بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Effect of Age on Success Rate of IVF Treatment among Infertile Sudanese women

In Khartoum state

تأثير العمر على معدل نجاح التلقيح الاصطناعي لدى النساء السودانيات العقيمات في ولاية الخرطوم

A dissertation submitted in a partial fulfillment for the Requirement

of the master degree in Clinical Chemistry

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October 2017

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(To be completed after the college council approval)

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قال الله تعالى:

وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْنًا عَلَى وَهْنٍ وَفِصَالُهُ فِي عَامَيْنِ أَنِ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ (14) وَإِنْ جَاهَدَاكَ عَلَى أَنْ تُشْرِكَ بِي مَا لَيْسَ لَكَ بِهِ عِلْمٌ فَلَا تُطِعْهُمَا وَصَاحِبْهُمَا فِي الدُّنْيَا مَعْرُوفًا وَاتَّبِعْ سَبِيلَ مَنْ أَنَابَ إِلَيَّ ثُمَّ إِلَيَّ مَرْجِعُكُمْ فَأُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ (15)

صدق الله العظيم

سورة لقمان – الآيات (*14-15*)

Dedication

To my

Father

Mother

Brothers

Sister

Friends

and

College

Acknowledgments

All and first thanks to the almighty ALLAH. Then I would like to express my gratitude and ever last appreciation to my supervisor**Dr. NuhaEljailiAbubaker**for this guidance, helpful suggestions and sage advance for solving problems, valuable supervision as well as precious advice, insightful criticisms, support continues assistance through the whole process of this research . Also special thanks to all members of Sudan university of science and technology(SUST)Collage of graduate studies specially to staff members of clinical chemistry. I am really do not find the words that express my thanks and gratitude to my Father; Who taught me that the best kind of knowledge to have is that which is learned for its own sake and Mother; Who taught me that even the largest task can be accomplished if it is done one step at a time. Finally, I am gratefulto allwomen participate in this study .

Abstract

Infertility is the most widespread and considered as one of the most important unappreciated health problems, particularly in developing countries and about 10% of women failure to achieve a normal pregnancy. This study was conducted to estimate plasma FSH, LH and PRL among infertile Sudanese women.

The study included one hundred and fifty women participate in this study and divided into three groups according to the age of participant, group (1) (35-37) years included 44 women, group (2) (38-40) years included 59 women and group (3) (41-45)years included 47 women. One hundred and fifty blood samples were collected from these women during period from January to July 2017, chosen from Sudan Assisted Reproduction Center (SARC). Fluorescence immunoassay (FIA) method used to estimate plasma FSH, LH and PRL concentration, and results were analyzed using statistical package for social science (SPSS) computer program.

The study showed that, the means concentration of FSH were significantly increase in women group (2) and group (3) compared to group (1) of infertile Sudanese women . Mean ±SD :(10.32±7.03 versus 7.98±3.36 mIU|ml ,p. value=0.03). (11.19±6.40 versus 7.98 ± 3.36mIU|ml, p -value=0 .007) respectively, while there was insignificant different in FSH level between group (3) compared to group (2) (11.19±6.40 versus 10.32 ± 7.03mIU|ml ,p-value = 0.5).

Result of this study showed, there were insignificant different between

the means of LH and prolactin levels when compared between study

groups (p- value≥0.05). Mean ±SD for group3 versus group2:

(5.06±2.74 versus 5.46 ± 2.95mIU|ml) for LH, (22.62±16.05 versus 21.45 ± 9.70ng|ml) for PRL.group2 versus group1: (5.46 ±2.95 versus 5.30±2.24mIU|ml) for LH. (21.45 ±16.05 versus 19.94±12.92 ng|ml) for PRL . group3 versus group1:(5.06±2.74 versus 5.30 ± 2.24mIU|ml) for LH. (22.62±16.05 versus 19.94 ± 12.92ng|ml) for PRL.

Other factors shared age to increase risk of infertility in Sudanese women included: female factor (tubal block 20.6%, PCO 12% and fibroid 1.3%) , male factor (34.7%), both male and female factors (6.7%)and un explaining factor (24.7%).

The study also showed there was significant weak positive correlation between age and FSH concentration . (r =0.198, P. Value = 0.01), while there was no correlation between LH and age .(r = 0.016,P. Value = 0.84).The study showed there was insignificant weak negative correlation between age and PRL . (r= - 0.19, P .Value=0.23).

The success rate of IVF treatment,21.3% ofinfertile women under IVF treatment have positive result (β -HCG)(10.6% in group (1) ,8.67% in group (2) and 2.0% in group (3)).while 78.7% of infertile women under IVF treatment have negative result (β -HCG).

It is concluded that the plasma level of FSH was significantly increase in infertile Sudanese women , while the plasma levels of LH and PRL showed insignificant different, also there was significant positive correlation between FSH and age, and success rate of IVF decrease with age.

مستخلص الدراسة

العقم هو الأكثر انتشارا ويعتبر واحدا من أهم المشاكل الصحية ، وخاصة في البلدان النامية، وحوالي 10٪ من النساء فشلن في تحقيق الحمل السريري. أجريت هذه الدراسة لتقدير مستوى هرمونات FSH, LH and PRL في البلازما لدى النساء السودانيات العقيمات . وشملت الدراسة مائة وخمسين امرأة مشاركة في هذه الدراسة وقسمت إلى ثلاث مجموعات حسب عمر المشارك، المجموعة (1) (35-37) سنة شملت 44 امرأة، مجموعة (2) (38-40) سنة شملت 59 امرأة والفئة (3) (41-45) سنة شملت 47 امرأة. تم جمع مائة وخمسين عينة دم من هؤلاء النساء خلال الفترة من يناير إلى يوليو 2017، وتم اختيارهم من مركز مساعدة الإنجاب السوداني (سارك). تم استخدام الطريقة المناعية لتقدير تركيزFSH, LH and PRL في البلازما، وتم تحليل النتائج باستخدام حزمة إحصائية للعلوم الاجتماعية (SPSS) برنامج الكمبيوتر.

وأظهرت الدراسة أن متوسط تركيز هرمون FSH زاد بشكل ملحوظ في مجموعة النساء (2) والمجموعة (3) مقارنة مع المجموعة (1) .متوسط ± الانحراف المعياري : (10.32 ± 7.03 مقابل 7.98 ± 3.36وحدة | مل، الاحتمال الاحصائي للمقارنة = 0.03). (11.19 ± 6.40 مقابل 7.98 ± 3.36 وحدة| مل، الاحتمال الاحصائي للمقارنة = 0 .007) على التوالي، و لم يكن هناك اختلاف ملحوظ في مستوى المعنوية بين المجموعة (3) مقارنة مع المجموعة (2) (11.19 ± 6.40 مقابل 10.32 ± 7.03 وحدة | مل، الاحتمال الاحصائي للمقارنة = 0.5).

اظهرت نتائج الدراسة انه لم يكن هناك اختلافات ملحوظة بين متوسط مستويات الهرمون LH and PRLعند المقارنة بين مجموعات الدراسة (الاحتمال الاحصائي للمقارنة ≤ 0.05).متوسط ± الانحراف المعياري للمجموعة 3 مقابل المجموعة 2:(5.06 ± 2.74 مقابل 5.46 ± 2.95) وحدة | مل بالنسبة لل LH (22.62 ± 16.05 مقابل 21.45 ± 9.70) نانو غرام | مل بالنسبة للPRL. متوسط ± الانحراف المعياري للمجموعة 2 مقابل المجموعة 1: (5.46 ± 2.95 مقابل 5.30 ± 2.24) وحدة | مل بالنسبة لل LH. (21.45 ± 16.05 مقابل 19.94 ± 12.92) نانوغرام | مل بالنسبة لل PRL.المجموعة 3 مقابل المجموعة 1: (5.06 ± 2.74 مقابل 5.30 ± 2.24) وحدة | مل بالنسبة لل LH, (22.62 ± 16.05 مقابل 19.94 ± 12.92) نانوجرام | مل بالنسبة لل PRL.

ومن العوامل الأخرى التي تشارك العمر لزيادة خطر الإصابة بالعقم لدى النساء السودانيات في إطار علاج التلقيح الاصطناعي: العوامل الانثوية (انسداد الانابيب 20.6%, تكيسات المبايض 12% وتليف جدار الرحم 1.3%) عامل زكوري (34.7%) , وعوامل زكورا واناثا (6.7%) وعوامل غير معروفة (24.7%).

وأظهرت الدراسة أيضا وجود علاقة ارتباط معنوية بين العمر و تركيز FSH للنساء تحت علاج التلقيح الاصطناعي. (معامل بيرسون للارتباط = 0.198، ومستوى المعنوية. = 0.01) في حين لم يكن هناك علاقة ارتباط بين LH والعمر . (معامل بيرسون للارتباط= 0.016 = ومستوى المعنوية. فالو = 0.84).

وأظهرت الدراسة وجود علاقة ارتباط سلبي ضعيف غير معنوي بين العمر و PRL. (معامل بيرسون للارتباط= 0.19 - ، ومستوى المعنوية= 0.23).

