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A study on significant biochemical changes in the serum of infertile women

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KEYWORDS

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Blood samples;
Serum Ferritin;
Vitamin D and C.

A B S T R A C T

The aim of the study is to investigate the levels of hormone, Lipid, Iron and Vitamins in the serum of the primary infertility women. There are many biological causes of infertility, including some that medical intervention can treat. The blood samples collected were analysed for hormone (LH,FSH,PROLACTIN,ESTRADIOL),LIPIDS (cholesterol, triglycerides, HDL VLDL,LDL), Iron, Haemoglobin, Serum, Ferritin and vitamins (D,E,C).The results were analysed with graph pad prism software and are tabulated as follows. The level of LH, FSH, PROLACTIN and ESTRADIOL were found to be increased in the test group on comparison with control. The level of CHOLESTEROL, LDL, VLDL were found to be increased in the test group on comparison with control. The level of TRIGLYCERIDES and HDL were found to be decreased in the test group on comparison with control. The level of IRON and HAEMOGLOBIN and were found to be decreased in the test group on comparison with the control. The level of Serum Ferritin was found to be increased in the test group on comparison with the control. The level of Vitamin D and C were found to be decreased in the test group on comparison with the control. The level of Vitamin E was found to be increased in the test group on comparison with the control.

Introduction

Infertility is a major problem today. As recently as 1980 only an estimate of 10-15 couples were affected. Now most survey puts the percentage of couples remaining childless involuntarily at above 20% in developing countries.

Infertility is defined as a couple's inability to become pregnant within one year of unprotected intercourse. In any given year, about 15 percent of couples in North America and Europe who are trying to conceive are infertile. Most couples are

advised to seek help after a year but some with more complex pre-existing are advised to do so earlier.

Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full.

Classification

Infertility is basically classified into two primary infertility and secondary infertility. Primary infertility is infertility in a couple who have never had a child.

Secondary infertility is failure to conceive following a previous pregnancy. Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause.

Infertility is often perceived as a predominantly female disorder, even though male-factor infertility is equally prevalent, and half of infertile couples fail to reproduce because of problems with the man's fertility.

It is well known that fertility in women decreases with increasing age, as illustrated by the following statistics:

Infertility in married
women ages 16-20 = 4.5%

Infertility in married
women ages 35-40 = 31.8%

A Concept of Infertility

The desire to reproduce is an intensely motivating human force. Because of its personal nature, couples may also experience strong religious, cultural and societal pressures to conceive. Therefore, it is understandable that people experiencing infertility often perceive it as a serious life crisis. Societal and parental pressures for propagation of the family name can place a

psychological burden on the infertile couple. This central role of reproduction in the human experience has contributed greatly to the desire of couples to overcome infertility. Child bearing is an important aspect of most marriages. For most couples, the conception and raising of children are the expected outcomes of their sexual relationship. To some extent, it has led to the rapid evolution of technologic advances in reproductive biology. The physical, psychological and financial challenges of assisted reproductive technology may further impact the couple (Monga *et al.*, 2004; Seibel *et al.*, 1997).

Fertility in both men and women is at its maximum in the mid-twenties and, in women, declines after the age of 30 years (Willocks and Neilson, 1991, p. 181). The fertility of a marriage is a sum of the fertilities of the two partners. Low fertility in one can to some extent be balanced by high fertility in the other, whereas low fertility in both partners may result in infertility. This explains why some couples fail to reproduce, yet when they separate and each takes a new mate, they both proceed to have children (Tindall, 1987).

Intra Uterine Insemination

Intrauterine insemination (IUI) places sperm directly inside the woman's uterus, which may increase her chances of becoming pregnant. IUI, in combination with gonadotropins, may be recommended if the woman does not ovulate or when there is another known issue, such as a low sperm count, difficulty ejaculating, or a narrow cervical opening, or if the couple's infertility is unexplained. IUI in combination with gonadotropins may increase overall pregnancy chances, but it also increases the chances for multiple pregnancies.

The male partner is usually instructed to obtain a semen sample by masturbating and ejaculating into a sterile container. The man should avoid ejaculating two to three days before collecting the sample. The semen is then prepared in a laboratory to separate the active sperm from the inactive sperm and seminal fluid. The process is similar if donor sperm is used.

