ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

Ph.D THESIS

Zahraa Ahmed Hussein AL-ZAIDI

PERSPECTIVE STUDY OF CYTOKIN LEUKEMIA-INHIBITORY FACTOR LIF AND INTERLEUKIN-11 IL-11 GENE EXPRESSION AND THEIR EFFECT ON INFERTILE TURKISH WOMEN

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Doctor of Philosophy by the board of jury on 25/04/2022.

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ABSTRACT

Ph.D THESIS

PERSPECTIVE STUDY OF CYTOKIN LEUKEMIA- INHIBITORY FACTOR LIF AND INTERLEUKIN-11 (IL-11) GENE EXPRESSION AND THEIR EFFECT ON INFERTILE TURKISH WOMEN

Zahraa Ahmed Hussein AL-ZAIDI

ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES DEPARTMENT OF BIOTECHNOLOGY

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In this study three different groups consist of 25 Turkish women whose suffering from primary infertility for different reasons, polycystic ovary syndrome PCOS, tubal factor infertility (TFI), and unexplained infertility (UI), as well as a group of control healthy fertile women underwent genetic assessment analysis, in Kayseri Province/ Turkey.

For investigating the pathophysiology of infertility for these groups, this study linked to two main cytokine genes interleukin 11 IL-11 and leukemia inhibitory factor LIF, since they have fundamental roles in regulating the blastocyst implantation process in the uterine epithelium. The expressions of LIF and IL-11 genes were evaluated by quantitative real-time polymerase chain reaction (qRT–PCR) in patients and healthy controls.

As a result of the genetic test, all three groups showed promising results regarding LIF gene, which appeared at very small levels compared to the control group, indicating the great importance of this gene in healthful pregnancy.

On the other hand, regarding IL-11, the two groups TFI, and UI, were significantly linked to the lower level of the gene expression, while the PCOS group has no significant difference when compared to control group, indicating the crucial role of this gene with successful embryo implantation

Keywords: Infertility, Interleukin-11, leukemia inhibitory factor, polycystic ovary syndrome, Tubal factor, Unexplained infertility.

ÖZ

DOKTORA TEZİ

SİTOKİN LÖSEMİ-İNHİBİTÖR FAKTÖR LIF VE INTERLEUKIN-11 IL-11 GEN EKSPRESYONU VE KISIR TÜRK KADINLARINA ETKİSİNİN PERSPEKTİF ÇALIŞMASI

Zahraa Ahmed Hussein AL-ZAIDI

ÇUKUROVA ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
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Bu çalışmada, polikistik over sendromu PCOS, tübal faktör infertilitesi (TFI) ve açıklanamayan infertilite (UI) gibi farklı nedenlerle primer infertiliteye sahip olan Türk kadınları 25 kişiden oluşan 3 farklı gruba ve sağlıklı doğurgan kadınlardan oluşan kontrol grubuna ayrılarak bu gruplarda Kayseri/Türkiye'de genetik değerlendirme analizi yapılmıştır.

Bu gruplarda infertilitenin patofizyolojisini araştırmak üzere bu çalışma uterus epitelindeki blastosist implantasyon sürecini düzenlemede temel bir role sahip iki ana sitokin geni olan interlökin 11 IL-11 ve lösemi inhibitör faktör LIF ile ilişkilendirmiştir. LIF ve IL-11 genlerinin ekspresyonları, hastalarda ve sağlıklı kontrollerde kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (qT-RT PCR) Light Cycler ile değerlendirildi.

Genetik test sonucunda LIF geni için her üç grubun da kontrol grubuna göre çok küçük düzeylerde olması LIF geni ile ilgili umut verici bir sonuç olup, sağlıklı bir gebeliğin olmasında büyük önem taşıdığını göstermektedir.

Bununla birlikte, IL-11 gen ekspresyonu TFI ve UI gruplarında kontrole göre düşük iken, PCOS grubunda anlamLı bir fark elde edilememiştir.

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Anahtar kelimeler: Kısırlık, Interlökin-11, Lösemi inhibe edici faktör, Polikistik over sendrom, Tüp faktörü, Açıklanamayan kısırlık.

EXPANDED ABSTRACT

Infertility is a growing sociological and health condition that affects upwards of 10% of women around the world. A variety of factors can stymie the implantation process in women at any point. Successful implantation of blastocysts more into the female uterus is indeed an important path in mammalian reproduction that is governed by a complicated web of hormones, cytokines, and growth factors, as well as their interactions. The preparation of receptive endometrium and blastocysts is aided by a complex system of these molecules. Infertility in women is induced by several of the reasons, for instance, ovulation disorders, e.g. polycystic ovary syndrome (PCOS), fallopian tube dysfunction, e.g. tubal factor infertility(TFI), and occasionally, the reason for infertility is rarely discovered, e.g. unexplained infertility (UI).

This perspective study is carried out in Erciyes University hospitals, the School of Medicine, Department of Medical Genetics. The functional roles of endometrial cytokines, notably interleukin 11 IL-11 and leukemia inhibitory factor LIF, in the pathophysiology of infertility in Turkish women, were investigated.

75 women suffering from (2 to 7) years of primary infertility between 21 and 45 years of age were studied. The patient groups are divided into three TFI, 25 patients suffering from UI, additional to 25 control women group.

The genetic assessment for each group for both in IL-11 and LIF genes, were established by using reverse transcriptase technique (cDNA) as well as the amplification process, quantitative real-time PCR (qRT–PCR). Statistical analysis of the data was done using the GraphPad Prism and $P \leq 0.05$ values were considered statistically significant.

As a result of the statistical analysis, the expression level of LIF gene in the three patient groups were found to be highly statistically significant when compared with the expression level in the control group. This result suggests that infertile women's LIF gene expression was dysregulated in both the proliferative and

secretory phases, leading to defect in the endometrial LIF activity which can be the main cause of infertility and recurrent implantation failures.

On the other side, when the expression level of the IL-11 gene in the patient's group and the control group was compared, it was not found to be statistically significant with the PCOS group $P>0.05$, this result may be related to different modulated AKT/STAT3 signaling to regulate adipocyte proliferation with high BMI value which has a crucial effect on the expression of this gene.

IL-11 expression was statistically lower in the TFI and UI groups compared to the control group. Therefore, IL-11 may play a role in the regulation of implanting process between the endometrium and the blastocyst.

In conclusion, our findings show that low levels of LIF and IL-11 gene expression are possibly linked to a variety of primary infertility conditions, including PCOS, tubal factor, and unexplained infertility since they play a fundamental role in embryo implantation. Investigation of the probability of mutations within IL-11 and LIF genes and their relation to infertility are recommended for more confirming results.

GENİŞLETİLMİŞ ÖZET

İnfertilite, dünyadaki kadınların %10'undan fazlasını etkileyen, büyüyen bir sosyolojik ve sağlık durumudur. Kadınlarda implantasyon sürecini herhangi bir noktada çeşitli faktörler engelleyebilir. Memeli üremesinde blastosistlerin dişi rahmine daha başarılı bir şekilde yerleştirilmesi hormonlar, sitokinler, büyüme faktörleri ve bunların etkileşimLerinden oluşan karmaşık bir ağ ile yönetilen önemLi bir yoldur. Alıcı endometriyum ve blastosistlerin hazırlanmasında, bu molekülleri içeren bu karmaşık sistem etkilidir. Kadınlarda infertilite, polikistik over sendromu PCOS gibi yumurtlama bozuklukları, tübal faktör infetrilitesi gibi fallop tüpü disfonksiyonu ve açıklanamayan infertilite gibi infertilite nedeninin neredeyse hiç bulunamadığı durumLarda görülmektedir.

