.9 \pm 4.1$	<b>0.02</b>
	Control	60	$26.0 \pm 2.5$	
WHR	infertile	180	$0.99 \pm 0.03$	<b>0.007</b>
	Control	60	$0.76 \pm 0.06$	
Days of the cycle when was blood sampled	infertile	180	$2.6 \pm 0.6$	<b>0.9</b>
	control	60	$2.5 \pm 0.4$	

Significant at p- value  $<0.05$

Menstrual disturbances observed in women who participated in this study ,case and control groups were 65.6% and 28.3% respectively as revealed in table 2 ,but normal cycle mostly were reported 71.7% in control women, but minority were reported 34.4% in infertile women.

Table 2: Distribution of menstrual pattern in the control and case group

Groups	Regular mense		Irregular mense		Total NO. (Percent)%
	frequency	Percent%	Frequency	Percent%	
Control 60	43	71.7	17	28.3	60(100%)
Case 180	62	34.4	118	65.6	180(100%)

P<0.001 by chi-square test

Irregular cycle displayed three mode in case group like; secondary amenorrhea, oligomenorrhea and polymenorrhea, but the women in control group who had irregular mense had just two mode of period as ; oligomenorrhea and polymenorrhea. Infrequent period reported by 118 women in case group, composed of 34 women had secondary amenorrhea, while 56 women had oligominorrhea and women with polymenorrhea formed 28 women, whereas about 62 of infertile women had regular cycle, control group presented regular mense in about 43 women, while Infrequent period were just reported in 17 women.

As demonstrated in table 3, it was illustrated the detailed of the hormonal profile assay and other characteristics according to menstrual pattern, this enabled us to confirm the findings of

the unadjusted comparison showed in table 1, to reveal the variety of analyzed tests value owing to variation in pattern of menstrual cycle.

Significantly higher serum FSH levels were noted in the infertile women with regular menses, secondary amenorrhea, oligomenorrhea and polymenorrhea when their distributions were compared to resemble there in control group ( $p < 0.05$ , and  $p < 0.01$ , respectively).

There was decrease in LH level among all different group according to menstrual cycle pattern of infertile women as compared with control group but this decrease was statistically not significant. The mean FSH: LH ratio in women across the first 2-3 days of the menstrual cycle was significantly higher ( $p < 0.01$ ,  $p < 0.05$ ) across all cycle pattern in infertile women who had irregular menses when compared to the control group with menstrual abnormality also, as well as FSH: LH ratio increased in case women having regular mense as compared with control women who had regular period also, but this increase was statistically not significant. The infertile women who had regular period and who infected by secondary amenorrhea, oligomenorrhea and polymenorrhae had mean of prolactin level range ( $24.6 \pm 9.4$  ng/ml,  $32.6 \pm 41.5$  ng/ml,  $41.1 \pm 56.7$  ng/ml and  $23.5 \pm 5.4$  ng/ml) respectively, which was statistically higher in significance ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) than the control group which their prolactin value implied in normal range ( $3.8-23.2$  ng/ml) through all menstrual pattern. As exhibited in table 3, although the mean of BMI within the overweight range ( $25-29.9$  kg/m<sup>2</sup>) in both fertile and infertile women who infected with menstrual irregularity, but the mean of BMI ( $29.3, 28.2, 26.9$  kg/m<sup>2</sup>) was higher significantly ( $p < 0.05$ ) in infertile women who had secondary amenorrhea, oligomenorrhea and polymenorrhea respectively as compared with fertile women those defined as long and short cycle, while the women with normal period in both case and control group had desirable BMI ( $24.9 \pm 3.1$ ,  $24.9 \pm 1.99$  kg/m<sup>2</sup> respectively).

For infertile women those defined as having normal, long and short cycle were had WHR ( $0.80, 1.00, 0.99, 0.89$ ) respectively as compared to fertile women as they had regular and irregular mense were have ( $0.73, 0.78, 0.72$ ) respectively.