أما نسبة نجاح علاج التلقيح الصناعي، فقد بلغت 21.3٪ من النساء اللواتي تحت علاج التلقيح الصناعي اللواتي لهن نتيجة إيجابية في كشف الحمل (10.6٪ في المجموعة (1)، 8.67٪ في المجموعة (2)، 2.0٪ في المجموعة (3) .في حين أن 78.7٪ من النساء تحت علاج التلقيح الاصطناعي لهن نتيجة سلبية .

وخلصت الدراسة إلى أن مستوى FSH في البلازما زاد بشكل ملحوظ في النساء السودانيات العقيمات ، ولم يكن هنالك اختلاف ملحوظ لهرموني LH and PRL، كما كان هناك ارتباط إيجابي بين FSH والعمر، ونسبة نجاح التقيح الاصطناعي تنخفض مع العمر.

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Abbreviation	Full term
FSH	Follicle-Stimulating Hormone
LH	Luteinizing Hormone
PRL	Prolactin
β –HCG	Beta-Human Chorionic Gonadotropin
ART	Assisted Reproductive Technologies
IVF	In Vitro Fertilization
IUI	Intra Uterine insemination
AMH	Anti Müllerian Hormone
GnRH	Gonadotropin-Releasing hormone
TSH	Thyroid-Stimulating Hormone
TRH	Thyrotropin-Releasing Hormone
ICSI	Intra Cytoplasmic Sperm Injection
WHO	World Health Organization
CCCT	Clomiphene citrate challenge test
AFC	Antral follicle count
POF	Premature ovarian failure
HSG	Hysterosalpingogram
ICSH	Interstitial cell-stimulating hormone
DA	Dopamine
SO	Super Ovulation
GIFT	Gamete intra fallopian transfer
ZIFT	Zygote intra fallopian transfer

Chapter one

Introduction

Rationale

Objectives

1.INTRODUCTION

1.1:Introduction

Infertility is the failure to achieve a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse (Zegers*et al.*, 2009). It is considered as one of the most important unappreciated health problems, particularly in developing countries (Ombelet*et al.*, 2008) and has been acknowledged as a public health issue by the world Health Organization (WHO) (Vayena*et al.*, 2002). Infertility could be primary when both partners have never conceived in their lifetime , or secondary due to inability of couples or partners to conceive after a year when one or both partners have previously had a child or children (Schmidt *et al.*, 1985).

Ovarian reserve, or the total number of remaining oocytes within the ovary, declines with ovarian age, but this does not always equate with the age of the woman. A baseline measurement of serum FSH concentration, usually on day 3 of the menstrual cycle, is a fairly good predictor of ovarian reserve in women of reproductive years.(Schmidt *et al.*, 1985).

Hormonal imbalance (FSH, LH, and PRL) has been proposed as a common reason for infertility. Hormonal aberrations may result from problems with certain endocrine glands, such as the pituitary, thyroid, adrenal gland or ovaries. In female it often leads to the inability to ovulate, or release, an egg and in men it can lead to infertility by affecting the sperm – its development, shape, movement or quantity.(Schmidt *et al.*, 1985).

Follicle-stimulating hormone (FSH) is a member of the glycoprotein hormone family that has a central and essential role in reproduction. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland.

Luteinizing hormone (LH) is a glycoprotein hormone having two subunits.LH is secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH)secreted by the hypothalamus.(Schmidt *et al.,* 1985).

Prolactin (PRL) : Human prolactin (PRL; lactogenic hormone) is secreted from the anterior pituitary gland in both men and women . PRL is a single chain polypeptide hormone with a molecular weight of approximately 23 KDa .(Schmidt *et al.,* 1985).

HCG: Human chorionic gonadotropin (hCG) is a sialoglycoprotein initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall.(Schmidt *et al.,* 1985).

1.2:Rationale:

Infertility rates have increased over the years. At least 10-15% worldwide are affected by infertility. This is most due to an increase in the age of women and can be caused by problems related to the man or woman or can often be Unexplained, so the infertile couples need serious medical intervention involving the use of advancedassisted reproductive technologies (ART) procedures, such as In vitro fertilization (IVF) . The success of IVF depends on many factors, age is main factor, about 27.7% for women aged between 35-37, 20% women aged between38-39, and 13.6% for women aged between40-42.

FSH determination is fundamental to elucidating reproductive physiology, regulating fertility, and diagnosing and treating disorders of reproduction.
Measuring LH, FSH and PRL at day 2 0r 21 can indicate whether or not the hormonal state is compatible with pregnancy .

There are few published studies about this in Sudan, so this study may help to improve awareness of the natural age-related decline in female fertility with assisted reproductive technologies (ART) and provide recommendations for their management, and to review investigations in the assessment of ovarian aging.

1.3: Objectives

1.3.1: General Objective:

To study the effect of age on success rate of IVF treatment among infertile Sudanese women in Khartoum state .

1.3.2: Specific Objectives:

1.To compare mean concentration of FSH, LH and PRL in study groups.

2.To calculate the percentage of thesuccess rate of IVF among study groups.

3.Other factors shared age to increase risk of infertility inSudanese women.

4.To correlate between FSH, LH, PRL, and age.

Chapter two

Literature Review

2.Literature review

2.1Infertility:

Infertility is one of the most important and underappreciated reproductive health problems in developing countries. It is defined as the inability of a sexually active, non-contracepting couple to achieve conception within one or two years of unprotected sexual intercourse (Mosher *et al.*, 1990). Infertility is often traumatic, whether or not couples already have other living children. Infertility has been steadily increasing overtime in Nigeria compared with what was obtainable in the past (Mosher *et al.,* 1990). The reason is the trend for women to delay child bearing due either to various reasons including education or occupational career. Thus, the causes of infertility are grouped by sex and include male factors (35%), female factors (ovulatory dysfunction –20%, tubal dysfunction –30%, abnormal cervical mucous–5%), and those of unknown etiology (10%).(D. Madhukar*et al.*, 2009).

2.1.1 Types of Infertility:

2.1.1.1 Primaryinfertility when both partners have never conceived in their lifetime . Approximately 15% of couples attempting their first pregnancy meet with a failure.

2.1.1.2 Secondary infertility: is inability of couples or partners to conceive after a year when one or both partners have previously had a child or children,10% face secondary infertility. (Schmidt *et al.,* 1985)

2.2 :Ovulation and the menstrual cycle:

During their reproductive years, women have regular monthly menstrual periods because they ovulate regularly each month. Eggs mature inside of fluid-filled spheres called “follicles.” At the beginning of each menstrual cycle when a woman is having her period, a hormoneproduced in the pituitary gland, which is located in the brain, stimulates a group of follicles to grow more rapidly on both ovaries. The pituitary hormone that stimulates the

ovaries is called follicle-stimulating hormone (FSH). Normally, only one of those follicles will reach maturity and release an egg (ovulate); the remainder gradually will stop growing and degenerate.Pregnancy results if the egg becomes fertilized and implants in the lining of the uterus (endometrium). If pregnancy does not occur, the endometrium is shed as the menstrual flow and the cycle begins again. In their early teens, girls often have irregular ovulation resulting in irregular menstrualcycles, but by age 16 they should have established regular ovulation resulting in regular periods. A woman's cycles will remain regular, 26 to 35 days, until her late 30s to early 40s when she may notice that her cycles become shorter. As time passes, she will begin to skip ovulation resulting in missed periods. Ultimately, periods become increasingly infrequent until they cease completely. When a woman has not had a menstrual period for 1 full year, she is said to be in menopause.

As women age, fertility declines due to normal, age-related changes that occur in the ovaries. Unlike men, who continue to produce spermthroughout their lives, a woman is born with all the egg-containing follicles in her ovaries that she will ever have. At birth there are about one million follicles. By puberty that number will have dropped to about 300,000. Of the follicles remaining at puberty, only about 300 will be ovulated during the reproductive years. The majority of follicles are not used up by ovulation, but through an ongoing gradual process of loss called atresia. Atresia is a degenerative process that occurs regardless of whether you are pregnant, have normal menstrual cycles, use birth control, or are undergoing infertility treatment.(American Society for Reproductive Medicine , 2012).

2.3:Fertility changes with age

Both males and females become fertile in their teens following puberty. For girls, the beginning of their reproductive years is marked by the onset of ovulation and menstruation. It is commonly understood that after menopausewomen are no longer able to become pregnant. Generally, reproductive potential decreases as women get older, and fertility can be expected to end 5 to 10 years before menopause.

In today's society, age-related infertilityis becoming more common because, for a variety of reasons, many women wait until their 30s to begin their families. Even though women today are healthier and taking better care of themselves than ever before, improved health in later life does not offset the natural age-related decline in fertility. It is important to understand that fertility declines as a woman ages due to the normal age-related decrease in the number of eggs that remain in her ovariesThis decline may take place much sooner than most women expect.(American Society for Reproductive Medicine , 2012).

2.4 :Fertility in the aging female:

A woman's best reproductive years are in her 20s. Fertility gradually declines in the 30s, particularly after age 35. Each month that she tries, a healthy, fertile 30-year-old woman has a 20% chance of getting pregnant. That means that for every 100 fertile 30-year-old women trying to get pregnant in 1 cycle, 20 will be successful and the other 80 will have to try again. By age 40, a woman's chance is less than 5% per cycle, so fewer than 5 out of every 100 women are expected to be successful each month. Women do not remain fertile until menopause. The average age for menopause is 51, but most women become unable to have a successful pregnancy sometime in their mid-40s. These percentages are true for natural conception as well as conception using fertility treatment, including in vitro fertilization (IVF). Although

stories in the news media may lead women and their partners to believe that they will be to able use fertility treatments such as IVF to get pregnant, a woman's age affects the success rates of infertility treatments. The age-related loss of female fertility happens because both the quality and the quantity of eggs gradually decline.(American Society for Reproductive Medicine, 2012).