Bu perspektif çalışma Erciyes Üniversitesi hastaneleri, Tıp Fakültesi Tıbbi Genetik Anabilim Dalı'nda gerçekleştirilmiştir. Türk kadınlarında infertilite patofizyolojisinde endometriyal sitokinlerin, özellikle interlökin 11 IL-11 ve lösemi inhibitör faktörün LIF fonksiyonel rolleri araştırılmıştır. Çalışmada 21-45 yaşları arasında 2-7 yıldır primer infertiliteye sahip 75 hasta kadın incelenmiştir. Hasta grupları her grup 25 kadın içermek üzere infertilite nedenine göre üç kategoriye ayrılmıştır. Hasta grupları; PCOS, tubal faktör, açıklanamayan infertilite şeklinde olup kontrol grubu da 25 kadından oluşmaktadır.

İnterlökin 11 ve LIF genleri için her grupta ters transkriptaz tekniği (cDNA) ve ayrıca amplifikasyon işlemi olan quantitative real time PCR (qRT-PCR) kullanılarak genetik değerlendirme yapılmıştır. Verilerin istatistiksel analizi Graphpad Prism 8 kullanılarak yapılmıştır ve $P \leq 0.05$ değerleri istatistiksel olarak anlamLı kabul edilmiştir.

İstatistiksel analiz sonuçlarında üç hasta grubunda (PCOS, tubal faktör ve açıklanamayan infertilite) LIF geninin ekspresyon düzeyi kontrol grubuyla karşılaştırıldığında ileri düzeyde yüksek bulunmuştur. Bu sonuç, infertil kadınlarda LIF gen ekspresyonunun hem proliferatif hem de sekretuar fazlarda düzensiz

olduğunu ve infertilite ve tekrarlayan implantasyon başarısızlıklarının ana nedeni olabilecek endometrial LIF aktivitesinde defekt olabileceğini göstermiştir. Diğer taraftan hasta grubu ve kontrol grubundaki IL-11 geninin ekspresyon düzeyi karşılaştırıldığında, PCOS grubu ile istatistiksel olarak anlamLı bir fark bulunamamıştır (P>0.05). bu tutarsızlık, bu genin ekspresyonu üzerinde çok önemLi bir etkiye sahip olan adiposit proliferasyonunu düzenlemek için farklı modüle edilmiş AKT/STAT3 sinyalleriyle ilgili olabilir. IL-11 ekspresyonu, kontrol grubuna kıyasla TFI ve UI gruplarında istatistiksel olarak daha düşüktü. Bu nedenle IL-11, endometrium ve blastosist arasındaki implantasyon sürecinin düzenlenmesinde rol oynayabilir.

Sonuç olarak, bulgularımız, düşük seviyelerde LIF ve IL-11 gen ekspresyonunun, embriyo implantasyonunda temel bir rol oynadıkları için PCOS, tubal faktör ve açıklanamayan infertilite dahil olmak üzere çeşitli primer infertilite koşullarıyla muhtemelen bağlantılı olduğunu göstermektedir. Daha doğrulayıcı sonuçlar için IL-11 ve LIF genlerindeki mutasyon olasılığının ve bunların infertilite ile ilişkisinin araştırılması önerilir.

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LIST OF SYMBOLS AND ABBREVIATIONS

% : Percentage
°C : Degree Celsius
Bp : base pair
DNA : Deoxyribonucleic Acid
Ml : Microlitres
PCR : Polymerase Chain Reaction
PCOS : Polycystic Ovary Syndrome
UI : Unexplained Infertility
LIF : Leukaemia Inhibitory Factor
IL : Interleukin
OSM : Oncostatin M
CNTF : Ciliary Neurotrophic Factor
NCBI : National Center for Biotechnology Information
CT-1 : Cardiotropin-1
BMI : Body Size Index
WHO : World Health Organization
ART : Assisted Reproductive Techniques
IVF : *In Vitro* Fertilization
ICSI : Intracytoplasmic Sperm Injection
IUI : Intrauterine Insemination
AMA : Advanced Maternal Age
SHBG : Sex hormone binding globulin
GH : Growth Hormone
IGFBP : Insulin-like growth factor binding protein
FSH : Follicle Stimulating Hormone
HH : Hypogonadotrophic Hypogonadism
EDC_S : Endocrine Disrupting Contaminants

ACTH : Adrenocorticotrophic Hormone
TFI : Tubal Factor Infertility
PID : Pelvic Inflammatory Disease
EGF : Epidermal Growth Factor
CAM : Cell Adhesion Molecule
KDa : Kilo Dalton

1. INTRODUCTION

Fertility refers to a woman's capacity to become pregnant; if the female is unable to conceive after a year of unprotected sexual intercourse, it is deemed infertile. Inability to conceive in 12 months for patient's female below 35 years of age, and struggling to conceive in a six-month duration for those over 35 years of age is a stricter concept of infertility(Chua et al., 2020). Infertility affects one in seven to one in five (14-20%) in female's reproductive years around the world. Today, more women are deferring their pregnancy plans until later in life. The mean age at the first pregnancy in Turkey is 22.9 years, and it is rising. Within the past ten years, the age range 25–29 has had the highest age-specific fertility rate (before that, the age group 20–24) (Özerdoğan & Yilmaz, 2018). Ovulatory abnormalities, tubal disorders, endometriosis, chromosomal defects, and UI are some of the triggers of infertility. Female age affects pregnancy physiologically and genetically, resulting in a smaller ovarian follicular reservoir, ovulation disruptions, and a rise in meiotic errors inside the oocyte. Female fertility begins to decrease at the age of 30 since a female below the age of 30 has a 20% chance of getting pregnant every month, this percentage decreases to 5% just after the age of 40 (Soma-Pillay et al., 2016). Obesity and overweight, which is a serious health condition and a common issue between females of reproductive age, Its characterized by irregular and unnecessary fat accumulation, which has a negative impact on the overall health. The elevated peripheral aromatization of androgens to estrogens affects gonadotropin production in overweight women. Obese women have the highest risk of having an unsuccessful pregnancy due to lower implantation and conception rate, increased miscarriage rates, as well as elevated mother and fetus complications of pregnancy (Kutchi et al., 2019).

Other aspects that are thought to be having a growing detrimental impact on women's conception include the increased incidence of polycystic ovary syndrome PCOS, which would be aggravated by obesity. PCOS (polycystic ovarian syndrome)

is a widespread endocrine condition that impairs 6–10% of females of productive maturity age. In reality, research shows that females with PCOS get a higher rate of high blood pressure, diabetes, and heart disease than the general population. Patients with PCOS can experience abnormal or erratic menstrual cycles, excessive hair development, skin problems, or scalp baldness, unexpected weight gain or overweight, as well as infertility (Kalyanaraman & Pal, 2021).