The infertility rate ranged from 3.5% to 16.7% in more developed nations and from 6.9% to 9.3% in less-developed nations, with an estimated overall median prevalence of 9%. In 17 studies sampling 6410 women, the proportion of couples seeking medical care was, on average, 56.1% (range 42–76.3%) in more developed countries and 51.2% (range 27–74.1%) in less developed countries. The proportion of people actually receiving care was substantially less, 22.4%. Based on these estimates and on the current world population, 72.4 million women are currently infertile; of these, 40.5 million are currently seeking infertility medical care. Infertility affects a relatively large number of couples at some point in their reproductive lives - globally, between 50 and 80 million couples (WHO, 1994). Moreover, in pronatalist cultures such as those of India, and South Asia more generally, the consequences of infertility for women can be devastating. One estimate of overall primary and secondary infertility in South Asia drawn on the basis of women at the end of their reproductive careers (aged 45-49) suggests a rate in the range of 10 percent: 8 percent in India, 10 percent in Pakistan, 11 percent in Sri Lanka, 12 percent in Nepal and 15 percent in Bangladesh. (Jejeebhoy, 1998). Based on the census reports of India 2001, 1991, 1981 researchers show that childlessness in India has risen by 50 per cent since 1981. It is not because couples are choosing not to

have children but primarily due to growing infertility

Causes of infertility

The following causes of infertility may only be found in females. For a woman to conceive, certain things have to happen intercourse must take place around the time when an egg is released from her ovary; the systems that produce eggs and sperm have to be working at optimum levels; and her hormones must be balanced.

Some women are infertile because their ovaries do not mature and release eggs. In this case synthetic FSH by injection or via a pill can be given to stimulate follicles to mature in the ovaries.

Problems affecting women include endometriosis or damage to the fallopian tubes (which may have been caused by infections such as chlamydia). Other factors that can affect a woman's chances of conceiving include being over or underweight, or her age as female fertility declines sharply after the age of 35. Sometimes it can be a combination of factors, and sometimes a clear cause is never established.

Common causes of infertility includes: Female infertility

- tubal blockage
- Pelvic inflammatory disease
- age-related factors
- uterine problems
- previous tubal ligation
- endometriosis
- advanced maternal age
- non-consummation
- tubal spasm and hypogonadism
- Spontaneous abortion

Hyperemesis and psychological vomiting
Pre-eclamptic toxemia
Amenorrhoea and anovulation
Anorexia nervosa

Male infertility

The main cause of male infertility is low semen quality. Another possible cause is a low sperm count. Infertility associated with viable, but immotile sperm may be caused by primary ciliary dyskinesia.

Combined infertility

In some cases, both the man and woman may be infertile or sub-fertile, and the couple's infertility arises from the combination of these conditions. In other cases, the cause is suspected to be immunological or genetic; it may be that each partner is independently fertile but the couple cannot conceive together without assistance.

Unexplained infertility

In the US, up to 20% of infertile couples have unexplained infertility. In these cases abnormalities are likely to be present but not detected by current methods. Possible problems could be that the egg is not released at the optimum time for fertilization, that it may not enter the fallopian tube, sperm may not be able to reach the egg, fertilization may fail to occur, transport of the zygote may be disturbed, or implantation fails. It is increasingly recognized that egg quality is of critical importance and women of advanced maternal age have eggs of reduced capacity for normal and successful. Also, polymorphisms in folate pathway genes could be one reason for fertility complications in some women with

unexplained infertility. The number of infertile couples seems to be increasing because many couples postpone the start of a family. Delaying pregnancy decreases the number and quality of available eggs and allows a greater length of time for women to develop unwanted sequelae of conditions such as endometriosis, uterine fibroids, and pelvic inflammatory disease (PID) (Brugh *et al.*, 2002).

Hormone profile in infertility

Luteinizing hormone (LH)

LH is a glycoprotein hormone having two subunits. The alpha subunit is similar to those of FSH, hCG and TSH. The beta subunit is different from those of the other glycoprotein hormones and confers its biochemical specificity

LH is a heterodimeric. Each monomeric unit is a glycoprotein molecule; one alpha and one beta subunit make the full, functional protein. Its structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The protein dimer contains 2 glycopeptidic subunits, labeled alpha and beta subunits, that are non-covalently associated (i.e., without any disulfide bridge linking them

In females, LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells. With the rise in estrogens, LH receptors are also expressed on the maturing follicle, which causes it to produce more estradiol. Eventually, when the follicle has fully matured, a spike in estrogen production by the follicle

stimulates a positive feedback loop in the hypothalamus that stimulates the release of LH from the anterior pituitary.

This increase in LH production only lasts for 24 to 48 hours. This "LH surge" triggers ovulation, thereby not only releasing the egg from the follicle, but also initiating the conversion of the residual follicle into a corpus luteum that, in turn, produces progesterone to prepare the endometrium for a possible implantation. LH is necessary to maintain luteal function for the first two weeks of the menstrual cycle. If pregnancy occurs, LH levels will decrease, and luteal function will instead be maintained by the action of HCG (a hormone very similar to LH but secreted from the new placenta).

Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) determinations are performed on oligomenorrhoeic and amenorrhoeic women. High levels of FSH are consistent with women who have premature ovarian failure, while basal FSH levels are associated with poor ovarian reserve (Willet, 1996). FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle.