2.5 :Egg quantity:

The decreasing quantity of egg-containing follicles in the ovaries is called "loss of ovarian reserve." Women begin to lose ovarian reserve before they become infertile and before they stop having regular periods. Since women are born with all of the follicles they will ever have, the pool of waiting follicles is gradually used up. As ovarian reserve declines, the follicles become less and less sensitive to FSH stimulation, so that they require more stimulation for an egg to mature and ovulate. At first, periods may come closer together resulting in short cycles, 21 to 25 days apart. Eventually, the follicles become unable to respond well enough to consistently ovulate, resulting in long, irregular cycles. Diminished ovarian reserve is usually age-related and occurs due to the natural loss of eggs and decrease in the average quality of the eggs that remain. However, young women may have reduced ovarian reserve due to smoking, family history of premature menopause, and prior ovarian surgery. Young women may have diminished ovarian reserve even if they have no known risk factors.(American Society for Reproductive Medicine, 2012).

There are medical tests for ovarian reserve, but none have been proven to reliably predict the possibility of becoming pregnant. These tests do not determine whether or not a woman can become pregnant, but they can determine that age-related changes of the ovaries have begun. Women with poor ovarian reserve have a lower chance of becoming pregnant than women with normal ovarian reserve in their same age group. No single test nor any combination of tests is 100% accurate.Tests of day-3 FSH, antimüllerian

hormone, and estrogenlevels involve blood sampling on the 2nd, 3rd, or 4th day of the menstrual cycle. High levels of FSH or estrogen indicate that ovarian reserve is low. However, many women with diminished ovarian reserve will have normal levels of FSH on day 3, so a normal day-3 FSH does not confirm normal ovarian reserve. Other tests of ovarian reserve that are sometimes utilized include the clomiphene citrate challenge test (CCCT) and ultrasound assessment of follicle numbers, called the antral follicle count.(American Society for Reproductive Medicine , 2012).

2.6 :Infertility evaluation and advanced Maternal Age:

Infertility usually is diagnosed if a woman has not become pregnant after 1 year of unprotected intercourse (i.e., no contraceptive measures used). However, if she is 35 or older, the evaluation should begin after 6 months of trying unsuccessfully to conceive. If a couple has an obvious medical problem affecting their ability to conceive, such as absence of periods*(*amenorrhea), sexual dysfunction, a history of pelvic disease, or prior surgery, they should begin the infertility evaluation immediately. Fertility tests may include ovulation detection and evaluation of the fallopian tubes, cervix, and uterus. The male partner will have a semen analysis. Most testing can be completed within 6 months, and appropriate treatment can be started immediately after the evaluation is completed.

Women who have a medical disorder, such as high blood pressure or diabetes, should talk with their clinical care provider before attempting pregnancy. It is important that health problems are under control. The clinical care provider may suggest a change in medication or general health care before pregnancy as there are increased risks for older women. Conditions such as high blood pressure or diabetes develop more commonly in women who conceive after age 35.Special monitoring and testing may be recommended during pregnancy. Preconception counseling is often beneficial as well. Children born to women over age 35 have a higher risk of chromosomal problems.

Women can choose to discuss these risks with their clinical care provider or a genetic counselor prior to attempting pregnancy. Prenatal testing may be performed after conception to check for certain birth defects. Amniocentesis and chorionic villus sampling are two methods of prenatal testing. Blood testing and ultrasound also may be used as screening tests for certain birth defects. Many parents want to know as much about the pregnancy as possible so they can make informed decisions .(American Society for Reproductive Medicine , 2012).

2.7: Infertility factors :

A.The age factor:

Delaying pregnancy is a common choice for women in today's society. The number of women in their late 30s and 40s attempting pregnancy and having babies has increased in recent years. If you've chosen to delay pregnancy, due to college or career for example, you may not realize that your fertility begins to decline significantly in your mid 30s and accelerates 10 in your late 30s. Some women even begin to experience a decline in their fertility in their late 20s and early 30s **.**Fertility declines with age because fewer eggs remain in your ovaries, and the quality of the eggs remaining is lower than when you were younger. Blood tests are now available to determine your ovarian reserve, a term which reflects your age-related fertility potential. In the simplest of these tests, the hormones follicle-stimulating hormone (FSH) and estradiol are tested in your blood on the second, third, or fourth day of your menstrual period. An elevated FSH level indicates that your chances for pregnancy may be lower than routinely expected for your age, especially if you are age 35 or older. In addition, an AMH level (anti-müllerian hormone level) may also be ordered to provide additional information about your

ovarian reserve. A lower AMH level indicates decreased ovarian reserve. (American Society for Reproductive Medicine, 2012).

Abnormally high FSH or low AMH levels do not mean that you have no chance of successful conception. However, they may indicate that success rates may be lower, that more aggressive treatment may be warranted, and/ or that higher medication doses may be needed. Another commonly used option to detect ovarian reserve is the use of transvaginal ultrasound to determine antral follicle count (AFC), which is when each follicle in both ovaries are counted. An AFC is performed during the first 3-4 days of the menstrual cycle. (American Society for Reproductive Medicine , 2012).

Older women tend to have a lower response to fertility medications and a higher miscarriage rate than younger women. The chance of having a chromosomally abnormal embryo, such as one with Down syndrome, also increases with age. Because of the marked effect of age on pregnancy and birth rates, it is common for older couples to begin fertility treatment sooner and, in some cases, to consider more aggressive treatment than younger couples. Possible treatments for age-related infertility in women include fertility drugs plus IUI or IVF. In cases where the treatments fail or are predicted to have a low chance of success, egg donation is an option. Egg donation has a high chance of success, regardless of your FSH level. More recently, embryo donation has also become a viable option for many couples. For couples who have not succeeded with fertility treatments or who choose to forgo treatment, adoption is an important option. (American Society for Reproductive Medicine , 2012).

B. The ovulation factor:

Problems with ovulation are common causes of infertility, accounting for approximately 25% of all infertility cases. Ovulation involves the release of a mature egg from one of your ovaries. After ovulation, the ovary produces the

hormone progesterone. During the 12 to 16 days before menstruation begins, progesterone prepares the lining of your uterus into an optimal environment for implantation and nurturing of the fertilized egg. If you have regular menstrual cycles, you are probably ovulating. Cycle lengths of approximately 24 to 34 days (from the beginning of one period to the beginning of the next period) are usually ovulatory. If you only have a period every few months or not at all, you are probably not ovulating or are ovulating infrequentlyTo predict ovulation before it takes place, in order to schedule intercourse or insemination, for example, you may use an over-the-counter ovulation prediction kit. These urine test kits are designed to detect the surge of luteinizing hormone (LH)that occurs just before you ovulate. The LH surge stimulates one of the ovaries to release an egg and produce progesterone. Ovulation prediction kits usually detect the LH surge about a day or a day and a half before ovulation, giving you and your partner advance notice of an egg that will be released (ovulation). However, not all women who ovulate will have an LH surge that will be detected using these kits. You may find these kits difficult and frustrating, and false positive and false negative results do occur occasionally. (American Society for Reproductive Medicine , 2012).

In a normal cycle, progesterone levels are highest about seven days after ovulation. Your physician may perform a blood test to measure the level of progesterone in your blood at this time. Generally, blood progesterone is tested on day 19 to 23 of a 28-day menstrual cycle. An elevated progesterone level helps to confirm ovulation and the adequacy of ovarian hormone production. Your physician may perform a pelvic ultrasound examination to evaluate ovulation, but this is not done routinely. This examination may indicate whether your ovaries are producing follicles. These follicles are fluid filled sacs located just beneath the ovary’s surface that contain the immature eggs. Ultrasound may also help to document a follicle’s collapse and

subsequent release of fluid, implying release of an egg. (American Society for Reproductive Medicine , 2012).

If you are not ovulating, your physician may order special tests to determine the reason and then prescribe certain drugs to induce ovulation. Your medical history and physical exam will help determine which tests are appropriate. Both oral and injectable medications are available to help induce your ovulation. (American Society for Reproductive Medicine , 2012).

Causes of ovulatory dysfunction include :

Polycystic ovary disease– characterized by excessive androgen production by either the adrenals or the ovaries, which become enlargedand contain multiple cysts.

Hyperprolactinemia– Hyperprolactinaemia is associated with reduced secretion of gonadotrophins and hence anovulation.

Hypothalamic anovulation– ovulation is dependent on the presence of a functioning hypothalamic-pituitary-gonadal axis, any alteration

in the GnRH pulse generator alters gonadotropin secretion & eventual response at the level of the ovary.

Premature ovarian failure (POF) – Premature ovarian failure is a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles before the age of40 years (secondary amenorrhea). It is defined by abnormally low levels of estrogen and high levels of FSH, much before the age of onset of menaupasue, which demonstrate that the ovaries are no longer responding to circulating FSH by producing estrogen and developing fertile eggs. (American Society for Reproductive Medicine, 2012).