Tubal dysfunction is most often caused by a pelvic inflammatory disease, which accounts for the majority of all cases, and that it can damage the fallopian tube in a variety of ways. Ectopic pregnancy and sterilization are two major effects of tubal disruption. It is defined as preventing a successful pregnancy as well as entire term birth by blocking the descent of a fertilized or unfertilized oocyte into the uterus via the fallopian tubes (Egbe et al., 2020).

On the other side, UI seems to be a form of idiopathic fertility problems in which the reason is unclear. UI accounts for about 30% of all infertility couples. With UI, the disorders are almost certainly present, but current methods cannot detect them. The probable issues, that the egg is not delivered in an appropriate way for fertilization, or that it does not penetrate the fallopian tube at all. Other factors such as sperm not being able to enter the egg, fertilization failing, zygote transportation being disrupted, and embryo implantation failing, all of these reasons could be the main cause for UI (Buckett et al., 2019 & Sadeghi et al., 2015).

Embryo implantation is critical for a productive pregnancy as molecular mechanisms events (for example, growth factors and cytokine interpretation) contribute to the formation of decidua, adhesion, and invasion of trophoblast, as well as placenta development (Mori et al., 2016). Mostly within the mid secretory period, or through the 19th to the 23rd day since the menstrual cycle, the embryo implantation process occurs in the receptive endometrium.

Cytokine pathways play a crucial role in the reproductive cycle and during pregnancy. Cytokines affect uterine activities on the first day of menstruation, implantation, conception, and Parturition (Ingman & Jones, 2008). Cytokines are

frequently involved in the regulation of trophoblast invasiveness.

On the other hand, maternal hormones respond to the modulatory impact of cytokines on fetal maintenance. As a result of the disequilibrium in cytokine systems of pro-inflammatory versus anti-inflammatory cytokines, abnormal pregnancy, and infertility, including unexplained infertility, could occur (Guzeloglu-Kayisli et al., 2009).

Blastocyst implantation is indeed a highly controlled process and special characteristic of mammalian reproduction. The activity of growth factors and cytokines over their receptors plays a major role in steroidal control of uterine function. Leukemia Inhibitory Factor LIF, a part of the interleukin (IL)-6 group, is such cytokine that is needed for effective implantation. LIF is a 40–50 kDa glycoprotein with a variety of physiological activities. It is found in a variety of embryonic and mature tissues, with a significant degree of expression in the uterus. Throughout the luteal of normal menstrual cycle and the implantation process is almost effective, LIF's mRNA and it is protein are expressed in the endometrial glands. Low concentrations of LIF in uterine flushing have been observed to be associated with infertility in one research, and relatively high levels of LIF had been suggested to be predictive of implantation effectiveness in another study (Huang et al., 2018).

Another cytokine that is involved in the female fertility system, which is IL-11 that primarily expressed in the uterus by the luminal and glandular endometrial epithelium, as well as decidualized stromal cells, mostly during the receptive phase (whenever the uterus is receptive to an implanting blastocyst). IL-11 is a member of the IL-6 cytokine group which involves IL-6, LIF, OSM, CNTF, and cardiotropin-1 (CT-1). IL-11 is thought to be essential for decidualization in the endometrium. According to some studies, IL-11R interactions are significant in the decidualization and reduction of miscarriage (Bhurke et al., 2018).

Given this, this work aims to study the predictable pathophysiology of the two specific types of cytokine (i): Leukemia Inhibitory Factor LIF, (ii): interleukin-

11 IL-11, in reproductive failure women. As well as examining their relationship with the main cause of infertility as three categories: (i) infertility due to polycystic ovary syndrome PCOS, (ii): infertility due to Tubal factor, and (iii): infertility due to Unexplained reason. The relevance of patient's age, as well as the body size index (BMI) also will be considered in this. To achieve this, aim the following steps should be made:

(i): specific questionnaire will be taken from each patient and control includes the (Age, BMI, menstrual cycle situation, medication taken up, etc...)

(ii): Examine the relationship between the cytokines gene expression LIF and IL-11 with the predictable pathophysiology of infertility related.

2. LITERATURE REVIEW

Reproduction is a prerequisite for maintaining the existence of any species. Nevertheless, infertility is a prevalent human condition, affects millions of reproductive-age individuals worldwide, and it impacts their families and communities. The failure to have children between couples with medical, social, psychological, financial, and demographic implications is considered to be a significant public health issue. A commission sponsored by The Lancet and the Guttmacher Institute reports that infertility affects around 49 million to 180 million couples globally and Within 20 years, the prevalence has not decreased. As shown in 2017 WHO estimates, it impacts close to 28 % among reproductive-age individuals internationally are infertility (Polis C., 2017) .Turkey Demographic and Health Survey (Hacettepe University Institute of Population Studies, 2017) declared that the prevalence of infertility in Turkey decreased significantly between 1993 and 2017. the proportion of married women between the ages of 15-49 who stated that it is not possible to have children and who have no children in 2017 was 3.9%, this decrease is a result of improvements in maternal health services in Turkey Assisted Reproductive Techniques (ART) such as in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and intrauterine insemination (IUI). ART use was found as 1.9% in 2008 and 4.1% in 2013 (Sarac & Koc, 2018). Although several underlying issues of human infertility were already resolved Via ART, concerning the effectiveness of these procedures, the implantation mechanism is still a rate-limiting step. Proper preparation of the receptive endometrium and the normally developed active embryo is a condition for effective implantation. Consequently, the molecular basis communication between the embryo and the uterus must continue to be understood with regards to pre-implantation and implantation processes.

2.1. Fertility and Infertility

Fertility is the inherent life-giving capacity. It is different from fecundity, Which is known as reproductive potential; Infertility is a lack of fertility, while sterility is considered a lack of fecundity (Feyisa et al., 2020). 79 % of the population was estimated to be fertile, 18 % infertile and about 3% subfertile. The prevalence of embryo wastage and pregnancy loss is comparatively high in humans; it has been reported to be 30 % before implantation, approximately 30 % of early pregnancy loss, and more than 10 % of clinical pregnancies (Mustafa et al., 2019). Infertility in humans is the fact of not getting pregnant without using birth control, during a year of sexual intercourse. It is predicted that about 72 million women between 20 and 44 years of age worldwide are presently infertile (Nations Department of Economic & Affairs, 2020). Male infertility has been estimated to be responsible for 20-30% of cases of infertility, whereas 20-35 % is directly attributable to female infertility, and 25-40 % was mostly due to combined problems in both areas (Gowramma G S, Shantharam Nayak, 2019). The highly complicated coordination and synchronization of interactions in the hypothalamic-pituitary-ovarian axis regulate female fertility; therefore, female fertility can be affected by various diseases or disorders of the reproductive tract, the neuroendocrine system, and the immune system, or by other general illness. The major influences of female infertility According to the ESHRE Capri guidelines for diagnosis and treatment workshop are ovulation disorders (most specifically, Polycystic ovary syndrome PCOS), infertility by tubal causes, endometriosis, and Unsolved infertility (Rooney & Domar, 2018).

