The Association between Follicular Fluid Leptin, Insulin Resistance and ICSI Outcome in Women with Unexplained Infertility

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Abstract: Background: Causes of infertility cannot be present in 25% of the infertile patients. The idiopathic causes of infertility which usually occur after 1 year from doing all the standard investigations like tests for tubal patency, capability for normal ovulation, and analysis of semen , all these testes were normal but the pregnancy not occur in spite of protected intercourse. Various studies have been done to explore the etiology of infertility, one of the most effective and important factors for infertility the high concentration of leptin in infertile subjects as recorded in many studies. **Objective:** The purpose of this study was to investigate if follicular fluid leptin concentrations and insulin resistance are correlated with ICSI success. **Methods:** This was a prospective study of 34 women with unexplained infertility who underwent intracytoplasmic sperm injection (ICSI) in assisted reproductive technology unit of Ain Shams University Hospital during the period from August 2017 to August 2018. A fasting blood sample was withdrawn before the regimen of ovarian hyperstimulation . Serum glucose and insulin were measured to calculate insulin resistance using HOMA-IR. Controlled ovarian hyperstimulation was done using long luteal GnRHa protocol. At the time of oocyte retrieval, bloodless aspirated follicular fluid samples were obtained via puncture of the dominant ovarian follicles (18-22 mm) in diameter. Following oocyte isolation, the human follicular fluid was collected, centrifuged and frozen till total leptin determination was performed as a single batch using ELISA kit. Clinical pregnancy was assessed via trans-vaginal US assessment at 7 weeks after embryo transfer to identify the presence of fetal sac & embryonic heart pulsations. **Results:** Pregnant cases had significant lower age, BMI and infertility duration than non-pregnant cases although clinical pregnancy had significant lower follicular fluid leptin and insulin resistance, however there was no significant variation among pregnant and non-pregnant women with respect to number of oocytes retrieved, good quality embryos, fertilized and metaphase II oocytes, total dose of gonadotrophins used, days of stimulation, follicular number, size, and endometrial thickness at day of ET. **Conclusion:** This study reported that follicular fluid leptin had significant positive correlations with BMI, fasting glucose, fasting insulin, HOMA-IR. This study demonstrated that clinical pregnancy is associated with lower follicular fluid leptin as it has significant moderate diagnostic performance in predicting clinical pregnancy.

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1. Introduction

Generally, infertility has been usually considered as one of the vital, severe and expensive health issues in diverse societies ***(1).***

Causes of infertility cannot be present in about 25% of the infertile couples. Both male and female are examined for fertility, where unknown causes of infertility is described with normal semen parameters together with a normal uterine cavity , maximal ovulatory functions, presence of patent tubes and failure of achieving pregnancy after at least 1year of unprotected intercourse ***(2).***

Assisted reproductive technique (ART), specially ICSI can be of beneficial effect in cases of unknown etiology of infertility to fertilize all oocytes. The routine use of ICSI in couples with unexplained infertility offers significant benefit ***(3).***

Various studies dealing with the etiology of infertility from different aspects, one of these factors the high concentration of leptin hormone in the serum which considered as one of main factors for associated with infertility in many researches (***1).***

It is well known the leptin hormone is related to adipokine family and synthesized by white adipose tissue. The mode of action of leptin may be include the regulation of food intake and energy homeostasis through it is effect on the central nervous system .The detection of obesity gene (leptin or ob gene) was cloned and sequenced for the first time in 1994. This gene is subjected for mutation which create a new gene responsible for diabetes and sever obesity in animals and human beings. Many organs in the body specially the brain containing leptin receptors. In several studies, they found that the level of leptin in the serum is liked with body fat mass extent and allocation, and play a role in supplying the CNS with peripheral stimulation on the sufficiency of nutritional condition for adequate reproductive task ***(4).***

In addition to the role of leptin hormone in inducing obesity, leptin is concerned with regulating of several physiological tasks, like metabolism, growth and reproduction. Where, leptin hormone is responsible for regulation and stimulation of hypothalamic-pituitary-gonadal axis, via its direct

effect on the hypothalamus in the brain and regulate the release of GnRH from the hypothalamus ***(5,6).***