Prolactin

Prolactin also known as lactotrope, is a protein that in humans is encoded by the PRL gene. Prolactin is measured in oligomenorrhoeic and amenorrhoeic women with or without galactorrhoea. Hyperprolactinemia is associated with

ovulatory disturbances and luteal phase defects, thus affecting infertility (Willet, 1996).

Estradiol

Estradiol (E2 or 17 β -estradiol, also oestradiol) is a sex hormone. Estradiol is abbreviated E2 as it has two hydroxyl groups in its molecular structure. Estrone has one (E1) and estriol has three (E3). Estradiol is about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect. Except during the early follicular phase of the menstrual cycle, its serum levels are somewhat higher than that of estrone during the reproductive years of the human female. Thus it is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity.

Lipid

Lipid profile or *lipid panel*, is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for infertility, and other diseases.

Lipid panels are commonly ordered as part of a physical exam along with other panels such as the complete blood count (CBC) and basic metabolic panel (BMP).

Iron

Women who do not get sufficient amounts of iron may suffer anovulation (lack of ovulation) and possibly poor egg health, which can inhibit pregnancy at a rate 60% higher than those with sufficient iron stores in their blood.

When the blood does not get enough iron, anemia, or an insufficient number of red blood cells, may develop. Since it is these red blood cells that deliver oxygen to all of the body's tissue and organs including the ovaries and uterus, this deficiency may cause the eggs stored in the ovaries to weaken over time and become unviable. Worse yet, should conception occur, anemia makes it impossible for the growing fetus' cells to divide and grow properly. This may result in a miscarriage in some cases.

Vitamin D

A steroid hormone that influences virtually every cell in your body and has been positively linked to health conditions ranging from cancer to heart disease, may significantly boost fertility in both men and women.

A new report has shown that exposure to sunlight boosts fertility in both men and women by increasing their levels of vitamin D, a benefit that appears to work on multiple levels. "Vitamin D and folate deficiency are known to be associated with infertility in women,

Vitamin E

Vitamin E has many biological functions, the antioxidant function being the most important. Other functions include enzymatic activities, gene expression, and neurological function(s). It has also been suggested that the most important function of vitamin E is in cell signalling (and, that it may not have a significant role in antioxidant metabolism).

Vitamin C

Vitamin C improves hormone levels and increases fertility in women with luteal

phase defect, according to a study published in "Fertility and Sterility". As for men, vitamin C has been shown to improve sperm quality and protect sperm from DNA damage; helping to reduce the change of miscarriage and chromosomal problems. Vitamin C also appears to keep sperm from clumping together, making them more motile.

Materials and Methods

Collection and processing of sample

The blood samples were collected from the biochemistry lab of LIFE CARE DKJ HOSPITALS Chennai. A total number of 30 blood samples of infertile women patient on the day 2 of the menstruation were collected. The samples were brought to the laboratory, serum were separated by using centrifugation.

The samples collected for estimation of hormone level in women during menstruation. Women who have regular menstruation and clinical abnormalities were chosen and served as test group.

The anticoagulated blood sample was collected for the assay of haemoglobin was used for the assay of the haemoglobin for the infertility patients. Serum sample was also used for the assay of the lipid level in the patients the sample was collected in the morning in the fasting condition the serum sample was also collected and used for the assay of the vitamin levels in the blood.

The patient who are with primary infertility were included for the study of the biochemical changes and the haemolysed blood samples are exclude and not used for the analysis of the hormone levels.

Hormone profile

Estimation of lutenizing hormone (lh)

The Access GLH assay is a sequential two step immunoenzymatic (“sandwich”) assay

Sample was added to a reaction vessel along with paramagnetic particles coated with goat anti - mouse: anti - HLH complexes and TRIS buffered saline with protein.

The GLH binds to the immobilized mouse anti - μ LH on the solid phase.

Alkaline phosphatase conjugated goat anti - GLH is then added, which binds to the previously bound μ LH on the particles.

A second separation and wash step removes unbound conjugate.

Then the chemiluminiscent substrate Lumi-phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer.

The light production is directly proportional to the concentration of in the sample is determined from a stored multi point calibration curve.

Estimation of follicle stimulating hormone

The Access μ FSH assay is a sequential two step immunoenzymatic (“sandwich”) assay

Sample was added to a reaction vessel along with paramagnetic particles coated with goat anti - mouse: anti - μ FSH complexes and TRIS buffered saline with protein.

The μ FSH binds to the immobilized mouse anti - μ FSH on the solid phase.

Materials bound to the solid phase one held in a magnetic field while unbound materials are washed away

Alkaline phosphatase conjugated goat anti - μ FSH is then added, which binds to the previously bound μ FSH on the particles.

A second separation and wash step removes unbound conjugate.

Then the chemiluminiscent substrate Lumi-phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer.

The light production is directly proportional to the concentration of in the sample is determined from a stored multi point calibration curve.