Table 2.1. Etiology of female infertility

Symptoms	Causes
Anovulatory infertility	Premature ovarian failure (POF) and early menopause polycystic ovary PCOS.
Tubo-peritoneal infertility	Tubal factor infertility Endometriosis.
Autoimmunity	Recurrent pregnancy loss
Unexplained infertility	

2.2. Oogenesis and Oocyte Biology

In the first fetal development stage, the oogenesis process begins. As (oogonia), the primordial germ cells move to the ovarian cortex. Oogonia undergo multiple mitotic division processes, and the overall number rises to several million by the 20th week of pregnancy in the fetal ovary. Consequently, in the phase of ovarian follicular atresia, many of these oogonia degenerate whereas others expand and undergo nuclear variations and are termed as primary oocytes. The primary oocytes are subjected to the first meiotic division and arrested at meiosis prophase-I (Jones et al., 2013). With regards to minimizing the number of chromosomes from diploid to haploid, which occurs only within germ cells, the crossing over of chromatin predictions a genetic diversity within each gamete. The primary oocytes already are encapsulated with follicular cells and become primordial follicles through the 28th week of pregnancy. The source of gametes from which all mature oocytes can grow is primordial follicles. At this stage, meiosis arrests primary oocytes and does not proceed until the day after sexual puberty (Jaffe & Egbert, 2017).

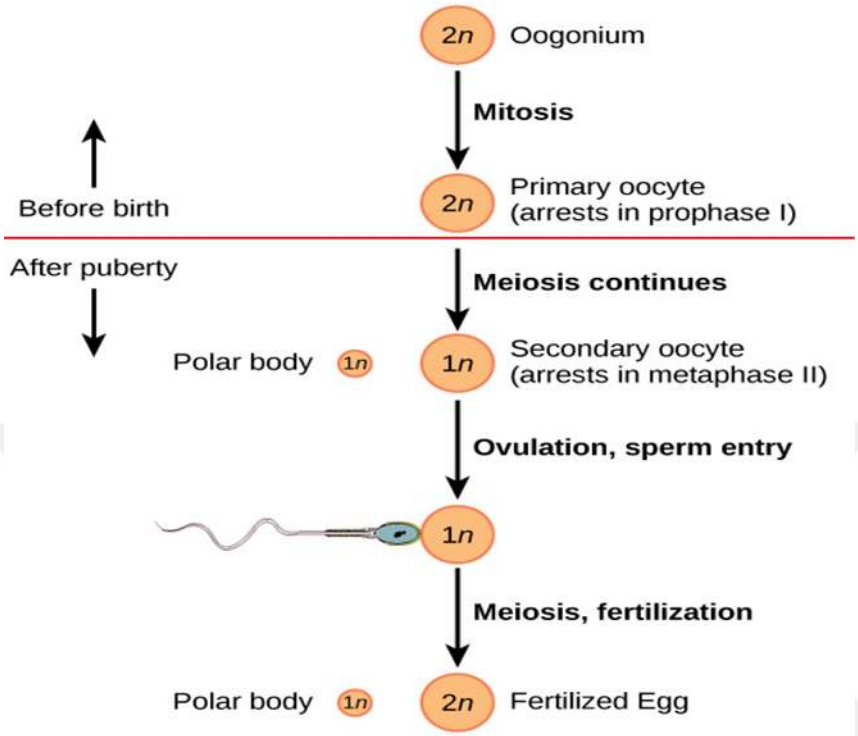

Figure 2.1. The oogenesis process occurs in the outer layer of the ovary (Sánchez & Smitz, 2012).

2.3 Basics of Infertility

According to their nutritional and lifestyle, environmental, career conditions, and infectious diseases e.g. (gonorrhea and chlamydia) the frequency of infertility differs between populations. Infertility, furthermore, includes both male and female partners and involves an extensive list of medical tests for diagnosis. Infertility testing requires all medical and sexual histories, a physical evaluation including female pelvic ultrasound.

2.4. Infertility Sociodemographic Factors

- **Age**

Along with maternal and infant health and quality of life, maternal age at first birth is perhaps the most significant indicator of the degree of female general fertility. Nowadays, more women appear to delay pregnancy plans to older age (KAVAS, 2018). In females, it is recognized that the quality and quantity of oocytes decreases with age, with the percentage of reduction in fecundity being clinically significant by the mid-30s since the rate of miscarriage and the incidence of crucial chromosomal disorders follow an opposite pattern (Igarashi et al., 2015). In particular, advanced maternal age (AMA; described as ≥ 35 years) has only a minor effect on the rate of fertilization, with a moderate effect on the blastocyst stage of embryo growth, But it results in a significant effect on the incidence of blastocyst aneuploidy (Ubaldi et al., 2017). In addition to the increasing reduction of the ovarian reserve, female aging often entails compromised competence of oocytes/embryos due to faulty physiological pathways, including the energy output and equilibrium, metabolism, epigenetic regulation, cell cycle control points, and expanded meiotic mis-segregation (Capalbo et al., 2017). The median age for primary birth in Turkey is 22.9 years and growing. In the age group (25-29) the highest age-specific fertility rate has been recorded in the last 10 years (Özerdoğan & Yilmaz, 2018).

- **Body size**

A common concern among women of reproductive age is obesity, which would be an important public health problem. Rates of overweight and obesity provide an unhealthy and inappropriate accumulation of fat that adversely impacts the body's health. Related to the World Health Organization (WHO), if the mass of the body (BMI) is equal to or greater than 25 kg/m2, this is considered to be overweight.

Whereas it is marked obesity if the (BMI) is equal to or higher than 30 kg/m2 (World Health Organization, 1994). Obesity, particularly in females, plays a significant role in reproductive disorders. Anovulation, menstrual irregularities, infertility, problems with assisted reproduction, miscarriage, and adverse pregnancy was correlated with overweight (Broughton & Moley, 2017). In overweight women, due to the extreme increasing peripheral aromatization of androgens to estrogens, gonadotropin secretion is influenced, as well as Insulin resistance and hyperinsulinemia contribute to hyperandrogenemia in obese women. Sex hormone-binding globulin (SHBG), growth hormone (GH), and insulin-like growth factor-binding protein (IGFBP) are reduced; otherwise, the levels of leptin are increased. Such variations can clarify the impaired ovulatory function and thus, reproductive health (Nghiem et al., 2018). The incidence of overweight women in Turkey has gradually increased. For instance, 41.0% of all Turkish women are obese, referring to Turkish Nutrition and Health Statistical data (YÜKSEL, 2019).

- **Smoking**

The biological believability of the harmful effect of smoking on the ovaries, oocytes, as well as reproductive tract, is provided by several sources of evidence. Mostly in the ovary and/or follicular fluid of smokers, numerous known toxins have been recognized. Chemicals in cigarette smoke tend to exacerbate follicular depletion, an earlier age of menopause, and reproductive failure (Qiu et al., 2017). The mean level of basal follicle-stimulating hormone (FSH) in tobacco users is dramatically higher than in non-smokers. With one survey, in active smokers, basal FSH levels were 66 % greater than in nonsmokers; while in passive smoking 39% higher than in nonsmokers (Dhalwani et al., 2015). Only about one-third of the estrogen excretion in smokers during the luteal phase was observed than in non-smokers, perhaps because components of tobacco smoke inhibit aromatase of granulosa cells leading to Inducing oxidative estrogen metabolism.

Based on the extant research indicate that cigarette smoking has been consistently linked to relatively early menopause. The adverse effects on the maturation of ovarian follicles and the acceleration of follicular depletion indicate that the possibility of menopause is twice as high for tobacco users compared to a non-smoker in women over the age of 44-55(Qiu et al., 2017). Women in the 35-44 age range with a maximum of 19.6% in Turkey smoke tobacco(*Türkiye'nin Sigara İçme Istatistikleri*, 2018).