Leptin hormone play an important role on the regulation of reproductive system from the onset of puberty to pregnancy, where there are a close association between fertility of animal and energy homeostasis .On the other hand, studies on experimental animals revealed that high level of leptin secretion may have unfavorable actions on the fertility of animals ***(7).***

Though the physiological and pathophysiological consequences of this association among leptin and ovarian function in human beings are now widely studied, especially in assisted reproductive technology (ART) , a little number of researches have tried to assess the interest for determining the serum and/or follicular leptin levels (FFL) in infertile females who will doing in vitro fertilization technique , however the obtained data are contradict ***(8).***

Some investigators found that leptin receptors have been located in the granulosa and thecal cells of the ovary and between these cells leptin suppress steroid secretion through antagonizing insulin-like growth factor I(IGF-I) ***(9).***Moreover, leptin hormones were found another role in the ovarian tissues , where it enhances the proliferation and survival of trophoblast cells by means of an autocrine effect and an anti-apoptotic action. The secretion of luteinizing hormone (LH) have a positive impact on leptin gene expression in placenta as recorded by some researchers ***(4).***

This can be related with the differences in level of leptin in the blood during pregnancy. Certainly, the concentration of leptin in the serum are gradually elevated during the 1st weeks of pregnancy to reach a peak at the 28th week of pregnancy. Subsequently, the concentration of leptin is steadily dropped and record the lowest values at parturition . These data postulate that leptin may not merely play a key position among the endometrium and embryo during implantation, but also it is essential for regulating the normal placental function, and also, it have an important role in maintaining fetal growth and normal pregnancy ***(10).***

Some researchers have postulated that leptin might exert a double task in the control of reproductive functions . They reported that leptin can induce agonistic and antagonstic effects , where at lower level of leptin, can induce a negative impact on endocrine system, with respect to controlling reproduction, whereas, when leptin at high level than normal, it have a negative impact on the ovarian function and growth of foeti ***(1).***

Aim of the Work

This study aims to investigate if follicular fluid leptin concentrations and insulin resistance are correlated with ICSI success.

2. Patients and Methods

This was a prospective study of 34 women with unexplained infertility who underwent ICSI in assisted reproductive technology unit of Ain Shams University Hospital after approval of the research ethical committee during the period from August 2017 to August 2018.

Inclusion criteria:

- Women in reproductive period (age 18-38 years).
- Unexplained infertility.
- Absence of any underlying complex disorders as diabetes,obesity and cardiovascular disease.

Exclusion criteria:

- Male factor infertility.
- Presence of an abnormal uterine cavity due to endometrial polyps, myomas, endometrial synechiae,septate uterus etc.
- Women with PCOS.
- Patient with poor ovarian reserve.
- Patient with ovarian hyperstimulation.
- Poor responders to controlled ovarian stimulation.

All the patients were subjected to:

- Full medical history including age, obstetric history, menstrual history, duration of infertility, number of previous assisted reproductive technique (ART) attempts.
- Physical examination (general including weight and height, abdominal and pelvic).
- BMI was calculated as weight (kg)/square of height (m2) and categorized as low (<18.5 kg/m2), normal (18.5-24.99 kg/m2), or high (25 kg/m2).Women with high BMI values was further categorized as overweight (25.0-29.99 kg/m2) or obese (R30.0 kg/m2).
- Investigations as LH, FSH &E2 levels on cycle day 3.
- Before beginning any of the controlled ovarian hyperstimulation protocols, a blood sample was taken after an overnight fast and immediately sent to the hospital's chemistry laboratory for measuring Serum glucose and insulin.
- The homeostasis-model assessment of insulin resistance (HOMA-IR) was calculated by multiplying fasting insulin (μU/mL) and glucose (mg/dL) levels and then dividing this product by the constant (405).The patient was considered to have normal insulin resistance if the score is below 3 and considered to have moderate insulin resistance if it is between 3 to 5 and sever insulin resistance if it is above 5.
- Controlled ovarian hyperstimulation with long luteal GnRH agonist (GnRH-a) down regulation protocol in the form of triptoreline acetate (Decapeptyl 0.1mg/ml- Ipsen, S.P.A, Milan, Italy) subcutaneous daily injections from the mid-luteal phase of the previous menstrual cycle at day 21 onwards till the day of human chorionic gonadotropin administration.
- Human menopausal gonadotropins daily intramuscular injections were given to enhance

ovarian stimulation from day 2 of cycle in the form of (Fostimone, IBSA, Luganon, Switzerland). The doses of gonadotropins were continuously adjusted according to ovarian response.