Estimation of prolactin

The Access Prolactin assay is a simultaneous one step immunoenzymatic (“sandwich”) assay

Sample was added to a reaction vessel along with polyclonal goat : anti PRL alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti - PRL antibody.

The serum to plasma heparin PRL binds to the monoclonal anti - PRL or the solid phase, while the goat anti - PRL - alkaline phosphatase conjugate reacts with a different antigenic site or the serum PRL

After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away.

Then the chemiluminiscent substrate Lumi-phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer.

The light production is directly proportional to the concentration of in the

sample is determined from a stored multi point calibration curve.

Estimation of estradiol

The Access Estradiol assay is a competitive binding immune enzymatic assay.

Sample was added to a reaction vessel along with paramagnetic particles coated with goat anti rabbit : rabbit anti – estradiol and TRIS buffered saline with protein solution.

After 20 minutes, estradiol alkaline phosphate conjugate is added.

Estradiol in the sample competes with the estradiol – alkaline phosphatase conjugate for binding sites on a limited amount of specific anti - estradiol antibody.

Resulting antigen :antibody complexes are bound to the capture antibody on the solid phase

After incubation in a reaction vessel materials bound to the solid phase are held in a magnetic field while unbound materials are washed away.

Then the chemiluminiscent substrate Lumi-phas 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of estradiol in the sample the amount of analyte in the sample is determined from a stored multi point calibration curve.

Lipid profile

Estimation of cholesterol

200µlitre of pipes buffer cholesterol oxidase peroxidise cholesterol esterase were taken in a tube 10µlitre of the sample

was added to the reagent mixture and the colour developed was read at 540 nm after ten minutes of incubation a standard with a concentration of 200mg/dl was also treated in the same manner 10µlitre of distilled water and 1000µlitre of the reagent mixture serves as blank.

Estimation of triglycerides

200µlitre of each of buffer lipoprotein lipase GLYCEROL -3-Phosphate-OXIDASE GLYCEROL KINASE and peroxidise were taken in a tube 10µl of the sample was added to the reagent mixture and the colour developed was read at 540nm after ten minutes incubation a standard with a concentration of 200mg/dl was also treated In the same manner 10µl of distilled water and 1000µl of the reagent mixture serves as a blank.

Estimation of HDL

500ul of Reagent 1 was added to test tubes. 5000ul of serum and 500ul of standard was then added to the tubes. the tubes were incubated for 10minutes at room temperature. The pink colour developed was read at 546 nm. The results are expressed in mg/dl.

Estimation of LDL

$LDL = \text{Triglycerides} / \text{factor } 5$

Estimation of VLDL

The ratio of LDL to HDL

Iron profile

Estimation of iron

To 1ml of plasma, 1ml water and 2ml protein precipitant were added. mixed thoroughly and allowed to stand for 5

minutes and centrifuged. 2ml supernatant was taken and 2ml chromogen solution was added. Mixed well and allowed to stand for 5 minutes. The optical density is measured at 535 nm. A standard curve is constructed using, 0.1, 0.5, 1.0, 1.5 and 2.0 ml of working standard and treating them similar to test sample. A standard with volume range of 0.1, 0.5, 1.0, 1.5 and 2.0 ml were treated in similar manner.

Estimation of haemoglobin

Two test tubes were taken and labelled as blank "b" test "t" to the "t" test tub 10µl of freshly collected blood was added to that 2.5 ml of darkins reagent is added to both the test tubes and kept in incubation for ten minutes and then read at 540 nm using green filter. Simultaneously a standard was prepared by taking 10µl of cyanomethaemoglobin standard and 2.5ml of darkins reagent .

Estimation of serum ferritin

1.0ml of working reagent was added to 30µl of sample .the optical density was measured at 540nm the readings were taken after 10seconds and 5minutes of inserting the cuvette into the instrument.

Vitamin D

Vitamin D assay is a quantitative determination of 25- hydroxyvitaminD in human serum was done by chemiluminescent assay. (wayne; 2000)

Vitamin E

100 µl of the serum sample was taken and to it equal volume of ethanol was added Vortex the mixture well for 2 minutes. To that 400 µl of hexane was added and again vortex for 2 minutes and centrifuged at

2500rpm for 10mts. 300 µl of the clear supernatant. Was removed and added to it. Nitrogen gas was passed to it until it completely gets dried. 100 µl of ethanol was added to the dry tube and vortexed for 1 minute. 50 µl was loaded into HPLC and the values are calculated against the standard area.

Vitamin C

0.5 ml of heparinized plasma was added to 2 ml of freshly prepared metaphosphoric acid the plasma-metaphosphoric acid mixture was centrifuged for 10 min at 2500 rpm. 1.2 ml of the clear supernatant was taken for analysis 1.2 ml of each concentration of working standard solution was taken in series of test tube 1.2 ml of metaphosphoric acid to two tubes for use as blank. 0.4 ml of DTCS reagent was added to all tubes the contents, and incubate the tubes in a water bath at 37° C for 3 hrs. The tubes were then cooled for 10min in an ice bath. 2 ml of cold sulphuric acid, was added to all the tubes in a vortex mixer. (The temperature of the mixture must not exceed room temperature). The optical density of both standard and test was read at 520nm..