There was statistically no significant difference ( $p > 0.05$ ) among those values of thyroid hormone based on different in menstrual pattern among case and control group.

Table 3: Distribution of variables according to menstrual pattern in vase and control grup

Variables	Groups	Regular Case=62 Control=43	Amenorrhea Case=34 Control=0	Oligomenorrhea Case=56 Control=10	Polymenorrhea Case=28 Control=7
LH mIU/ml	Case=180	5.0±1.7	5.0±2.3	5.7±2.4	5.8±1.9
	Control=60	6.3±2.1		6.2±3.5	6.0±1.7
FSH mIU/ml	Case,180	11.1±1.6*	16.1±7.5**	18.1±8.1**	15.3±6.9*
	Control,60	7.8±1.7		7.2±2.1	7.4±2.7
FSH/LH ratio	Case,180	11.1±1.6*	3.2±1.4**	3.1±1.7**	2.6±1.3*
	Control,60	1.2±1.6		1.1±0.9	1.2±1.8
PRL ng/ml	Case,180	24.6±9.4*	32.6±15.5***	41.1±20.7***	23.5±10.4
	control	20.4±6.4		23.1±8.9	10.3±0.7
T4 mIU/ml	Case	90.0±13.9	86.5±15.5	85.7±14.2	88.4±25.6
	control	82.4±11.1		91.3±11.0	92.5±14.6
T3 mIU/ml	Case	1.6±0.4	1.6±0.5	1.6±0.5	1.8±0.6
	control	1.6±0.4		1.8±0.4	1.9±0.6
TSH mIU/ml	Case	2.3±1.4	2.5±1.7	2.6±1.3	2.3±1.3
	control	2.1±1.1		1.8±0.7	1.5±0.8
BMI (kg/m <sup>2</sup> )	Control	24.9±3.1	29.3±2.8*	28.2±10.1*	26.9±12.5*
	case	24.9±1.9		26.4±4.1	25.7±2.0
WHR	Control	0.80± 0.08	1.00± 0.34**	0.99± 0.04*	0.89 ±0.03*
	case	0.73± 0.12		0.78 ±0.32	0.72± 0.04

P\* < 0.05 and p < 0.01\*\* and p < 0.001\*\*\* in comparison of the distribution (Mann-Whitney U test) to the respective group in control.

To show the potentially confounding bias of prolactin level might be led to infertility, owing to the subjects of this study were further divided into three subgroups depending upon their mean serum prolactin concentrations as; hyperprolactinemic,

groups	Prolactin status and numbers of subjects	Prolactin concentrations Mean ± SD
Infertile women NO.=180	Hyperprolactinemia n=100	38.0± 3.7**
	Euprolactinemia n=65	23.2 ± 4.9
	Hypoprolactinemia n=15	2.5± 0.8*
Control women NO.=60	Hyperprolactinemia=15	25.5 ±1.6
	Euprolactinemia =35	22.5± 5.3
	Hypoprolactinemia n=10	3.6± 0.03

euprolactinemic and hypoprolactinemic as revealed in table 4.

Table 4: Distribution the mean concentration of prolactin among subjects of study P < 0.05\*, p < 0.01\*\* in comparison of the distribution (mann-whitney U test) to the respective group in controls

Prolactin levels in hyperprolactinemic subgroup ( $38.0 \pm 3.7$  ng/ml) were reported significantly highly increased ( $p < 0.01$ ) in case group as compared with prolactin level ( $25.5 \pm 1.6$  ng/ml) in hyperprolactinemic subgroup, those the fertile women, as well as the infertile women who infected with hypoprolactinemia had significantly lower of prolactin level ( $2.5 \pm 0.8$ ,  $p < 0.05$ ) than women those detected hypoprolactinemic ( $3.6 \pm 0.03$ ) in control group.