- Ovarian response was monitored by transvaginal ultrasonography which was performed every other day from day 8 using (transvaginal probe of 7.5 MHz of Mindray China DP 8800 ultrasound machine) in ART unit. The size and number of the growing follicles were accurately registered in the patients' sheets.
- Human chorionic gonadotropin (Profasi 1000IU-Serono, Aubonne, Switzerland) intramuscular injection was administered when there were at least 3 oocytes measuring ≥ 18 mm.
- Trans-vaginal US-guided oocyte retrieval under general anesthesia was performed 34-36 hours after HCG injection.
- Follicular Fluid samples:

At the time of oocyte retrieval, bloodless aspirated follicular fluid samples were obtained via puncture of ovarian follicles into individual tubes. Following oocyte isolation, the human follicular fluid (FF) will be centrifuged for 20 minutes at 1000×g at 2 - 8°C to remove cellular components and debris. Collected samples will be transferred to sterile polypropylene tubes and frozen at −20°C until total leptin determination was performed as a single batch. The quantitative determination of follicular leptin was done using the commercially available ELISA kit (Human LEP (Leptin) ELISA kit, Assay kit co., Ltd, USA) with assay range:0.2ng/ml-60ng/ml.

Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti LEP antibody was pre-coated onto 34-well plates and the biotin conjugated anti- LEP antibody was used as detection antibodies.

The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer.

TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the LEP amount of sample captured in plate. Reading the O.D. absorbance at 450nm in a microplate reader, and then the concentration of LEP was calculated.

- Oocytes were subjected to enzymatic decoronisation by hyaluronidase (Fertipro NV, Beernem, Belgium). Each sperm, selected according to their mobility and morphology, was transferred to a drop of polyvinylpyrolidone (PVP, Irvine Scientific, USA).
- Fertilization of the oocytes was done by microinjection using micromanipulators mounted on an inverted microscope (Olympus IX71). Each oocyte was then cultured in an incubator (Galaxy, R 170-200P, UK) containing culture medium (Global LOT, Life Global, USA).
- Fertilization was assessed 16-18 hours after injection of oocytes. Normal fertilization was confirmed when 2 clearly distinct pronuclei were present or presence of 2nd polar body.
- Fertilization rate was calculated as the percentage of transformation of microinjected oocytes into 2 pronuclei.
- On day 5 after oocytes retrieval, patients were in lithotomy position. The cervix was exposed with a bivalve speculum and then the mucus in the cervical canal was removed by a cotton swab.
- All ET were performed without any anesthesia or sedation with moderate bladder filling.
- The embryo transfer catheter used was (Labotect Embryo Transfer Catheter Set, Labotect GmbH, Germany). Labotect is a soft catheter that's less likely to induce endometrial trauma and uterine contractions. Embryo loading was done by by using a ‘‘three-drop technique’’. First an air bubble was loaded in the catheter followed by media containing embryos then another air bubble was loaded to bring the total volume to 30 µL.
- Abdominal ultrasound guided embryo transfer using (Shenzen Mindray bio medical electronic model DP 8800 plus) was performed. During transfer, all patients had the upper part of the endometrium thicker on abdominal ultrasonography
- The tip of the inner catheter was placed approximately 1.5–2 cm from the fundal endometrial surface. The medium containing the embryos was gently released into the uterine cavity
- The catheter was slowly withdrawn and examined by the same embryologist under a stereomicroscope to be sure that there were no retained embryos. After the procedure, the patients were kept supine for approximately 60 min.
- Implantation rate was measured as the number of gestational sacs found by trans-vaginal US divided by the number of embryos transferred x 100.
- Progesterone supplement for luteal support was given in the form of (Prontogest, Macryl, Egypt) 400 mg daily vaginal pessaries started 1 day before embryo transfer and continued till a pregnancy test was performed 9 days after embryo transfer (biochemical pregnancy).
- Outcome measure(s): The primary outcome will be biochemical pregnancy rate based on serum

quantitative beta-hCG level at 9 days after ET. Secondary outcome clinical pregnancy rate using trans-vaginal US examination after 7 weeks of embryo transfer to detect the presence of fetal sac & embryonic heart pulsations.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 18.0, IBM Corp., Chicago, USA, 2009. Descriptive statistics were done for quantitative data as minimum& maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data, median and 1st& 3rd inter-quartile range for quantitative non-normally distributed data, while it was done for qualitative data as number and percentage. Inferential analyses were done for quantitative variables using Shapiro-Wilk test for normality testing, independent t-test in cases of two independent groups with normally distributed data. While correlations were done using Pearson correlation for numerical normally distributed data. ROC curve was used to evaluate the performance of different tests differentiate between certain groups. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