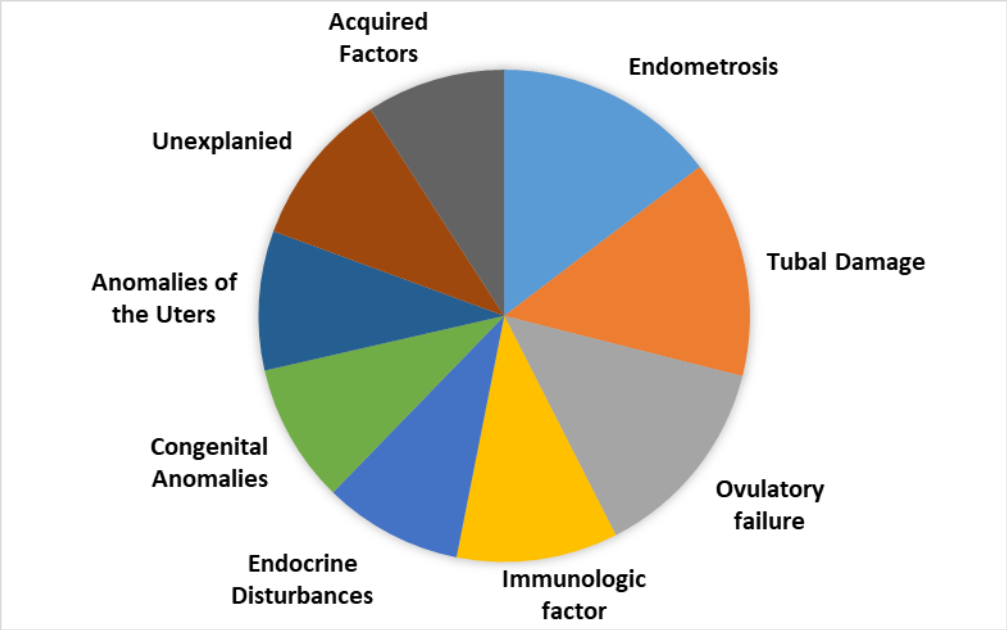

Figure 2.2 The main causes of Female infertility (Barbieri, 2019)

2.5. Infertility Physiological factors

2.5.1 Ovulation disorders

- **Hypothalamic dysfunction**

A significant cause of anovulation is hormonal imbalance, not quite enough follicles will be developed by women with a hormonal imbalance to ensure the production of an ovule.

Inefficient ovarian stimulation by LH and FSH gonadotrophins contributes to hypogonadotropic hypogonadism (HH), either because of inadequate pituitary activation of hypothalamic GnRH or because of extremely limited secretion due to pituitary compromise. Decreased levels of FSH, LH, and estrogen will decrease the consistency of the eggs that the body produces or trigger them to avoid fully releasing. As a result, hypothalamic disorder patients cannot menstruate at all. Females would be unable to conceive without a balanced and normal menstrual period (Mikhael et al., 2019).

- **Hyperprolactinemia**

Another central cause of ovulation disorders is (Hyperprolactinemia). The accumulation of abnormally elevated levels of prolactin in the blood constitutes hyperprolactinemia. For females, regular levels are lower than 500 mIU/L. It may be triggered by diseases that affect the pituitary gland and hypothalamus. Physiological conditions such as pregnancy, breastfeeding, persons with severe hypothyroidism, anxiety, strength training, and some drugs may be induced a high level of prolactin. Women with hyperprolactinemia may have difficulty getting pregnant or their breasts may begin to produce milk outside of pregnancy (galactorrhea) (Kaiser, 2012).

- **Polycystic ovary syndrome**

Polycystic ovary syndrome PCOS is a cluster of clinical symptoms found within a phenotypically heterogeneous group of women linked to ovarian dysfunction. The Rotterdam criteria are generically defined PCOS as a condition comprising of two out of three indicators relating to unusual or absent ovulation, morphological classification of the ovaries by ultrasound examination, and hyperandrogenism (Deans R, 2019)

.

Some groups claim that uncontrolled androgen secretion is the most important underlying disease since it is known to contribute to ovarian dysfunction (Hart & Doherty, 2015). The pathogenesis of PCOS is uncertain as to the potential causes of the genetic predisposition modulated by hyperinsulinemia or even the childhood development environment since there is relatively strong scientific proof from animal models for the programming impact of early life exposure to androgens.

2.5.1.1 Etiology and pathophysiology of PCOS

1- Prenatal exposure to androgens

Results obtained from the experimental evidence from animal studies along with clinical material of clinical processes in human populations, it is thought that excessive fetal exposure to maternal androgens contributes to the development of the PCOS phenotype in offspring/children (i.e., congenital adrenal hyperplasia) (Puttabyatappa et al., 2016). Female newborns to PCOS mothers had elevated concentrations of testosterone in the umbilical vein, which have been equivalent to male levels. Endocrine-disrupting contaminants (EDCs) that are androgenic, such as 3,4,4'-trichlorocarbanilide as well nicotine, create a hyperandrogenic state in the fetus. EDC exposure during prenatal development may be to blame for fetal programming changes (Hewlett et al., 2017).

2- Impaired ovarian steroidogenesis

Steroidogenesis in the ovary is characterized by a highly coordinated sequence of enzyme activities that convert a C27 steroid (cholesterol) into the three types of carbon-reduced steroid hormones: estrogen (C18), androgen (C19), and progestin (C21). The large percentage of cholesterol used in steroidogenesis comes from serum lipoproteins (Wickenheisser et al., 2006).

The theca and granulosa cells that work together produce ovarian steroids. LH stimulates theca cells to develop androstenedione by cholesterol, which will then be transformed to estrogens in the granulosa cells via the action of FSH-dependent

aromatase. To put it another way, any molecule of estrogen is generated from an androgen molecule (Baumgarten & Stocco, 2018). The overall number of theca cells and their steroidogenic quality, which are both deficient in PCOS women, are indeed the two main factors influencing the limited amount of androgen secreted by the ovary. Several of the follicles within the PCOS ovary have hypertrophy of the theca interna, resulting in an increased amount of steroidogenic cells. The induction of androgen production and secretion in ovarian theca cells is a stable phenotype (Richards et al., 2018).

3- Impaired adrenal androgen production

Adrenal steroidogenesis failure has additionally been related to the improvement of hyperandrogenemia in PCOS. The causes of adrenal androgen overload in PCOS are unknown, but it is been hypothesized that it is caused by the over-activity of adrenocortical products concerning adrenocorticotrophic hormone (ACTH) stimulation (Rosenfield & Ehrmann, 2016). Furthermore, adrenal steroidogenesis impairment results in diminished cortisol levels and reduced negative feedback on (ACTH), which leads to a rise in adrenocorticotrophic hormone (ACTH) synthesis to ensure stable serum cortisol levels and, as a result, induce adrenal androgen release. consequently, hyperandrogenism in Women with PCOS is influenced by a synergic abnormality of steroidogenesis of both the ovary and the adrenal glands (Baptiste et al., 2010).