Result and Discussion

The blood samples collected were analysed for hormone (LH,FSH, PROLACTIN, ESTRADIOL), LIPIDS (cholesterol, triglycerides, HDL VLDL,LDL) Iron Haemoglobin Serum ferritin and vitamins (D,E,C).

The results were analysed with graph pad prism software and are tabulated as follows.

The level of LH, FSH, PROLACTIN and ESTRADIOL were found to be increased in the test group on comparison with control.

The level of CHOLESTEROL, LDL, VLDL were found to be increased in the test group on comparison with control

The level of TRIGLYCERIDES AND HDL were found to be decreased in the test group on comparison with control

The level of IRON and HAEMOGLOBIN and were found to be decreased in the test group on comparison with the control

The level of Serum ferritin was found to be increased in the test group on comparison with the control

The level of Vitamin D and C were found to be decreased in the test group on comparison with the control

The level of Vitamin E was found to be increased in the test group on comparison with the control

Elevated FSH concentrations in the hypergonadotropic group were not associated with significant changes in E2 and P4, but an increase in LH concentrations was found on days 1, 8, and 22 (Iain *et al* 2001).

The mean serum level of LH / FSH ratio for patients was (0.8) and for control group was(0.8). The difference is not significant yet others studies reported higher or lower ratio and they stated that this variation might be due either to primary central disorders involving (GnRH) secretion or secondary pituitary sensitization to (GnRH) by an abnormal feed back signals from ovaries (Lobo *et al* 2000). The PRL level in (60%) of infertile female showed a significant increase compared to that of healthy control while lower significant values than control was depicted in the rest of the patient .High prolactin level are found in(30%)of women with different kind of amenorrhea leading to infertility (Yen *et al* 1986).

About(60%)of patients of high level of prolactin showed lower levels of FSH and LH than healthy control. These results are in agreement with the present study and also other studies who suggested that a decline in gonadotrophine in hyperproctanemic patient showed the association between gonadatrophine deficiency and hyperprolactinemia which might be an indirect sign of functional hypothalamic pituitary interruption due to the inhibitory effect of PRL (M chleilly *et al* 1987).

Elevated day 3 FSH/LH ratio is associated with an inferior outcome in IVF treatment cycles and may be used as an additional predictor for decreased ovarian response (A Shrim *et al* 2003).Increasing age in the ovulatory group was associated with fewer follicles (p=0.0001) ,Lower peak estradiol (p=0.0001), and fewer pregnancies (p=0.04).

The infertile was increased with hormonal imbalances (FSH, LH, and PRL) that have an impact on ovulation and menstruation. found that excess weight is in not only link to increased risk of chronic disease, but also shown to increase the risk of reproductive problems (Catalano *et al* 2002).

Mean prolactin levels were elevated consistently across the menstrual cycle in the unexplained-infertility group compared with those in normal controls but reached significance only in the early and late follicular and midluteal phases of the cycle. (Richard *et al* 1985).

Table.1 Lipid profile

SI.NO	PARAMETER	CONTROL	TEST	P VALUE	F VALUE
1	CHOLESTEROL	144±25	160.2 ± 23.5	0.0625	2.912
2	TRIGLYCERIDES	97 ± 13	78.2 ± 13.33	P<0.0001	13.71
3	HDL	52 ± 5	46.2 ± 4.43	0.0002	9.653
4	LDL	76 ± 22	91.4 ± 14.62	0.0234	4.014
5	VLDL	17 ± 6	28.6 ± 2.96	P<0.0001	33.31

Values are expressed as Mean ±SD
P value ≤ 0.001 is considered to be significant.

Table.2 Iron profile

SI.NO	PARAMETER	CONTROL	TEST	P VALUE	F VALUE
1	IRON	77 ± 25	44.6 ± 10.87	P<0.0001	17.85
2	HEAMOGLOBIN	10 ± 2	9.02 ± 0.576	0.1090	2.305
3	SERUM FERRITIN	166 ± 3	168 ± 3	0.1649	1.860