Fertility hormones disturbance :

The presence of elevated LH/FSH concentrations with a decreased estradiol concentration is diagnostic of hypergonadotrophichypogonadism. The co-existence of decreased LH, FSH and estradiol values is diagnostic of hypogonadotrophichypogonadism.

Increasing levels of prolactin can cause a woman to progress from a deficient luteal phase to overtamenorrhea, usually associated with complete GnRH suppression.(American Society for Reproductive Medicine, 2012).

C. The tubal factor:

Because open and functional fallopian tubes are necessary for conception, 8 tests to determine tubal openness (patency) are important. Tubal factors, as well as factors affecting the peritoneum (lining of the pelvis and abdomen), account for about 35% of all infertility problems. A special x-ray called a hysterosalpingogram (HSG) **,**can be performed to evaluate the fallopian tubes and uterus . During an HSG, a special fluid (dye) is injected through cervix, fills your uterus, and travels into your fallopian tubes. If the fluid spills out the ends of the tubes, they are open. If the fluid does not spill out the ends, then the tubes are blocked. If the HSG shows blocked fallopian tubes, physician may perform a laparoscopy to assess the degree of tubal damage.(American Society for Reproductive Medicine, 2012).

If the tubes are found to be blocked, scarred, or damaged, surgery can sometimes correct the problem. But surgery does not guarantee that the tube, even if opened up or cleared of scar tissue, will function properly. Although some tubal problems are correctable by surgery, women with severely damaged tubes are so unlikely to become pregnant that in vitro fertilization (IVF) offers them the best hope for a successful pregnancy. Because very badly damaged tubes may fill with fluid (hydrosalpinx) and lower IVF

success rates, your physician may recommend removal of the damaged tubes prior to IVF.(American Society for Reproductive Medicine, 2012).

D. The male factor :

In approximately 40% of infertile couples, the male partner is either the sole or a contributing cause of infertility. Therefore, a semen analysis is important in the initial evaluation. Treatment for male factor infertility may include antibiotic therapy for infection, surgical correction of varicocele (dilated or varicose veins in the scrotum) or duct obstruction, or medications to improve sperm production. In some men, surgery to obtain sperm from the testis can be performed. In some cases, no obvious cause of poor sperm quality can be found. Intrauterine insemination (IUI) or IVF may then be recommended. Direct injection of a single sperm into an egg (intracytoplasmic sperm injection [ICSI]) may be recommended as a part of the IVF process. If no sperm are present on semen analysis or not found at surgical extraction, physician may discuss using a sperm donor. Insemination with donor sperm may also be considered if IUI is not successful or if you and your partner do not choose to undergo IVF. (American Society for Reproductive Medicine, 2012).

Because of complex and incomplete knowledge of the underlying causes, most infertile men are described as :

Azoospermic: No sperm in the semen.

Oligozoospermic: A low number of sperm in the semen (low sperm count).

Asthenozoospermic: Sperm have poor or low motility.

Teratozoospermic: Sperm have abnormal morphology .(American Society for Reproductive Medicine, 2012).

E.The peritoneal factor:

Peritoneal factor infertility refers to abnormalities involving the peritoneum (lining of the surfaces of your internal organs) such as scar tissue (adhesions) or endometriosis **.**Endometriosis is a condition where tissue that normally lines the uterus begins to grow outside the uterus. This tissue may grow on any structure within the pelvis including the ovaries and is found in about 35% of infertile women who have no other diagnosable infertility problem Endometriosis is found more commonly in women with infertility, pelvic pain, and painful intercourse. Endometriosis may affect the function of the ovaries, your ovarian reserve, the function of the fallopian tubes.(American Society for Reproductive Medicine , 2012).

F. Unexplained infertility:

In approximately 10% of couples trying to conceive, all of the above tests are normal and there is no easily identifiable cause for infertility. In a much higher percentage of couples, only minor abnormalities are found that should not be severe enough to result in infertility. In these cases, the infertility is referred to as "unexplained". Couples with unexplained infertility may have problems with egg quality, fertilization, genetics, tubal function, or sperm function that are difficult to diagnose and/or treat. Fertility drugs and IUI have been used in couples with unexplained infertility with limited success. If no pregnancy occurs within three to six treatment cycles, IVF may be recommended and has been shown to be the most effective treatment for unexplained infertility.(American Society for Reproductive Medicine, 2012).

2.8:Assessment of ovarian aging:

Ovarian aging will have begun before women notice any clinical changes to their menstrual cycles; therefore, they are often unaware that they may be at greater risk of infertility. Ovarian reserve testing has been explored as a means to determine a woman's fertility potential and provide an assessment of ovarian aging. Although chronological age alone serves as a good marker of

ovarian reserve, some women will experience a decline in their natural fertility. sooner than average, while some older women may maintain above average ovarian function. Identification of these two groups, in which ovarian reserve is inconsistent with chronological age, may be useful for counseling and planning treatment.(American Society for Reproductive Medicine ,2012). Many tests of ovarian reserve have been tried. However, testing has mainly been performed on infertile populations, with little data on the distribution in the normal fertile population. Ovarian reserve testing cannot be used to predict infertility or time to infertility; therefore, its application to the general population as a screening tool is untested. Most studies have used these tests to try to predict a woman's ovarian response and prognosis with fertility treatment and IVF. Overall, markers of ovarian reserve have been shown to correlate with egg quantity and response to ovarian stimulation, but not with egg quality. (American Society for Reproductive Medicine, 2012).

2.8.1 Female fertility hormones:

Follicle Stimulating Hormone (FSH) Luteinizing Hormone (LH), and Prolactin (PRL).The hypothalamus produces gonadotropin-releasing hormone (GnRH) which stimulates anterior pituitary production of FSH and LH; FSH stimulates ovarian follicle development and estrogen production during the follicular phase; the midcycle peak of LH stimulates ovulation (ovulation occurs about 24 hours after peak LH) and thereafter LH stimulates progesterone production in the luteal phase by the corpus luteum.(Clinipath Pathology Newsletter, 2011).

2.8.1.1:Follicle Stimulating Hormone (FSH):

The most commonly used test of ovarian reserve is the cycle day 3 or basal FSH level. An elevated basal FSH level (> 14 IU/L) is the first sign of ovarian aging that can be detected in women, and usually occurs in women aged35 to

40. Physiologically, the follicular pool is reduced to approximately 10% of the levels present at puberty.The rise in basal FSH is due to a loss in ovarian feedback (inhibin-A and B) as the available follicular cohort diminishes. Basal FSH levels are easy to obtain, and no special skills are required to perform the test or interpretthe results; therefore, it is easily accessible. However, basal FSH levels have been shown to be predictive for poor response to ovarian stimulation and for non-pregnancy only when the levels are extremely elevated.Although a high threshold may improve the usefulness of the test.in predicting a poorer prognosis, only a small number of women will have abnormal tests at this threshold. In addition, it has been associated with a false positive rate of 5%. Elevated basal FSH levels are also less predictive of pregnancy for women < age 35.(Kato, 1988).

2.8.1.2:Luteinizing hormone (LH):

is a glycoprotein hormone having two subunits. The alpha subunit is similar to those of follicle-stimulating hormone (FSH), human chorionic gonadotropin (hCG), and thyroid-stimulating hormone (TSH). The beta subunit is different from those of the other glycoprotein hormones and confers its biochemical specificity. LH is secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus. In males, LH is also called interstitial cell-stimulating hormone (ICSH). In both males and females, LH secretion is regulated by a balance of positive and negative feedback mechanisms involving the hypothalamic-pituitary axis, the reproductive organs, and the pituitary and sex steroid hormones. LH and the other pituitary gonadotropin, FSH, play a critical role in maintaining the normal function of the male and female reproductive systems (Bayer*et al* ., 1996).

2.8.1.3:Prolactin (PRL):

is one of several hormones that are produced by the pituitary gland. PRL has many different roles throughout the body, and most of those are clearly shown as clinical symptom. Perhaps the most important classical role of prolactin is to stimulate milk production in women after the delivery of a baby. Prolactin levels increase during pregnancy causing the mammary glands to enlarge in preparation for breastfeeding and ready to secrete colostrums closely after delivery. Later on the elevated prolactin levels help with the sustained production of milk during nursing. The somatomammotrop cells of the anterior pituitary gland synthesize and secrete prolactin, which is under the control of hypothalamic factors, mainly the tonic inhibition of Dopamine (DA). There are several other sources of PRL-like substances in the periphery such as placental lactogens, (similar to pituitary PRL), mammary gland (produced within the mammary epithelial cells), or PRL variants of immune cell origin (that modulates the immune system). (Gellersen*et al*., 1989; Andersen, 1990 ;Lkhider, 1996 ; Kurtz,1993; Gala , 1994; Montgomery,1990; Ben-Jonathan , 1996 and Yu-Lee, 1997).

It is important to underline that serum PRL in normal individuals is considered as almost entirely pituitary PRL sources, the above mentioned extra pituitary-PRL may contribute significant amounts but either carries as specific function and target mainly to the local environment acting via paracrine/autocrine manner. (Yu-lee, 1997 and Bachelot, 2007).