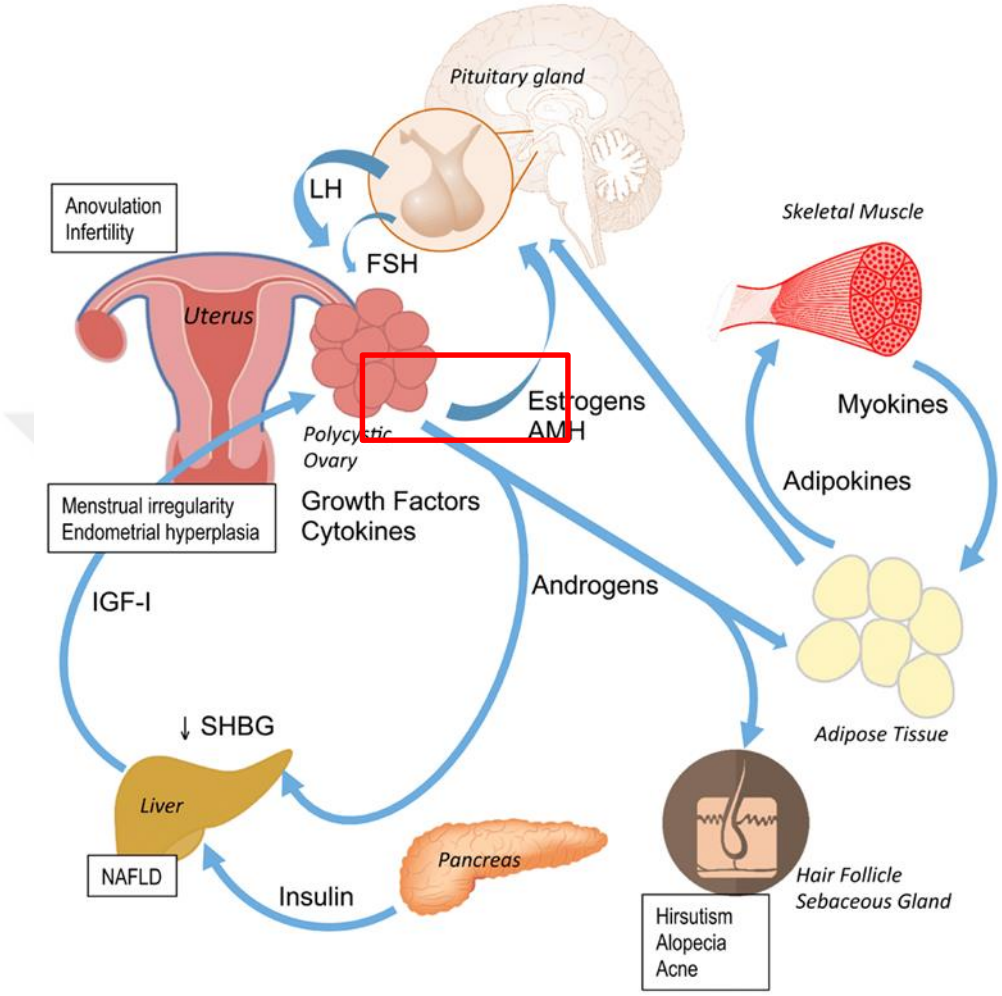

Figure 2.3. Numerous pathophysiological pathways of polycystic ovarian syndrome are depicted in this diagram. **AMH**, anti-Müllerian hormone; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone; **SHBG**, sex hormone-binding globulin; **IGF-I**, insulin-like growth factor I; **NAFLD**, non-alcoholic fatty liver disease (Rocha et al., 2019).

2.5.2. Tubal Factor

Infertility issues are influenced by the fallopian tube. The main frequent reason for fertility problems is TFI (tubal factor infertility), related to the occlusion and peritoneal pathology that creating adhesions, from 30 % to 35 % of old and young infertile women have been diagnosed with this disease. Blockages,

destruction, tissue damage, congenital abnormalities, as well as other triggers that hinder a fertilized or unfertilized ovum from descending into the uterus via the Fallopian tubes and preventing a successful pregnancy (Daniilidis et al., 2020). An infection, such as pelvic inflammatory disease (PID), has been considered the most prevalent cause of tube blockage.

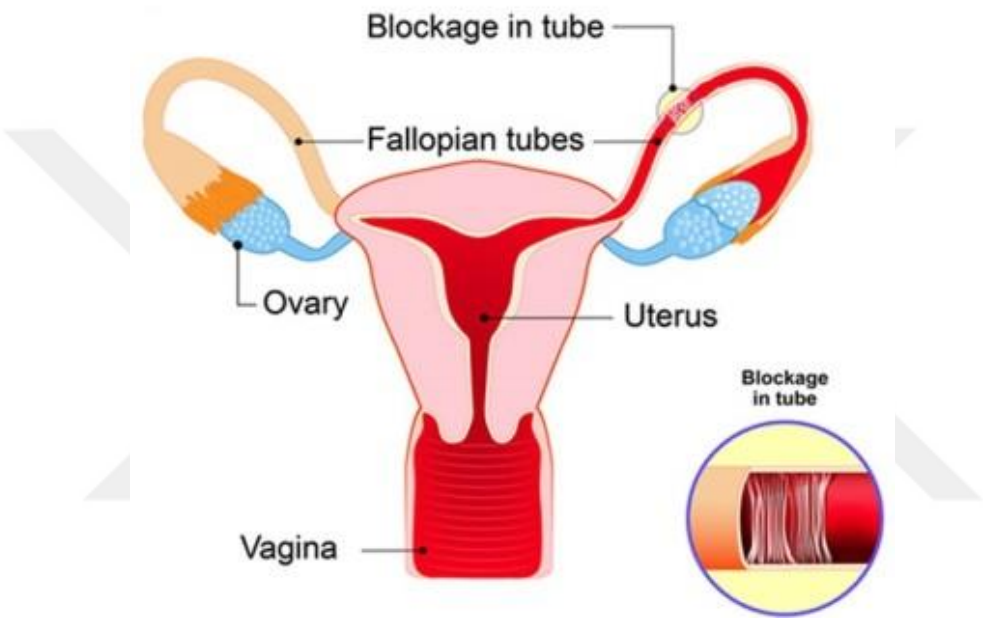

Figure 2.4. Blockage in Fallopian tube (Tubal Factor Infertility) (Traub et al., 2009)

Adhesions do not often obstruct the fallopian tube, but they may effectively make it defective by deforming or segregating it from the ovary (Soper, 2010). The fallopian tubes might become blocked in a variety of ways resulting in tubal factor infertility. Nevertheless, depending on where the blockage occurs in the fallopian tubes, there can be three different forms of blockages (Proximal, Medial, and Distal) (Patil, 2009). Chlamydia trachomatis is the most common reason for distal tubal obstruction (hindering the end of the tube that leads to the ovary). Tubal ligation operations, as this section of the tube, is a common primary goal of sterilization procedures, may cause midsegment tubal restriction. On the other side, during an infection, for incidence of septic abortion, the proximal tubal obstruction may result

(Eisenberg & Sciarra, 2009).