Values are expressed as Mean ±SD
P value ≤ 0.001 is considered to be significant

Figure.1

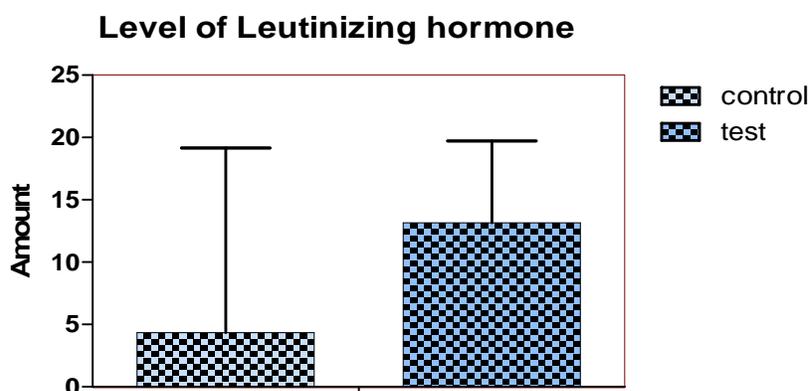


Figure.2

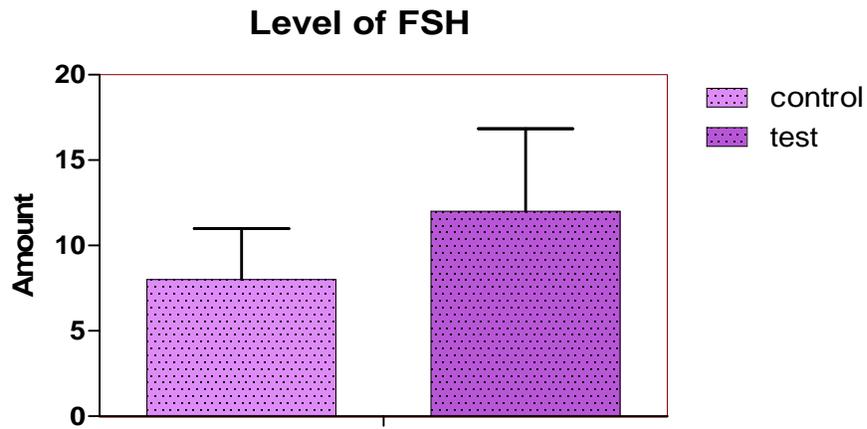


Figure.3

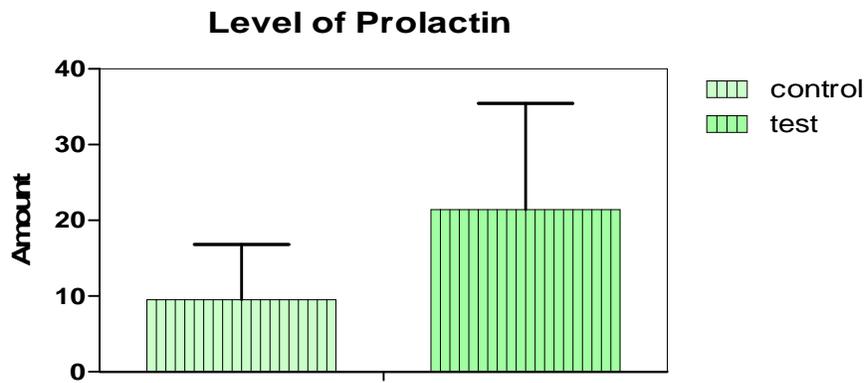


Figure.4

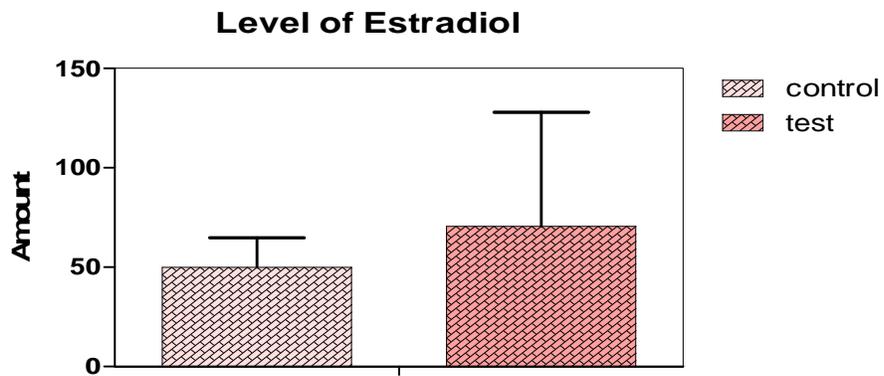


Figure.5

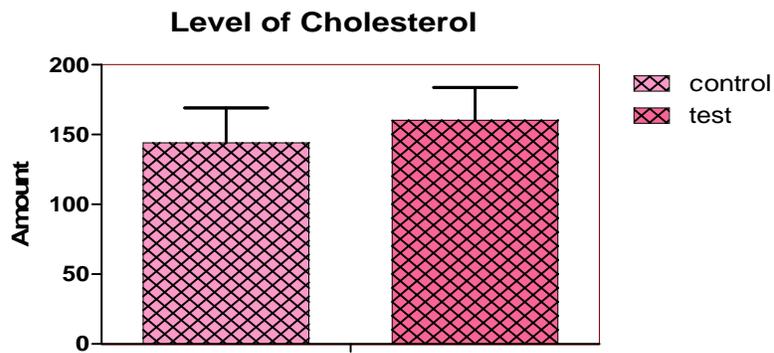


Figure.6



Figure.7

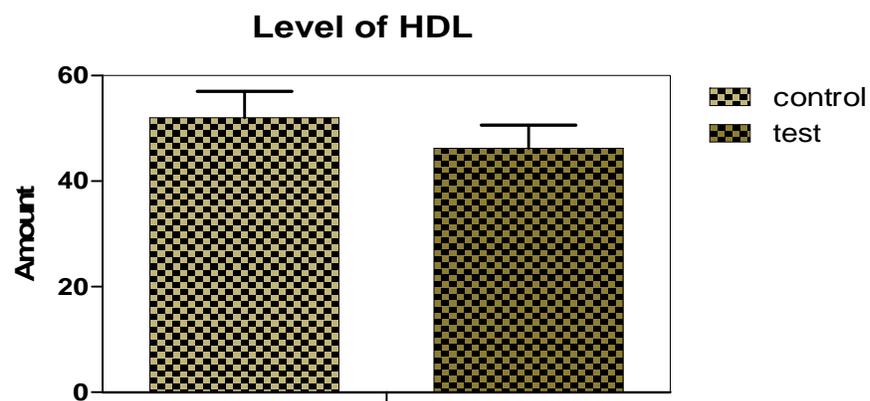


Figure.8

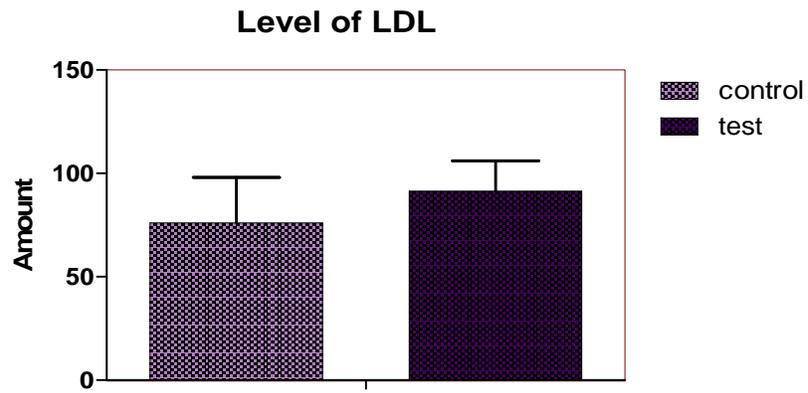


Figure.9

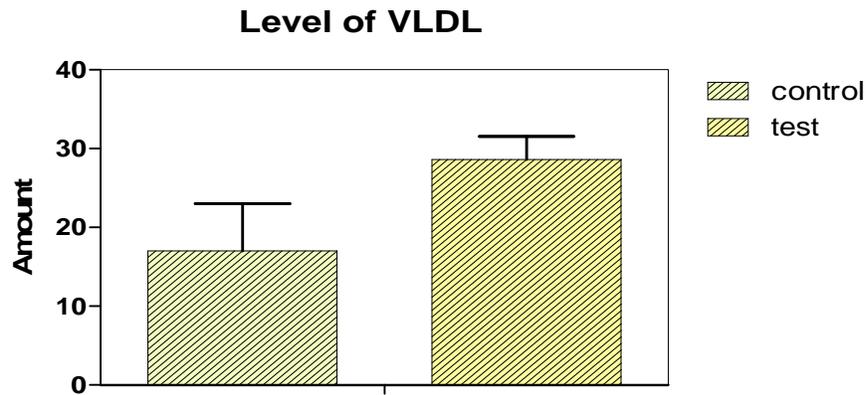


Figure.10

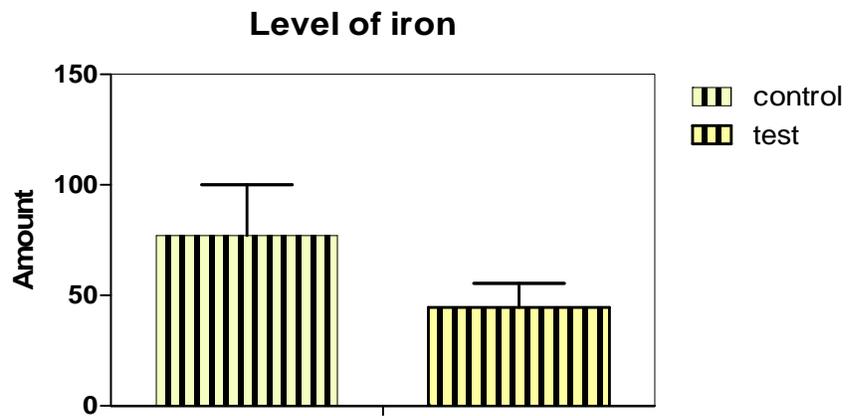


Figure.11

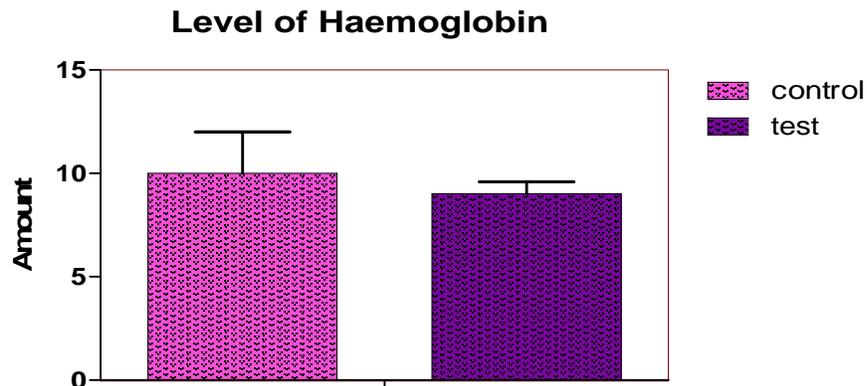
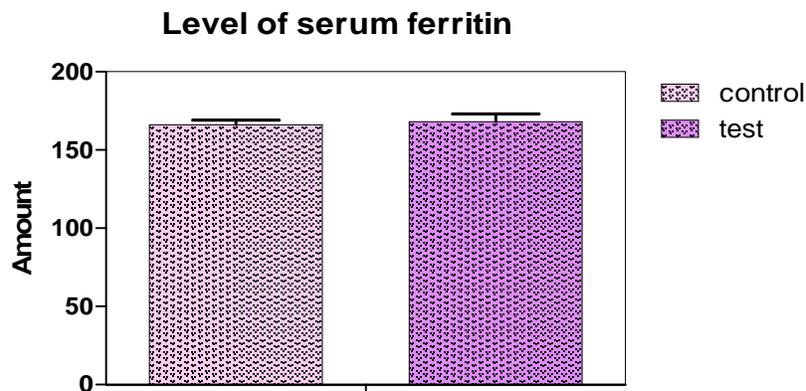


Figure.12



The hyperinsulinemic group had significantly lower high-density lipoprotein (HDL) (by 11%), higher total cholesterol to HDL ratio (by 23%), and greater triglycerides (by 57%) than the normoinsulinemic group. Six-month metformin therapy in hyperinsulinemic women was associated with a significant decrease of insulin level (by 35%), total cholesterol (by 11%), low-density lipoprotein (LDL) (by 12%), and

triglycerides (by 33%). (Banaszewska B *et al* 1987).