Generally, the lactogenic hormones play role also in regulation of reproductive function. On one hand, PRL is essential to maintain regular oestrus cycles. One of the other actions of PRL is to stimulate ovarian production of progesterone. That is required in the process of preparation for embryo implantation and it is dependent on a continued estrogen and progesterone secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents. (Binart,2000). On the other hand, high prolactin levels are associated with an

ovulation or may cause directly or indirectly infertility. In young women, hyperprolactinemia is probably one of the most common endocrine disorders related to pituitary function. Women who are not pregnant and are not breastfeeding should have lower levels of basal PRL (typically 10–28 μg/L in women and 5–10 μg/L in men are defined as "normal levels") If a non-pregnant woman has abnormally high levels of PRL, it may cause her difficulty in becoming pregnant. It is considered as the most frequent cause of an ovulatory sterility, although spontaneous pregnancy may occur occasionally. The prevalence of hyperprolactinemiavaries in different patient populations, stays below 1% (0.4% in an unselected normal population) but can be as high as 17% of women with reproductive disorders shown at the clinics (Crosignani , 1999).

2.8.1.4 :HCG:

Human chorionic gonadotropin (hCG) is produced in the earliest stages of pregnancy. During early pregnancy it plays a role in survival of the corpus luteum.(Baird ,2003) and in stimulating the thyroid gland (Grün ,1997) , and it also appears to have a significant role in the implantation of the blastocyst and protection of the embryo against immune attack at the fetal/maternalboundary. The hormone is initially produced by the embryo and, therefore, also acts as a marker for its presence . Levels of hCG following conception have been the subject of a great deal of research over the last twenty years, but with differing objectives.(Perrier ,2007).

The main thrust of research has been directed at patients undergoing in vitrofertilisation (IVF) or assisted conception since early results indicated that levels of hCG could be used as a marker for pregnancy outcome. This marker has the potential for predicting likely pregnancy outcome in both assisted conception and natural conception pregnancies at an earlier time and more predictably than other means of monitoring pregnancy. To a lesser degree the

levels of hCG have been investigated for their potential in estimating length of gestation.(Lohstroh ,2006).

HCG levels are also being investigated alongside other biochemical and physical markers for use in predicting genetic abnormality in pregnancy.

HCG levels rise rapidly in the earliest days of pregnancy and can be detected very early on in a pregnancy. The day that hCG is reported to be first detected depends upon the method of estimating conception and on the sensitivity of the assay for hCG.(Lohstroh ,2006).

Several studies have shown hCG detection in maternal urine 6 or more days after estimated day of fertilization(Nepomnaschy , 2008) . Lenton first detected hCG in plasma on day 8 after the LH surge (measured by standard RIA) but in only 5.3% of cases and in a recent prospective study by Cole, hCG detection in urine was detected as early as 4 days following ovulation (LH peak measure. However, these analyses rely on extremely sensitive measurements made in the region of the assay curve displaying high coefficients of variation, so some spread in first day of detection would be expected. For example, the Immunolite assay used by Cole only has a sensitivity of 1mIU/ml hCG, so measurements this early in pregnancy would be challenging the analytical capability of the assay .(Cole , 2008).

IVF pregnancies may not be the obvious place to find evidence for uniformity of hCG rise, because by their nature, they reflect pregnancies in which conception has usually been problematic. Therefore levels maynot necessarily be comparable to natural conceptions. However, this type of study does have the advantage of having greater precision in calculating gestational age in relation to hCG, as embryo implantation canbe considered a fixed time point.(Nepomnaschy ,2008).

2.9:Treatment options and alternatives:

(Assisted Reproductive Technologies)

If a cause for infertility is identified, the clinical care provider may suggest a specific treatment. However, sometimes no specific problem is found, and the infertility is labeled as "unexplained." With unexplained infertility, or when traditional treatments have failed, advanced infertility therapies such as super ovulation with timed intrauterine insemination (SO/IUI) or in vitro fertilization (IVF) may be suggested. In an SO/IUI cycle, fertility medications are administered to start the growth of multiple eggs in the ovaries. When these eggs are ready to ovulate, the partner's washed sperm is placed directly into a woman's uterus. This procedure is called intrauterine insemination (IUI) and causes minimal discomfort. IVF involves removing the eggs and fertilizing them with the male partner's sperm in the lab oratory and then transferring the resulting embryos to the uterus .(American Society for Reproductive Medicine ,2012).

2.9.1: In vitro fertilization (IVF):

The chances of pregnancy in infertile couples have been increased considerably by IVF (bing and Ouellette, 2009). Gamete intra fallopian transfer (GIFT) and zygote intra fallopian transfer (ZIFT) have been reported to be more effective than IVF, but are not frequently used techniques because tubal transfer of the gametes requires a laparoscopic procedure. IVF has an average success rate of 25-30% per cycle ; variation depends on the patient profile, female age being the most significant one. Success rate is inversely proportional to the increasing number of cycles and duration of infertility. However, previous pregnancy and live birth increases the chances of pregnancy in the future attempts. Success rate in most of the infertile women are similar ;however , women with multiple pelvic surgeries or history of severe endometriosis have 5% lower success rate . this may be attributed to

poor response to OS (Adamson and Baker, 2003).Despite tremendous progress in the field of assisted reproduction over the years, the current success rate of ART remains quite unsatisfactory and is limited to 30-40% (Gerris*et al* .,1999). Possible causes associated with poor ART success rate are being actively investigated worldwide (Swain and pool, 2008).

2.10: Chances of success with IVF:

Each couple has a unique set of circumstances, and the chances of treatment success vary widely. The success of IVF depends on many factors, especially the age and the quality of the embryos..(American Society for Reproductive Medicine, 2012).

Chapter three

Materials and Methods

3. Materials and Methods:

3.1 Materials:

3.1.1 study approach:

A quantitative method was used to estimate FSH, LH and PRL

concentration in infertile Sudanese women during the period from January to July 2017.

3.1.2 Study design:

Cross sectional study

3.1.3 Study area:

The study was conducted in Khartoum State in Sudan Assisted Reproduction Center (SARC).

3.1.4 Study population:

The study included infertile Sudanese women above 34 years.

3.1.5 Sample size:

The study included one hundred and fifty women participate in this study, group (1) (35-37) years included 44 women , group (2) (38-40) years included 59 women and group (3) (41-45) years included 47 women .

3.1.6 Inclusion criteria:

Infertile Sudanese women above 34 years under IVFtreatment were included .

3.1.7 Exclusion criteria:

The criteria of exclusion based on excluding any women under the age of 35 ,or under treatment by other reproductive techniques than IVF like IUI and ICSI, and women without infertility problems were excluded .

3.1.8 Ethical consideration:

Consent was taken regarding acceptance to participate in the study and re-assurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.1.9 Data collection:

Data were collected from records by using structural questionnaire, which was designed to collect and maintain all valuable information concerning each group examined.

3.1.10 sample collection and processing:

About 3 ml of venous blood (on the 2^{nd}or 3^{rd} day of the menstrual cycle) were collected from each participant women.

The sample collected under aseptic conditions in sterile Heparin containers and centrifuged for 5 minutes at 3000 RPM to obtain plasma for FSH ,LH, PRL (recommended to test the sample within 24 hours after collection).

3.2 Methods :

3.2.1 Estimation of FSH, LH PRL by using a fluorescence immunoassay (FIA) method:

3.2.1.1 Principle of method:

The tests use a sandwich immunodetection method ; the detector antibody in buffer binds to antigen in sample , forming antigen- antibody complexes , and migrates on to nitrocellulose matrix to be captured by the other immobilized – antibody on test strip .

The more antigen in sample form the more antigen – antibody complex and leads to stronger intensity of fluorescence signal on detector antibody , which are processed by instrument for ichroma tests to show FSH, LH, PRL concentrations in sample.

3.2.1.2 Procedure:

- One hundred and fifty micro liter for FSH and LH, and seventy fivemicro liter for PRL were transferred plasma of sample using a transfer pipette to tube containing the detection buffer.

-The lid of the detection buffer tube was closed and mixed the sample thoroughly by shaking it about 10 times.

- Seventy fife micro L of sample was pipette, mixed and loaded it in to the sample well on the cartridge for three hormones.

-The sample-loaded cartridge was leave at room temperature for 15 minutes.

-The sample was insertd in the cartridge holder of instrument for ichromatests .ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder . An arrow has been marked on the cartridge especially for this purpose .

-Ichroma tests was press to start the scanning process.

- Instrument for ichroma tests will be started scanning the sample-loaded cartridge immediately.

-The test result was red on the display screen of the instrument for ichromatests .

3.2.2Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.2.3 Statistical analysis:

Data obtained from this study was analyzed using statistical package for the social science (SPSS) version 20 T.test used for comparison and person correlation used for correlation .

Chapter four

Results

4.Results:

The results of the biochemical determinant of plasma FSH, LH and PRL in infertile Sudanese womenare given in tables and figures.

Table (4-1): illustrate the mean concentration of FSH,LH and PRL in group (2) compared to group(1) in infertile women.

The mean of FSH was significantly increase in group (2) compared to group(1) (p. value=0.03). While there were insignificant different in the mean of LH and PRL levels in group (2) compared to group (1) (p- value 0.31) and (p- value = 0.06). respectively .