3. Results

Table (1): Comparison between pregnant and non-pregnant regarding demographic characteristics

Variables	Pregnant (N=16)	Non pregnant (N=18)	P value
Age (years)	27.7±2.7	29.8±2.7	**0.026***
BMI (kg/m^2)	22.9±1.3	25.0±3.1	**0.020***
Infertility duration (years)	3.6±1.0	4.4±1.0	**0.016***

Data presented as Mean±SD Analysis using Independent t-test, *Significant

This table shows that cases with clinical pregnancy had significant lower age, BMI and infertility duration.

Table (2): Comparison between pregnant and non-pregnant cases regarding metabolic characteristics and hormone levels

Variables	Pregnant (N=16)	Non pregnant (N=18)	P value
D3FSH (mIU/mL)	7.1±1.1	7.0±1.9	0.778
D3LH (mIU/mL)	5.4±1.0	5.0±1.2	0.377
PRL (ng/mL)	12.9±1.9	12.4±2.7	0.538
E2 (pg/mL)	56.3±6.6	54.2±7.9	0.414
FF Leptin (ng/mL)	11.0±4.7	17.7±4.9	**<0.001***
Fasting insulin (µIU/mL)	5.5±1.0	6.3±1.2	0.051
Fasting glucose (mg/dL)	88.2±2.4	90.3±4.2	0.074
HOMA-IR	0.97±0.18	1.14±0.22	**0.025***

Data presented as Mean±SD , Analysis using Independent t-test, *Significant

This table shows that: cases with clinical pregnancy had significant lower FFL and IR.

Table (3): Characteristics of ovarian stimulation in pregnant and non-pregnant cases

Variables	Pregnant (N=16)	Non pregnant (N=18)	*P*-value
Total Gonadotropins dose (IU)	2171.9±522.2	2263.9± 565.4	0.627
Stimulation duration (days)	10.8±0.7	10.8±0.6	0.904
Follicular number	7.7±1.9	7.1±2.2	0.380
Follicular size (mm)	20.5±2.3	20.0±1.8	0.482
Oocytes retrieved	15.8±1.8	13.0±3.0	0.064
Fertilized oocytes	7.7±1.3	6.5±2.3	0.074
Metaphase II oocytes	6.2±1.4	5.1±2.1	0.073
Good quality embryos	1.6±0.5	1.7±0.5	0.721
Endometrial thickness (mm)	12.0±1.4	11.9±1.1	0.891

Variables presented as Mean±SD ,Analysis using Independent t-test, *Significant

The obtained data revealed that no statistically significant variation was observed among the pregnant and non-pregnant groups regarding total dose of gonadotropins used, days of stimulation, follicular number, size, oocyte retrieved, endometrial thickness at day of ET and fertilized and metaphase II oocytes.

Table (4): Correlation between FFL and other variables

Variables	R	P
Age	0.121	0.494
BMI	0.653	**<0.001***
Infertility duration	0.277	0.113
FSH	0.013	0.944
LH	0.003	0.987
PRL	-0.033	0.851
E2	0.008	0.964
Fasting insulin	0.375	**0.029***
Fasting glucose	0.534	**0.001***
HOMA-IR	0.466	**0.005***
Gonadotropine dose	-0.168	0.342
Stimulation duration	-0.134	0.451
Follicular number	0.021	0.906
Follicular size	-0.120	0.499
Oocytes retrieved	-0.673	**<0.001***
Fertilized oocytes	-0.574	**<0.001***
Cleaved oocytes	-0.493	**0.003***
§Endometrial thickness	0.045	0.805

^Pearson correlation, r: Correlation coefficient, *Significant

Table (4) and Figure (2) show that: There were significant positive correlations between FFL and BMI, fasting glucose, fasting insulin and HOMA-IR as well as significant negative correlations with fertilized and cleaved oocyte.