While normal range of prolactin level were found through euprolactinemic subgroup in both case and control group, and the difference between them statistically non-significant ( $p > 0.05$ ). While normal range of prolactin level were found through euprolactinemic subgroup in both case and control group, and the difference between them statistically non-significant ( $p > 0.05$ ).

## Discussion

As regards about LH level, there was significant reduction in the mean concentration of LH in infertile women, on the other hand table 1 also showed statistically highly significant level of FSH, and their FSH/ LH ratio relative to normal fertility women, this suggested the concentrations of FSH could fluctuate markedly as the ovarian sensitivity to gonadotropins varies (Mohan and Sultana, 2010) or might be resulted in an ovulatory cycle in these women perhaps due to variable in prolactin level or to diminished ovarian reserve in these infertile women, this was in agreement with earlier work (Mukherjee *et al.*, 1996) who reported that elevated day 3 FSH was predictive of a poor response to ovarian stimulation owing to low day 3 LH concentration, while the other study supposed the women showed sign of infertility as she had high or low level of FSH hormone (Norman, 2002).

As previous literatures had indicated, naturally ovarian reserve declined as women age, but some women experienced this decline earlier in life for unknown reason (Jensen *et al.*, 2011) as similar experience was found in our women study.

Highly level of prolactin was found in infertile women in comparison with the control group. This perhaps explained that promote excess of prolactin levels might be resulted from the delay of LH response to gonadotropin releasing hormone (GnRH) hence PRL concentration might be increased (Eniola *et al.*, 2012), these findings were in accordance with our results.

While another study suggested that hyperprolactinemia resulted from defect in the positive feedback of estrogen on LH and might caused LH or FSH suppression and hence interfering with the ovulation (Matsuzaki *et al.*, 1994) therefore, PRL seemed to play a critical role in human ovarian function due to the observation that hyperprolactinemia facilitated the modification of the luteal phase and menstrual cycle (Kauppila *et al.*, 1988), this might be in accordance with our observation about mense disturbance which was more frequent among infertile women as compared with control group.

After investigated the routine thyroid function testing in infertile women by measuring serum T3, T4, and TSH, all values were within normal range, this observation was in agreement with other previous studies (Agrawal and Kapoor, 1994; Singh *et al.*, 1990), and in a contrast with other studies which found that infertility resulted because of hypothyroidism or hyperthyroidism (Krassas *et al.*, 1999; Gerhard *et al.*, 1991) this was in discrepancy with those studies might be owing to other factors were possibly involved such as environmental and genetic differences, and the different habitual dietary of the women compared to women in our study. On the other hand, our study had some limitations, because limited only to women between 20-40 years in ages, wider range of age was more useful in gauging the distribution especially thyroid hormone influenced by age (Padubidri and daftary, 2003).

Body mass index (BMI) was a measurement that was associated with body fat. In our studied population, both cases and controls had higher than normal mean BMI ( $< 25$  kg/m<sup>2</sup>), which reflected the fact that obesity was a common finding in developing countries. This was

the result of a combination of reduced exercise, changes in dietary composition, and increased caloric intake (Heslehurst *et al.*,2007).

Many previous studies suggested that BMI influenced on the levels of reproductive hormones with some contradictory results, as reported by Rich-Edwards study(Rich-Edward *et al.*,1994).Although many multiparous women were obese, evidence found that obesity might affected fertility rates in women within fertile age and subsequent anovulatory infertility increased in women with increasing body mass index value. On the other hand Zaadstra *et al.*,in(1993) emphasised that the body fat distribution other than fat amount was associated with a decreasing chance of conception, this concept confirmed our results about WHR, that their mean more in infertile women than in fertile women. This might be related with central obesity as mention in previous research about the percentage of free testosterone fraction higher in women with central obesity than in those with peripheral obesity(Evans *et al.*,1983), therefore, condition of relative functional hyperandrogenism seemed to be associated with central obesity phenotype in women which would may play role in visceral fat enlargement in women(Pasquali *et al.*,1993).This maight interfered with many neuroendocrine and ovarian function, by reducing both ovulatory and fertility rates in otherwise healthy women.As a result this mechanism distributed the menstrual cycle in obese women and cause infertility(Hollmann *et al.*,1996).