2.5.3. Unexplained infertility

Throughout many cases, unexplained infertility (UI) corresponds to (a lack of diagnosis) done in women who have undergone all the usual examinations inclusive of checks of ovulation and tubal patency. The term "unexplained infertility" has been attributed to as many as 30–40 percent of infertile women (Deshpande & Gupta, 2019). Aberrations in endocrinological equilibrium, immunology, genetic and reproductive physiology have all been suggested as possible explanations of unexplained infertility (Zauner & Girardi, 2020). Almost every month of infertility above the average decreases the likelihood of pregnancy by 2%, or around 25% per year. The pregnancy rate drops by 9% every year a woman reaches the age of 30. Moreover, since insufficient ovarian reserve is the chief reason for infertility in women over 40, the concept of unexplained infertility differs with this group (*Fertility Treatment*, 2018). Women that are infertile for unexplained reasons have regular ovulatory cycles and hormonal statuses, no organ dysfunction, and no other symptoms.

However, defects in endometrial receptivity may be one cause of fertility issues in these women, in which Embryos cannot implant in the uterus if the endometrium is damaged, which could affect endometrial Numerous microarray investigations have demonstrated gene dysregulation in the endometria of females with fertility issues Competence (Messaoudi et al., 2019).The ovarian steroid hormones estrogen and progesterone are the number one regulators that manage uterine differentiation and proliferation, however, additionally, they affect the local manufacturing of cytokines as well as the growth factors (Shah et al., 2019). The extreme physiological correlation among each both the embryo and uterine tissues in women with (UI) has been studied notably over the past years, with a primary emphasis on defining the coordinating factors between them. Intrauterine insemination (IUI) is regularly used as a first-line remedy for women who are

experiencing infertility for no obvious reason. In vitro fertilization (IVF) is frequently recommended as a secondary therapeutic option, however, the best successful method of achieving pregnancy for women experiencing unexplained infertility still has to be proven (Ganguly et al., 2016).

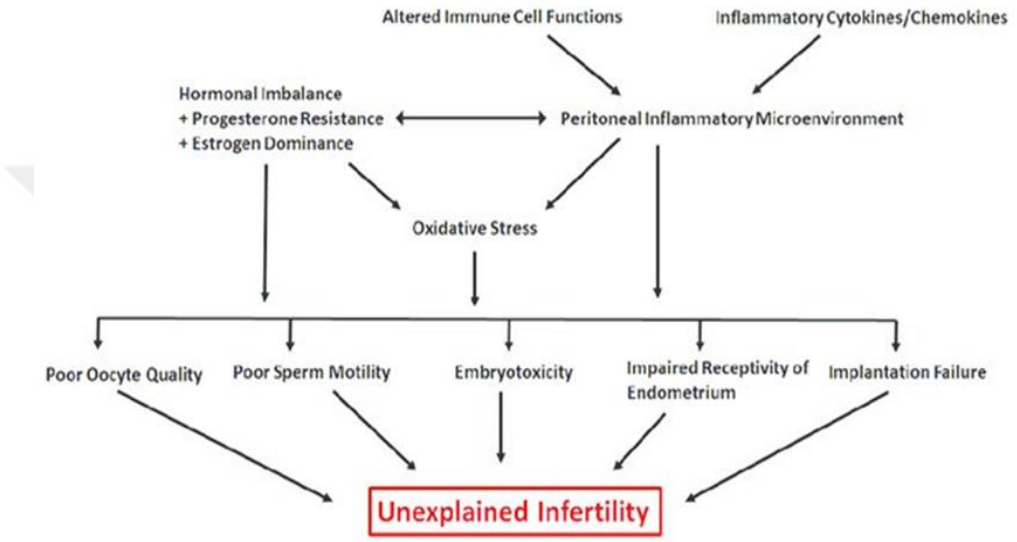

Figure 2.5. The factors that have a role in unexplained infertility (Miller et al., 2017).

2.6. Embryo Implantation

The implant process or (implantation) is the action through which the blastocyst connects to the underlying endometrial layer and eventually penetrates it, which is a dynamic and flexible process. The uterus would be only able to embrace the implanting embryo for a short time, which is known as the 'window of implantation, Beyond this time frame, the endometrium may be passive or even antagonistic to the embryo (Yoshinaga, 2018). This process depends on the coordinated growth of the embryo to the blastocyst phase and the progression of the uterus to the receptive stage. It is essential to create an efficient ‘interference' between maternal as well as embryonic tissues, that entails many endocrines, paracrine, and autocrine influences. A comprehensive framework of molecules is activated at the maternal-fetal interacting together under the control of ovarian

hormones as shown in Table (2.2), which play a critical role in facilitating the events (Diamanti-Kandarakis et al., 2009) Non-chromosomal pregnancy loss, which remains a significant real limitation of assisted reproductive treatments, can be caused by an alteration in implantation events quite early in the pregnancy. Several critical principles concerning implantation remain unresolved, despite important advances in reproductive studies (Hyde & Schust, 2015).

Table 2.2. Targeted disruption of a reproduction-associated hormone or cytokine in animals, either by gene deletion or peptide blocking.

Targeted molecule	Reproductive (implantation) function
ERα	Causing (infertile) due to ovarian failure, immature uterus, and estrogen resistance
ERβ	Causing (sub-fertile) by reducing ovarian efficiency
PRA	Causing (infertile) due to unable to promote and induce decidualization
LIF	Causing (infertile) due to implantation failure and unable to promote and induce decidualization
TGF-β1	Causing (infertile) due to Intrauterine natal lethality
IL-6	Causing (sub-fertile) due to There is a 50% reduction in viable implantation sites.
IL-11	Causing (infertile) due to defective decidualization
IL-15	Causing (infertile) due to (uNk) deficiency at implantation sites

ERα,β = estrogen receptor; **PRA** = progesterone receptor A;**LIF** =Leukemia inhibitory factor; **IL-6,11,15**= Interleukin; **(uNk)** = uterineNatural killer; **TGF-β1** = Transforming Growth Factor-β1

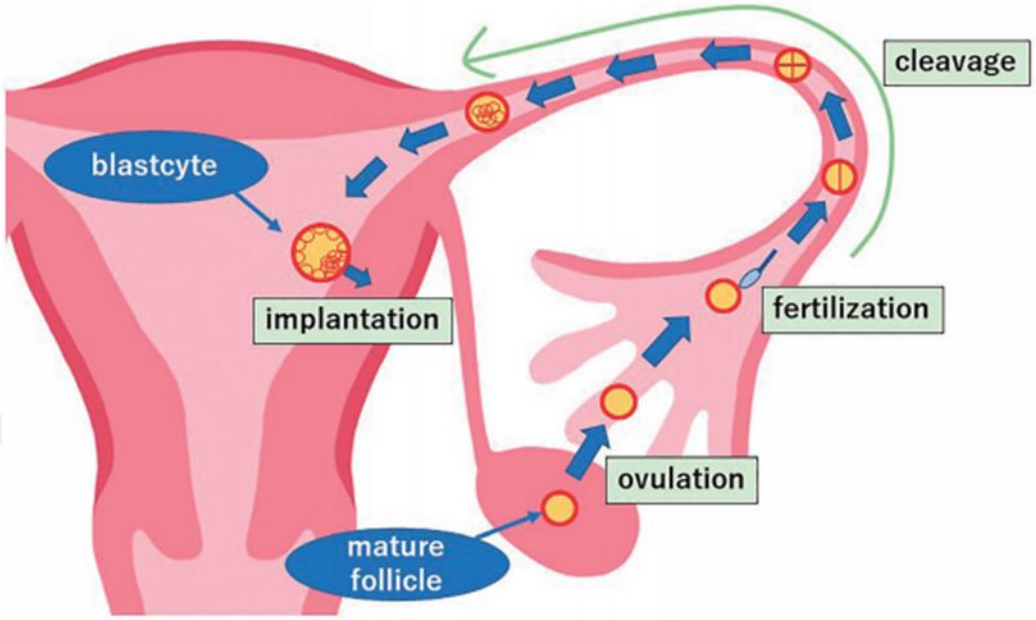

Figure 2.6 From ovulation to implantation, the process follows a set of steps (Kitazawa et al., 2020)

2.6.1. Mechanism of Embryo Implantation

There are three steps for embryo implanting:

(a) The blastocyst comes into contact with the endometrium's implantation position (apposition).