Table (5): Diagnostic performance of studied factors in prediction of clinical pregnancy

Factors	AUC	SE	P	95% CI	Cut off
BMI	0.653	0.097	0.129	0.463–0.842	--
FSH	0.568	0.101	0.501	0.370–0.765	--
LH	0.564	0.101	0.523	0.366–0.763	--
Prolactin	0.563	0.102	0.535	0.363–0.762	--
E2	0.578	0.100	0.438	0.382–0.775	--
FFL	0.832	0.071	**0.001***	0.693–0.970	**≤18.2**
F.insulin	0.668	0.094	0.094	0.484–0.853	--
F. glucose	0.656	0.097	0.121	0.467–0.846	--
HOMA-IR	0.733	0.090	**0.021***	0.556–0.909	--

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table (5) and figure (3): Only FFL had significant moderate diagnostic performance in predicting clinical pregnancy (its optimum cut off point gives moderate diagnostic characteristics). HOMA-IR had significant low diagnostic performance in predicting clinical pregnancy (its optimum cut off point gives low diagnostic characteristics).

Table (6): Diagnostic characteristics of FFL≤18.2 ng/mL in prediction of clinical pregnancy

Characters	Value	95% CI
Sensitivity	93.8%	69.8%–99.8%
Specificity	50.0%	26.0%–74.0%
Diagnostic accuracy (DA)	70.6%	52.5%–84.9%
Youden's index	43.8%	17.8%–69.7%
Positive Predictive value (PPV)	62.5%	40.6%–81.2%
Negative Predictive value (NPV)	90.0%	55.5%–99.7%
Positive likelihood ratio (LR+)	1.88	1.16–3.03
Negative likelihood ratio (LR-)	0.13	0.02–0.88
Diagnostic odd ratio (LR)	15.00	1.62–138.82
Kappa	0.426	0.159–0.692

CI: Confidence interval

FFL≤18.2 ng/mL had high sensitivity and PPV, but low other diagnostic characteristics in prediction of clinical pregnancy.

4. Discussion

In this study, we measure follicular fluid leptin in cases of unexplained infertility to demonstrate the association between follicular fluid leptin, insulin resistance and ICSI outcome.

In the current study, pregnant cases had significant lower age than non- pregnant cases.

This result is similar to ***Wunder (11)*** who demonstrated that age was significantly lower in the pregnant group than non-pregnant. In contrast, ***Llaneza-Suarez (9)*** found that there was no significant variation among pregnant and non-pregnant women with respect to the age. In the current study, pregnant cases had significant lower BMI than non- pregnant cases.

This result agrees with ***Akarsu (12)*** who showed that low levels of l/BMI were liked to high pregnancy outcome. This result disagrees with ***Llaneza-Suarez (9)*** who found no significant variation among pregnant and non pregnant regarding BMI. In the current study, pregnant cases had significant lower duration infertility than non-pregnant cases. This result is consistent with ***Mishra (13)*** who reported that pregnant cases had significantly lower duration of infertility than non - pregnant cases. This result disagrees with ***Hill (14)*** who found that there was no significant difference between pregnant and non-pregnant cases regarding infertility duration. In the current study, cases with clinical pregnancy had significant lower follicular fluid leptin. This result is consistent with ***Asimakopoulos (15)*** who reported that elevated FF leptin is associated with failure of conception in IVF cycles. This result is consistent with ***Anifandis (16)*** who reported that pregnancy success was maximal with lower FF leptin levels. This result agrees with ***Llaneza-Suarez (9)*** who reported that lower FF leptin levels were associated with a higher probability of having a pregnancy after IVF-ICSI. This result agrees with ***Lin et al. (2017)*** who reported that lower FF leptin may contribute to good pregnancy outcome. This result is inconsistent with ***Takikawa et al. (2010)*** who found that the concentration of FFL did not differ significantly between pregnant and non-pregnant cycles. This result disagrees with ***Almog et al. (2011)*** who reported that neither serum nor FF leptin was found to be correlated with cycle outcome parameters such as fertilization rate or pregnancy rate.