Mean ±SD for group2 versus group1:

(10.32±7.03 versus 7.98±3.36) mIU/ml for FSH.(5.46 ±2.95 versus 5.30±2.24) mIU/ml for LH.(21.45 ±16.05 versus 19.94±12.92) ng|ml for PRL.

Table (4-2): illustrate the mean concentration of FSH,LH and PRL in group (3) compared to group group(1) in infertile women .

The mean of FSH was significantly increase in group (3) compared to group(1) (p. value=0.007). While there were insignificant different in the mean of LH and PRL levels in group (3) compared to group (1) (p- value 0.69) and (p- value = 0.2).respectively .

Mean ±SD for group3 versus group1:

(11.19±6.40 versus 7.98 ± 3.36)mIU|ml for FSH.(5.06±2.74 versus 5.30 ± 2.24) mIU/ml for LH.(22.62±16.05 versus 19.94 ± 12.92)ng|ml for PRL.

Table (4-3): illustrate the mean concentration of FSH,LH and PRL in group (3) compared to group(2) in infertile women .

Therewere insignificant different in the mean level of FSH , LH and PRL in group (3) compared to group(2) (p. value=0.5) , (p- value 0.71) and (p- value = 0.6). respectively.

Mean ±SD for group3 versus group2:

(11.19±6.40 versus 10.32 ± 7.03)mIU|ml for FSH. (5.06±2.74 versus 5.46 ± 2.95) mIU|ml for LH.(22.62±16.05 versus 21.45 ± 9.70)ng|ml for PRL.

Table (4-4): Show the percentage of other factors shared age to increase risk of infertility in Sudanese women included: female factor (tubal block 20.6%, PCO 12% and fibroid 1.3%),male factor (34.7%) , both male and female factors(6.7%) and un explaining factor(24.7%).

Figure (4-1): Show correlation between FSH concentration and age .

There was a significant weak positive correlation between age and FSH concentration . (r =0.198, P. Value = 0.01).

Figure (4-2): Show Correlation between LH concentration and age .

There was no correlation (r = 0.016, P. Value = 0.84).

Figure (4-3): Show Correlation between PRL concentration and age .

There was insignificant weak negative correlation (r= - 0.19, P. Value=0.23).

Figure (4-4): show success rate of IVF treatment ,21.3% of infertile women under IVF treatment have positive result (β -HCG))(10.6% in group (1) ,8.67% in group (2) and 2.0% in group (3)). while 78.7% of women under IVF treatment have negative result (β -HCG).

Table (4-1): Comparison between mean concentration ofplasma FSH ,LH and PRL in group (2) and group(1) in infertile Sudanese women.

Variables	Group2 38-40 (years) N=59 Mean ± SD	Group1 35-37 (years) N=44 Mean ± SD	P.value
FSH (mIU|ml)	10.32±7.03	7.98±3.36	0.03
LH (mIU|ml)	5.46±2.95	5.30±2.24	0.31
PRL (ng|ml)	21.45±9.70	19.94±12.92	0.06

Results given in mean ± SD. P. Value ≤ 0.05 consider significant.

Table (4-2): Comparison between mean concentration ofplasma FSH ,LH and PRL in group (3) and group(1) in infertile Sudanese women.

Variables	Group3 41-45 (years) N=47 Mean ± SD	Group1 35-37 (years) N=44 Mean ± SD	P.value
FSH (mIU|ml)	11.19±6.40	7.98±3.36	0.007
LH (mIU|ml)	5.06±2.74	5.30±2.24	0.69
PRL (ng|ml)	22.62±16.05	19.94±12.92	0.2

Results given in mean ± SD. P. Value ≤ 0.05 consider significant.

Table (4-3): Comparison between mean concentration ofplasma FSH ,LH and PRL in group (3) and group(2) in infertile Sudanese women.

Variables	Group3 41-45 (years) N=47 Mean ± SD	Group2 38-40 (years) N=59 Mean ± SD	P.value
FSH (mIU|ml)	11.19±6.40	10.32±7.03	0.5
LH (mIU|ml)	5.06±2.74	5.46±2.95	0.71
PRL (ng|ml)	22.62±16.05	21.45±9.70	0.6

Results given in mean ± SD. P .Value ≤ 0.05 consider significant.

Table (4-4): Other factors shared age to increase risk of infertility in Sudanese women .

Association factors		Percent %
Male factor		34.7%
Female factors	Tubal block	20.6%
	PCO	12%
	Fibroid	1.3%
Both		6.7%
Un explaining factor		24.7%

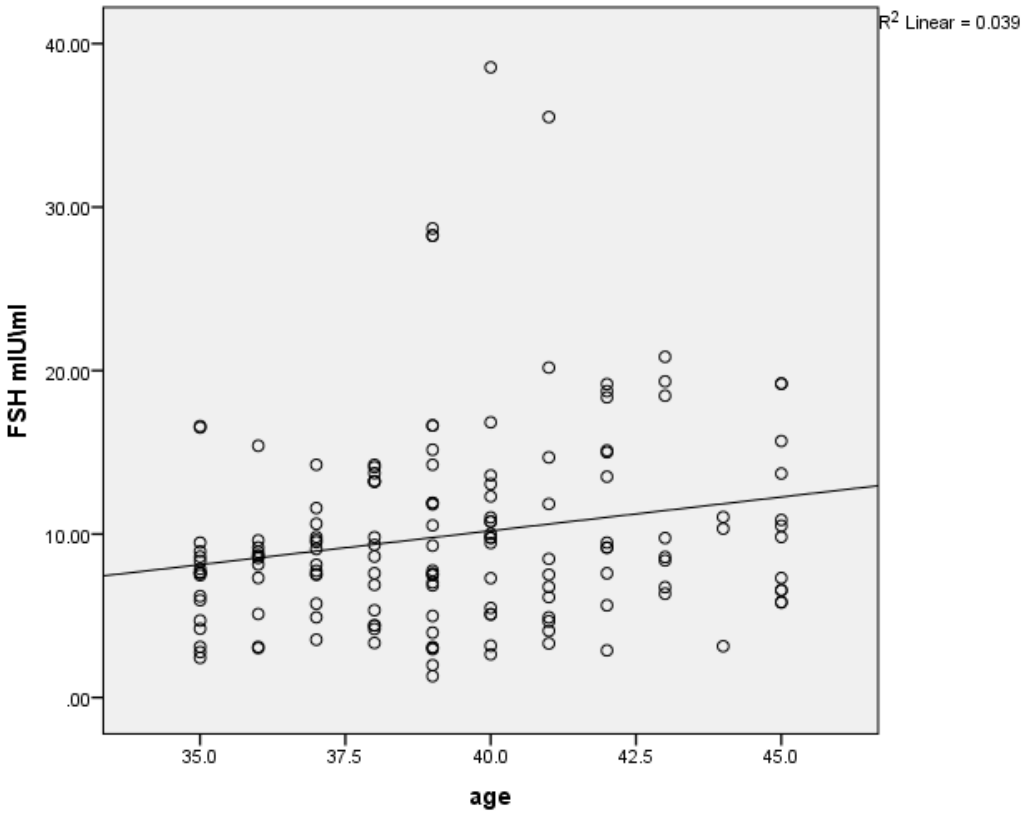

Figure (4-1): Correlation between FSH (mIU|ml) and age . (r =0.198, P. Value = 0.01).

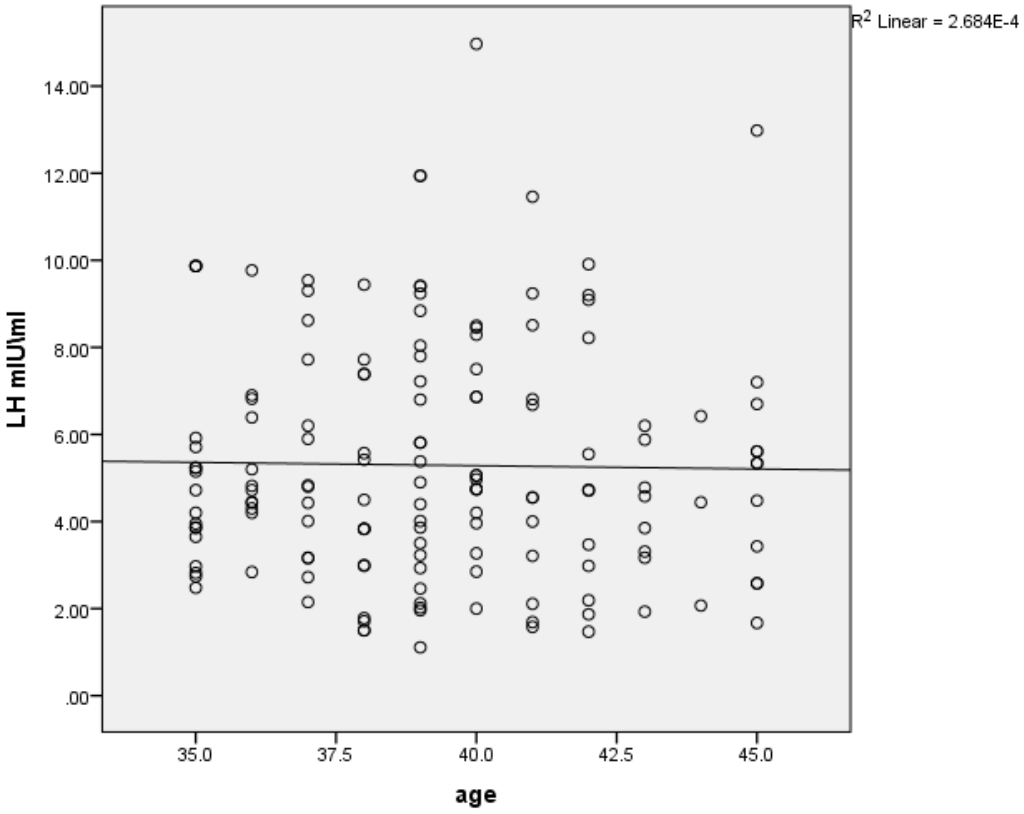

Figure (4-2): Correlation between LH(mIU|ml) and age . (r = 0.016, P. Value = 0.84).