Our observation about menstrual cycle changed including secondary amenorrhea, oligomenorrhea and polymenorrhea frequently more in infertile women whome were in agreement with other literature refered to menstrual cycle disturbance often provided a clue to problems such anovulation or ovarian failure( Norman,2002).

Our results recorded that secondary amenorrhea, oligomenorrhea , and polymenorrhea had main complaint elevated day 3 FSH with normal or low level of LH in case groups and followed by increase in FSH:LH ratio and became close to 3 value, our results agreed with other studies(Steeg *et al.*,2007;Toner *etal.*,1991). Those studies suggested that decreasing fecundity with increasing early follicular phase FSH represented standard clinical marker of reduced ovarian reserve and diminished responsiveness of the ovary to ovulation induction ,and possibility of impeding premature ovarian ageing and predictive of relative gonadotropin resistance(Waller *et al.*,1988; Steeg *et al.*,2007). The process of follicular depletion and decline in oocyte quality might be occurred, therefore proper menstrual cycle rarely observed with higher level of FSH and LH in infertile women(Mukherjee *et al.*,1996).

Previous literature agreed with our finding about whether primary ovarian insufficiency was afrequent disease (Unuane *et al.*,2011) ,for which this disease was being estimated in our study.

Our results of thyroid hormone agreed with literature mentioned that thyroid disorder in infertility patients had been discussed in many studies, but no consensus had been obtained(Akhter and Hassan,2009;Shalev *et al.*,1994) As similar, we found in our results that demonstrated thyroid hormone concentration within normal values though no clinical features of disease were present. But this was dissimilar with another studies(Sharma and Sharma,2012;farast *et al.*,2010). This might be owing to difference in habitual dietary salt intakes of our study that subjects compared to women in another studies, another explanation for these difference might be these irregularities in menstrual cycle sometimes precede the identification of thyroid dysfunction ( Mariuo *et al.*,2006).

It had long been recognized that extreme of weight-BMI more than 27 kg/m<sup>2</sup> associated with anovulation and consequent infertility, our result agreed with other studies where the patients had BMI greater, more frequently complained of prolonged cycle than control women ,this appeared to be associated with severe endocrine and metabolic abnormalities(Barbieri,2001;Cupisti *et al.*,2008). On the other hand, our results displayed that



fertile women with irregular menses also had undesirable BMI, the reproductive disturbances had influenced by the obesity particularly the abdominal phenotype ( Pasquali *et al.*,2003). Another previous research suggested that centrally distributed body fat might be more strongly associated with menstrual abnormalities and adverse hormonal profiles than measures of peripheral body fat or overall adiposity such as BMI,(Douchi *et al.*,2002;Hollmann *et al.*,1997). After investigated WHRs , the results revealed increased WHRs in infertile versus in control throughout menstrual cycle, this observation was in agreement with other literature indicated WHR of 0.7 for women showed strong correlated with fertility because they had optimal levels of estrogen and were less susceptible to ovarian dysfunction(Picon *et al.*,2007). Other investigator reported that a 0.1 unit increase in WHR decreased the probability of conception after adjustment of age, cycle length and regularity(Singh,1993). These finding agreed with current study that revealed women with upper body fat predominance defined by waist-hip ratio(WHR)>0.08 showed reduced fecundity compared with women with lower body fat predominance(WHR<0.73)

Our results suggested that the high levels of FSH and LH and their FSH/LH ratio were more frequent in women with higher of BMI and WHR. These finding agreed with previous studies that confirmed the selection of multiple growing follicles during ovarian stimulation with FSH required the serum FSH concentration to exceed a certain threshold, the threshold effect of FSH was lower among obese women, leading to a lower number of selected follicles, fewer collected oocytes and the need for higher concentration of FSH for stimulation(Bellver,2009). Furthermore, diminished ovarian reserve may be associated with obesity and just as the onset of menarche was earlier in obese women, so data suggested that onset of ovarian failure and increased production of FSH at menopause occurred several years earlier in obese than in normal weight women (Norman and Clark,1998).