In the current study, cases with clinical pregnancy had significant lower insulin resistance. These results agree with ***Llaneza-Suarez et al. (2014)*** who found a positive correlation between abdominal obesity, insulin resistance, and FFL concentrations and an inverse correlation with IVF-ICSI outcome.

In the this study, there was no significant difference between pregnant and non-pregnant cases regarding number of oocytes retrieved. This result agrees with ***Llaneza-Suarez et al. (2014)*** who demonstrated that there was no significant difference in the number of oocytes retrieved between pregnant and non-pregnant cases. This result agrees with ***Lin et al. (2017)*** who demonstrated that there was no significant difference in the number of oocytes retrieved between pregnant and non-pregnant cases. This result disagrees with ***Wunder et al. (2005)*** who demonstrated that the number of retrieved oocytes at pickup were significantly higher in the pregnancy group. In the current study, there was no significant difference between pregnant and non-pregnant cases regarding good quality embryos, fertilized and metaphase II oocytes. These results agree with ***Llaneza-Suarez et al. (2014)*** who reported that there was no significant difference between

pregnant and non- pregnant regarding fertilized oocyte and metaphase II oocyte.

In the current study, there was no statistically significant difference between the pregnant and non-pregnant groups regarding total dose of gonadotrophins used, days of stimulation, follicular number, size, and endometrial thickness at day of ET. These results agree with ***Akarsu et al. (2017)*** who found that there were no statistically significant differences between pregnant and non-pregnant women in terms of D3 FSH, D3 LH, gonadotropin dosage, Stimulation duration, the number of oocytes, the number of embryos transferred and endometrial thickness. This result is similar to ***Takikawa et al. (2010)*** who reported that no significant difference regarding the total dose of gonadotropin between the pregnant and non-pregnant cases. In the current study, Follicular Fluid Leptin had significant positive correlations with BMI, fasting glucose, fasting insulin, HOMA-IR.

This result is similar to ***Welt et al. (2003)*** who demonstrated that there were significant positive correlations between FFL and BMI. This result is consistent with ***Chakrabarti et al. (2012)*** who reported that leptin concentrations correlated positively with BMI. This result agrees with ***Li et al. (2012)*** who demonstrated that there were significant positive correlations between FFL and fasting insulin. This result disagrees with ***Placido et al. (2006)*** who found that follicular leptin is not correlated with BMI. In this study, follicular fluid leptin had negative correlation with fertilized, metaphase II oocytes and oocytes retrieved. This result is inconsistent with ***Placido et al. (2006)*** who found that positive correlation between follicular fluid leptin and fertilized oocyte. This result disagrees with ***Chakrabarti et al. (2012)*** who demonstrated that no significant differences between follicular fluid leptin and oocytes retrieved. In this study, there was no correlation between FFL and Infertility duration, day3FSH, day3 LH, total dose of gonadotropin, stimulation duration, follicular number and size. These results agree with ***Welt et al. (2003)*** who demonstrated that there were no correlation between leptin concentration and Day 3 FSH, Day 3 LH,Day 3 E2, Follicle number, size and total dose of gonadotropin. These results agree with ***Chakrabarti et al. (2012)*** who reported that there were no significant differences in the number of retrieved oocytes and FF leptin. These results agree with ***Li et al. (2012)*** who demonstrated that there were significant positive correlations between FFL and fasting insulin.

In contrast, ***Placido et al. (2006)*** found that no correlation between FF leptin and BMI and no significant correlation between leptin levels and follicular size found. In the current study, follicular fluid leptin had significant moderate diagnostic performance in predicting clinical pregnancy. The best cut-off value was≤18.2 (Youden index, 43.8%) with 93.8% sensitivity and50.0% specificity. ***Llaneza-Suarez et al. (2014)*** found that an optimal cut-off point for FFL for the prediction of IVF success, which was 16.0 ng/mL with a 78.3% specificity and a 54.2% specificity. ***Placido et al. (2006)*** found that FF leptin is considered the best predictive factor for fertilization with identification of a cut off value of 20.25 ng/ml.

Conclusion

This study reported that follicular fluid leptin had significant positive correlations with BMI, fasting glucose, fasting insulin, HOMA-IR. This study demonstrated that clinical pregnancy is associated with lower follicular fluid leptin as it has significant moderate diagnostic performance in predicting clinical pregnancy.

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