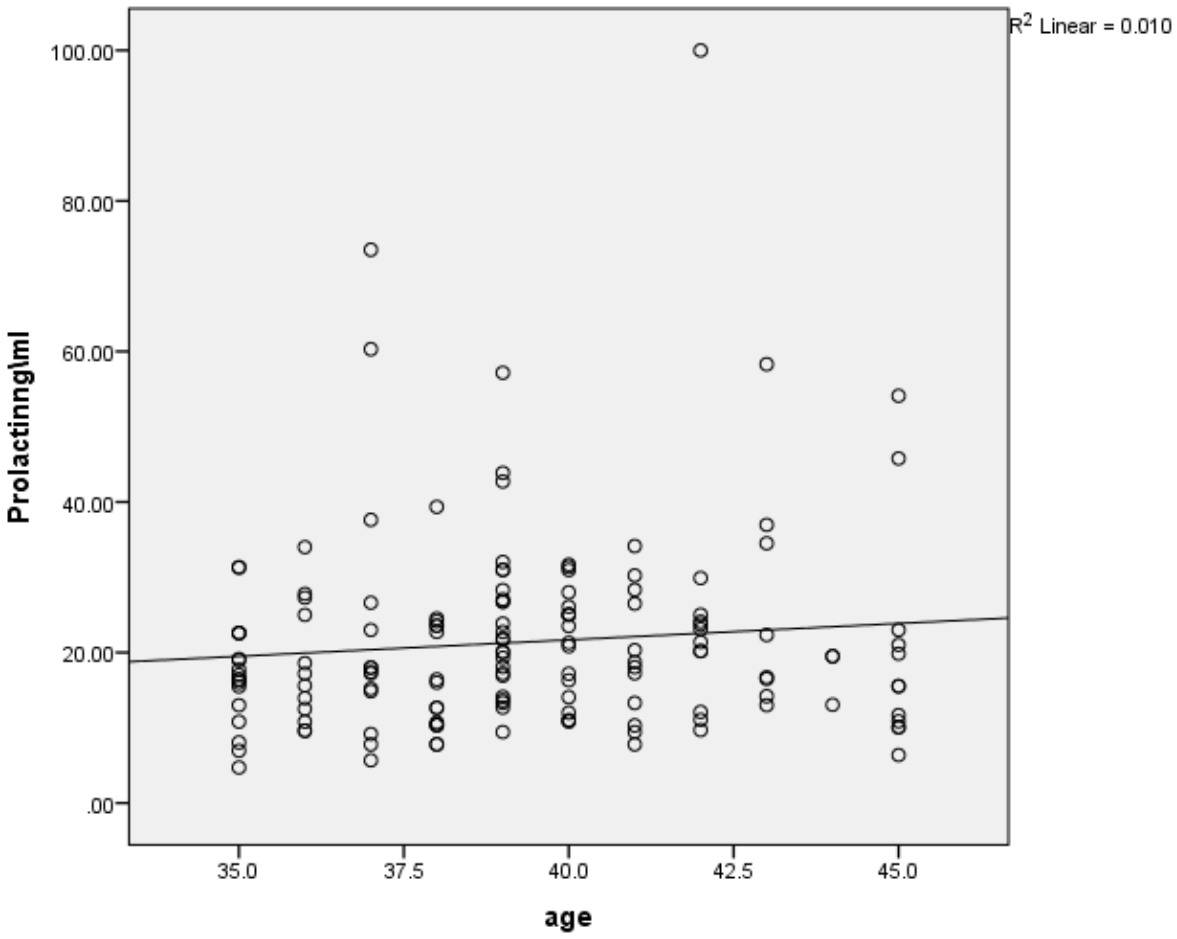

Figure (4-3): Correlation between PRL (ng|ml) and age . (r= - 0.19, P. Value=0.23).

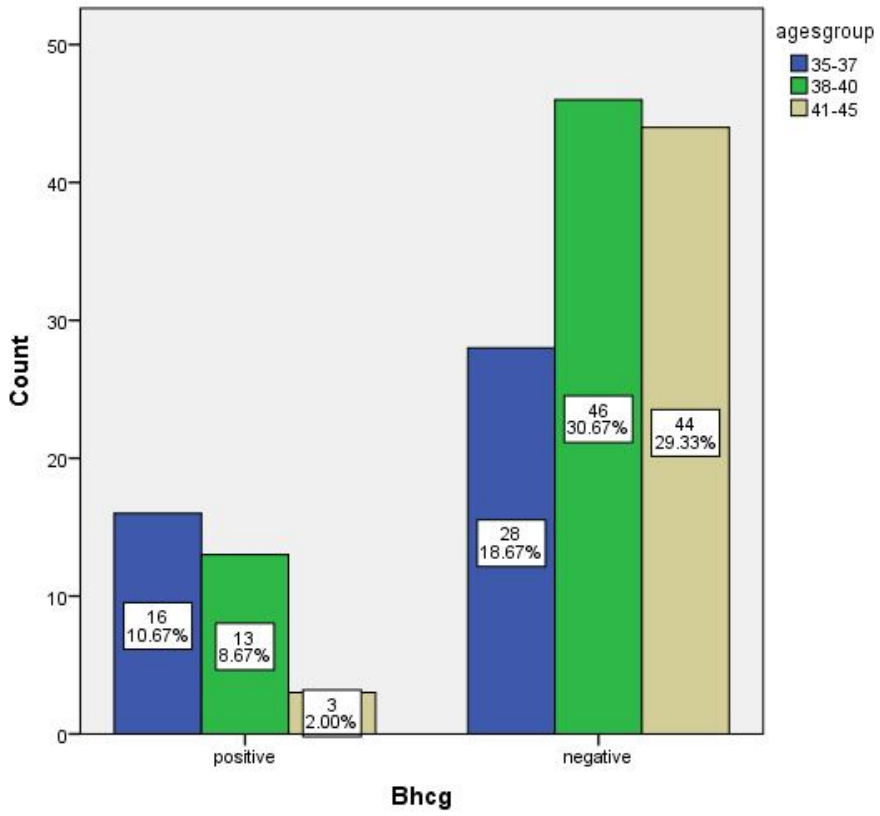

Figure (4-4) success rate of IVF treatment(positive β-HCG) in infertile Sudanese women .

CHAPTER FIVE

Discussion, conclusion and Recommendations

5. Discussion

5.1 Discussion

Female infertility is multi factorial, but primarily it is due to normal age, ovulation problems , blockage of Fallopian tube , (Scott *et al.,* 1989). This study was carried out to determine the levels of FSH, LH and Prolactin in infertileSudanesewomen.

The results of this study showed, the means concentration of FSH were significantly increase in both group (2) and group (3) compared to group (1) (p. value=0.03) (p -value=0 .007) respectively, while there was insignificant different in FSH level group (3) compared to group (2) (p- value = 0.5). This result agreed with another result carried by (Sherman *et al*.,1976; Ban *et al*., 2013;Aroma *et al*., 2014 and Balen*et al*., 1997). which showed that , FSH level was significant increase in women above 34 years when divided in to two groups (less than 35 years and above), the elevated level of FSH indicate reduced gonadal function ovarian reserve ; declines with age due to decline in inhibin-B, which is produced by the granulose cells in the early follicular phase. There is an inverse correlation between FSH and inhibin-B, which is likely due to a loss in negative feedback; the rise in FSH during the early follicular phase is one of the earliest signs of ovarian aging.(Klein *et al* .,1996).The result of this study showed, there were insignificant different between the mean of LH and prolactin levels in infertile when compared between study groups (p- value≥0.05). This result agreed with another result carried by (Ferdinand *et al*., 2012) ,which found, there was insignificant difference between prolactin concentration in different ages groups.Also the result disagreed with another result, which showed that the mean concentration of LH changed little with age. (Lee *et al*.,1988) .

Other factors shared age to increase risk of infertility inSudanese women included: female factor (tubal block 20.6%, PCO 12% and fibroid 1.3%),male factor (34.7%), both male and female factors (6.7%)and un explaining factor (24.7%).This result agreed with another result, which showed that40% of infertility problems from male and 60% from female. (American Society for Reproductive Medicine , 2012).