Each 2% increase in the intake of energy from trans unsaturated fats, as opposed to that from carbohydrates, was associated with a 73% greater risk of ovulatory infertility after adjustment for known and suspected risk factors for this condition. (Chavarro JE *et al* 2001).

Some trials have evaluated the use of supplementation with antioxidants vitamin C and vitamin E for prevention. Early

intervention at 16–22 weeks of pregnancy with supplementation of vitamin E and C resulted in significant reduction of

preeclampsia in the treatment group (Chappell *et al* 1999).

The level of lipid peroxidation did not differ between women with endometriosis-related infertility and fertile disease-free controls, suggesting that increased reactive oxygen species may not be one of the factors responsible for compromised fertility in patients with endometriosis (Amaral VF *et al* 2002). There is a potential relationship between abnormal lipoprotein metabolism and human female infertility.

Increased body mass index is associated with elevated triglycerides and free fatty acids in ovarian follicular fluid. Maturation within this lipid-rich environment is detrimental to oocytes (Yang X *et al* 2002). Lower serum retinol and alpha-tocopherol in men compared with their female partners could be related to their older age, higher body mass index and smoking habits. Low concentrations of these natural antioxidants were associated with abnormal semen parameters in men and anovulation in women. (Azemi MK *et al* 2001)

A trend towards higher levels of 1,25-dihydroxyvitamin-D(3) and Ca(2+) was observed in women with endometriosis, but differences did not reach statistical significance. As expected, serum concentrations of 25-hydroxyvitamin-D(3) and 1,25-dihydroxyvitamin-D(3,) but not Ca(2+), are influenced by the season (P < 0.001, P = 0.004, P = 0.57, respectively), while levels of the three molecules did not vary according to the phase of the menstrual cycle. (Somigliana E *et al* 2000).

A significant decrease in vitamin e was observed in patients with endometriosis, perhaps because antioxidants are consumed during oxidation reactions after ovulation

induction with exogenous gonadotropins the group of patients with endometriosis not only presented increase lipid peroxidation but also maintained lower vitamin e levels than the control group a fact that hypothetically could compromise oocyte quality in endometriotic patients however on the day of oocyte retrieval, serum vitamin E level were found to be similar in the two groups. (Carla Campous *et al* 1999).

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