This study was conducted to assess the role of prolactin concentration in maintain fertility as showed in table 4, the clinical implication expressed in hyperprolactinemia and hypoprolactinemia might associated with differences in reproductive function .

Our finding agreed about whether the level of circulating prolactin that involved in the regulation and maintain of normal reproductive function must be between 3-15ng/ml, when the levels were above or below this, associated with increased rate of infertility (Kauppila *et al.*,1988)

In published literature mentioning that elevation in the levels of prolactin might cause infertility in several different ways; prolactin might stop women from ovulating and women menstrual cycle would stop and secondary amenorrhea would be induced. In less severe case ,high prolactin might only disrupt ovulation. In this category experience might infrequent periods such as oligomenorrhea, women with the mildest case might ovulate regularly but do not produce enough of the hormone progesterone after ovulation, this known as a luteal phase defect which showed different PRL pulse amplitudes in the early follicular phase (Soules *et al.*,1991;Agarwal *et al.*,2010) were manifested by a short luteal phase in which the interval between LH peak and the onset of menses was  $\geq 10$  days thereby polymenorrhea might raising these explained according to as update internet study in 2013, as a result in shortening of the follicular phase, when gonadotropins were relatively high, and the corpus luteum phase flowed regularly shortening of the luteal phase ,this led to mense occurring faster than it should and this contributed in infertility .Another study explained this subject which disturbed pulsatile release of LH and prolactin interferes with the normal hypothalamic-pituitary-ovarian function .This could result in menstrual dysfunction ,ranging from inadequate corpus luteum progesterone secretion to oligomenorrhoea or secondary amenorrhea(Rossmann *et al.*,1988) .A similar response as our subjects might be occurring.

Although the physiological and clinical significance of hypoprolactinemia were not been studied extensively, but hypoprolactinemia reported adversely affect the luteal phase of menstrual cycle( Kauppila *et al.*,1988), as well as another studies indicated, prolactin might play stimulating role in oocyte maturation and the acquisition of development capacity, and found in the hypoprolactinemia cycle group the fertilization rate was significantly lower than normoprolactinemia (Oda *et al.*,1991).Other study indicated that menstrual disorders were associated with hypoprolactinemia in infertile women (Oseko *et al.*,1991), these finding were similar to our results.

Surprisingly, basal prolactin values in our results were relative increased and decreased in control women , this might be attributed up to prolactin that known to be a stress hormone, thus we interpreted the increased or decreased basal prolactin in the control women as stress-induced even drawing blood could sometimes itself cause someone to produce and immediate prolactin release, while infertility patients were more accustomed to medical examinations (Klibanski *et al.*,1984).However ,this relative prolactin values variation in control group might explain such clinically illogical situation found in the variable molecular heterogeneity of the peptide hormone,that the various forms were associated with varying bioactivity and immunoreactivity( Agarwa *et al.*,2010) .However our result were corresponding with another objects had been reported idiopathic hyperprolactinemia in mostly women with normal menstrual cycle and fertility( Egli *et al.*,1999).

Preliminary, the present data suggested a possible link between the body fat distribution, ovarian reserve and luteal defect and female infertility.

As a conclusion, this study provided important evidence that poor ovarian reserves may be used additionally in predictor for infertility in young women, also the present study indicated there was association between both overall and central obesity and prolactin level abnormality and menstrual cycle irregularity in young adult women , and body fat distribution played a critical role in human reproduction , as well as current study noted that thyroid function testing not as a routine measure in the infertile population .

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