Also the result showed that, there was a significant weak positive correlationbetween age and FSH level (r =0.198, P .Value = 0.01), while there was no correlation between age and LH level (r = 0.016, P. Value = 0.84).The result showed, there was insignificant weak negative correlation between age and PRL level . (r= - 0.19, P. Value=0.23). The finding obtained from especially designed questionnaire revealed that,10.6%of positive result of –β HCG in group (1) ,8.67% in group (2) and 2.0% in group (3) . This results agreed with another result carried by (Kimberly*et al*., 2011), which showed, assisted reproductive technology success were significantly decrease for women in their late 30s and 40s.Because of the decline in fertility and the increased time to conception that occurs after the age of 35. And other result carried by (Gunby*etal* ., 2011) reported that, Pregnancy rates collected from ART treatment cycles showthe significant impact of female age on success. The rate after IVF was 37.4% for women< 35 years of age, 26.5% for women aged 35 to 39 years, and11.4% for women aged ≥ 40 years, 1.9% for women aged 45 and over**.**

5.2 Conclusion

According to the results of this study it is concluded that:

Serum FSH is increased in infertile Sudanese women and FSH positively correlated to age, no correlation between age and LH, and there is weak negative correlation between PRL and age, and Success rate of IVF decrease with age.

5.3 Recommendations

From the finding of this study it is recommended that :

1.Infertility women should be monitor anti mollarian hormone(AMH) especially over the age of 40 under IVF treatment.

2. Estradiol (E_2) should be measure . (with FSH to determine ovarian reserve).

3.For infertility women in their 20s ; it is better to do IVF at this age to increase the success rate ; because age is main risk factor to the success rate of IVF.

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Appendices

Appendix I

Questionnaire

Sudan University of science and Technology

College of graduate studies

Effect of Age on Success Rate of IVF Treatment among Infertile Sudanese women

in Khartoum State

Number ( )

A. **General information**:

Name :……

Age:……

B. Association causes of infertility:

Tubal block ( ) PCO ( ) Fibroid ( ) Male factor ( ) Both ( ) un known ( )

C. **Hormone profile**:

FSH:…….mIU|ml .

LH:……mIU|ml .

PRL:……ng|ml.

B-HCG**:**

Positive ( ) Negative ( )

Document No. : INS-FS-EN (Rev. 08)
Revision date : July 20, 2016

Appendix II

Hormone

ichroma™ FSH

INTENDED USE

ichroma™ FSH is a fluorescence Immunoassay (FIA) for the quantitative determination of follicle stimulating hormone(FSH) in human serum/plasma. It is useful as an aid in management and monitoring of concentration of FSH.

For *in vitro* diagnostic use only.

INTRODUCTION

Follicle-stimulating hormone (FSH) is synthesized and secreted by gonadotrophs of the anterior pituitary gland. The alpha subunits of LH, FSH, TSH, and hCG are identical, and contain 92 amino acids. FSH has a beta subunit of 118 amino acids (FSHB), which confers its specific biologic action and is responsible for interaction with the FSH-receptor. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and Luteinizing hormone (LH) act synergistically in reproduction.

The most common reason for high serum FSH concentration is in a female who is undergoing or has recently undergone menopause. High levels of Follicle-Stimulating Hormone indicate that the normal restricting feedback from the gonad is absent, leading to an unrestricted pituitary FSH production. If high FSH levels occur during the reproductive years, it is abnormal. Conditions with high FSH levels include: Premature menopause also known as Premature Ovarian Failure, Poor ovarian reserve also known as Premature Ovarian Aging, Gonadal digenesis, Turner syndrome, Castration, Swyer syndrome, Certain forms of Congenital adrenal hyperplasia (CAH), Testicular failure.

Most of these conditions are associated with subfertility and/or infertility. Therefore high FSH levels are an indication of subfertility and/or infertility.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show FSH concentration in sample.

COMPONENTS

ichroma™ FSH consists of 'Cartridges', 'Detection Buffer Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human FSH at the test line, while chicken IgY at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human FSH-fluorescence conjugate, anti chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in phosphate buffered saline.
- The detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packaged in a Box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ FSH** as well as the Instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that Instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ FSH** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ FSH** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, sodium citrate, sodium heparin** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

Document No. : INS-LH-EN (Rev. 10)
Revision date : May 30, 2016

Appendix III

BIO TECHNOLOGY boditech

Hormone

ichroma™ LH

INTENDED USE

ichroma™ LH is a fluorescence Immunoassay (FIA) for the quantitative determination of Luteinizing hormone (LH) in human serum/plasma. It is useful as an aid in management and monitoring of determination of evaluating fertility issues, function of reproductive organs (ovaries or testicles), or detection of the ovulation.

For *in vitro* diagnostic use only.

INTRODUCTION

Human luteinizing hormone (LH, lutropin) is a glycoprotein hormone with two dissimilar subunits (α and β). LH has a molecular weight of approximately 29,000 daltons.[1] The α-subunit of LH contains 92 amino acid residues and is essentially identical to the β-subunits of follicle stimulating hormone (FSH, follitropin), thyroid stimulating hormone (TSH, thyrotropin), and human chorionic gonadotropin (hCG).[1-4] The β-subunit of LH contains 112 amino acid residues and is considerably different from that of FSH and TSH.[1,4,5] However, the β-subunits of LH and hCG are very similar. The structural similarities between LH and hCG are responsible for the observed similarity in biological properties.[1,5,6] In the female, hLH stimulates the final maturation of the follicle, follicular rupture, and ovulation.[7] Human LH is secreted by the gonadotropic cells of the anterior lobe of the pituitary gland in response to gonadotropin releasing hormone (GnRH) from the medial basal hypothalamus. Both hLH and hFSH are secreted in a pulsatile nature; however, this is less noticeable for hFSH perhaps due to the longer half-life in the circulation.[7] In a normal menstrual cycle negative feedback by estradiol suppresses hLH secretion in the follicular phase. As the follicle develops (in response to hFSH) estradiol production increases which triggers an increase in GnRH and an increased sensitivity of the pituitary to GnRH. A GnRH surge results in the preovulatory (mid-cycle) surge of hLH and ovulation. Following this surge, hLH is suppressed during the luteal phase due to negative feedback from progesterone and estradiol.[7-9] Variation in cycle lengths are observed in normally menstruating females due to variations in the length of the follicular phase. In the menopausal female, hLH levels are elevated in response to decreased production of ovarian estrogens and progestogens, which eliminates the negative feedback mechanism on the pituitary gland. As a result, ovulation and menstrual cycles decrease and eventually cease.[10] In the male, hLH is often referred to as interstitial cell-stimulating hormone and influences the production of testosterone by the Leydig cells of the testes.[11] At menopause, or following ovariectomy in women, concentrations of estrogens decline to low levels. The lowered concentrations of estrogens result in a loss of the negative feedback on gonadotropin release. The consequence is an increase in the concentrations of LH and FSH.[12,13,14] Concentrations of hLH and hFSH are commonly determined in investigations of menstrual cycle, fertility, and pubertal developmental abnormalities, such as premature ovarian failure, menopause, ovulatory disorders and pituitary failure.[15] The ratio of hLH/hFSH has been used to assist in the diagnosis of polycystic ovary disease. Low concentrations of hLH and hFSH may indicate pituitary failure while elevated concentrations of hLH and hFSH along with decreased concentrations of gonadal steroids may indicate gonadal failure (menopause, ovariectomy, premature ovarian syndrome, Turners Syndrome).[16] Low concentrations of gonadotropin are usually observed in females taking oral steroid based contraceptives.[17] In the male, elevated hLH and hFSH with low concentrations of gonadal steroids may indicate testicular failure or anorchia. In Klinefelter's syndrome hLH may be elevated due to Sertoli cell failure.[18]

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show LH concentration in sample.

COMPONENTS

ichroma™ LH consists of 'Cartridges', 'Detection Buffer Tubes' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human LH at the test line, while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human LH-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in CAPSO buffer.
- The detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packaged in a Box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (Cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ LH** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ LH** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ LH** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, sodium heparin, sodium citrate** should be avoided.

Appendix IV

BIO TECHNOLOGY boditech

Hormone

ichroma™ PRL

INTENDED USE

ichroma™ PRL is a fluorescence Immunoassay (FIA) for the quantitative determination of Prolactin (PRL) in human serum/plasma. It is useful as an aid in management and monitoring of hypothalamic-pituitary disorders.

For *in vitro* diagnostic use only.

INTRODUCTION

Human Prolactin (PRL: lactogenic hormone) is secreted from the anterior pituitary gland in both men and women. PRL is a single chain polypeptide hormone with a molecular weight of approximately 23 kDa. Normal women have slightly higher basal level of PRL than men; apparently, there is an estrogen-related rise at puberty and a corresponding decrease at menopause. During pregnancy, PRL level increases progressively to 10 and 20 times of normal value, declining to non-pregnant levels by 3-4 weeks post-partum.

The determination of PRL concentration is helpful in diagnosing hypothalamic-pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High PRL levels are commonly associated with galactorrhea and amenorrhea. PRL concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism. Also, PRL levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of PRL is episodic and demonstrates diurnal variation.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma™ tests to show PRL concentration in sample.

COMPONENTS

ichroma™ PRL consists of 'Cartridges', 'Detection Buffer Tubes', and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human PRL at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human PRL-fluorescence conjugate, Biotin-BSA conjugate- fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ PRL** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ PRL** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ PRL** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.