(b) The blastocyst's trophoblast cells adhere to the receptive endometrial epithelium (adhesion).

(c) invasive trophoblast cells penetrate the endometrial stroma by crossing the endometrial epithelial basal layer (invasion) (Yoshinaga, 2018).

Apposition: The blastocyst apposition at the uterine epithelium starts in the implantation process around 2-4 days just after the morula reaches the uterine cavity. Specific positioning of the blastocyst towards the lining of the uterus is highly required for effective implantation into maternal tissues(Kim & Kim, 2017). In the

female uterus, implantation takes place in the upper and posterior walls in the midsagittal plane. The blastocyst becomes an inner cell mass (embryo) and trophectoderm (placenta) all through the apposition stage. Decidualization is the mechanism by which stromal cells covering an implanting blastocyst differentiate into a specialized cell type defined as decidual cells (Salamonsen & Evans, 2018).Cytokines are peptides or glycoproteins that function as regulatory agents. Apart from hormones, cytokines typically serve as paracrine as well as autocrine triggers in local tissue, with endocrine mediator responses occurring only rarely (Bhurke et al., 2018). LIF (Leukemia-inhibitory factor) is a cytokine that belongs to the interleukin-6 family and is a significant mediator of estrogen response. In mice, knocking out the LIF gene causes infertility, which is marked by a deficiency in implantation and decidualization (Catalán et al., 2018). Fertile females have elevated levels of LIF expression around the period of implantation while infertile females have relatively low levels. The epidermal growth factor (EGF) signaling pathway is regulated by LIF, which promotes stromal proliferation. Cross-talk between endometrial epithelial CSF-1 (colony-stimulating factor-1) and trophectodermal CSF-1 receptors has been suggested to improve blastocyst attachment(Cheng et al., 2016).

Adhesion: The microvilli along the top layer of the outer surface trophoblast cells communicate with the uterine epithelial cells after the apposition of the available blastocyst at the uterine epithelium to start (adhesion) stage (Kim & Kim, 2017).Integrins, cadherins, selectins, and immunoglobulins are the four participants of the cell adhesion molecule (CAM) group. Cell-to-cell adherence is mediated by these interface ligands which are normally glycoproteins. Repair work of tissue integration, cell proliferation, morphogenic changes, cellular invasions, and tumor metastasis are some of their more well-known functions (Walmod et al., 2007).

Integrins are a family of transmembrane glycoproteins that serve as cell receptors and therefore are made up of various combinations of two non-covalently connected subunits. The connection between both the blastocyst and the

endometrium is appropriately increased mostly during the adhesion process to prevent detachment by flushing (Simon & Laufer, 2012) The existence of progesterone-regulated endothelial cadherin (E-cadherin) with both trophoblasts and endometrial epithelium suggests that it may play a key role in blastocyst adhesion to the endometrium (Wang et al., 2013). The glycoprotein receptor CD98 is primarily activated mostly on the membrane of endometrial cells, with which it is frequently connected not just through amino acid transportation but also through other mechanisms like cell fusion, which aids blastocyst adhesion. Management of primary endometrial epithelial cells with Human chorionic gonadotropin (hCG), 17-estradiol,LIF, or (Epidermal Growth Factor) EGF increases CD98 expression, which improves murine blastocyst adhesion, while siRNA-mediated depletion decreased blastocyst adhesion intensity (Domínguez et al., 2010).

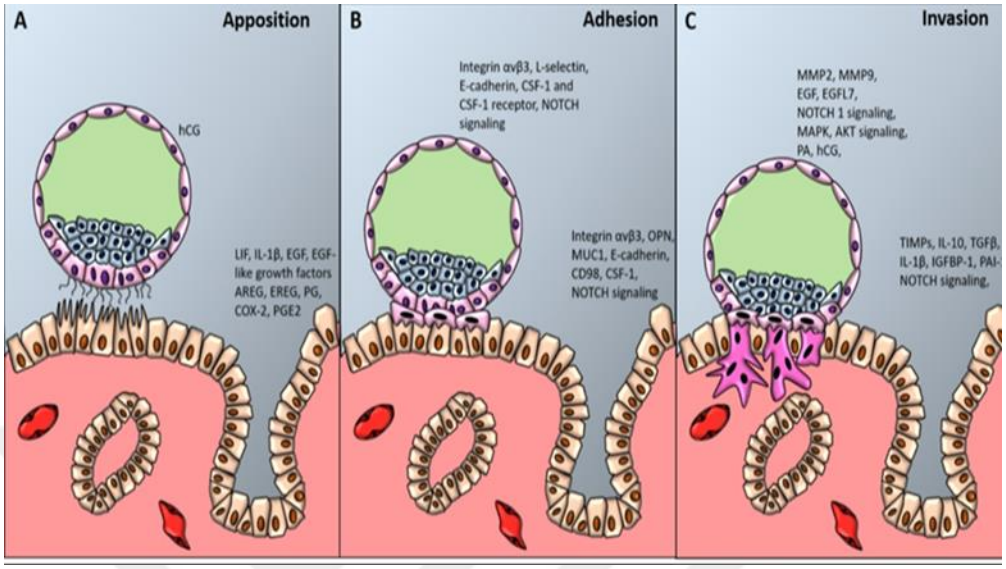

Figure 2.7. Apposition, adhesion, and invasion of blastocysts. A preimplantation-stage **(A, B)** and invading **(C)** blastocyst (approximately 9 to 10 days following conception as well as the mechanisms and indicators needed for uterine sensitivity and blastocyst apposition **(A),** adhesion **(B),** and invasion **(C)** are illustrated in the diagram. **hCG** (human chorionic gonadotropin), **OPN** (osteopontin), **AKT(** protein kinase B), **PA** (plasminogen activator), **TGF** (transforming growth factor-beta), **TIMPs** (tissue inhibitor of metalloproteinases), **PGE2** (prostaglandin E2), **CSF-1** (colony-stimulating factor-1), **NOTCH1** (Notch receptor 1), **MUC-1** (mucin-1), **MMPs** (metalloproteinases), **EGFL7** (epidermal growth factor-like domain 7), **MAPK** (mitogen-activated protein kinase), **PAI-1(** plasminogen activator inhibitor-1), **COX-2** (cyclooxygenase), **LIF** (Leukemia inhibitory factor) (Massimiani et al., 2020).

Invasion: Consequently, the invasion begins in the last stage which begins with extremely invasive trophoblast cells penetrating the uterine epithelium, Figure(2.7c) shows the Apposition, adhesion, and invasion of blastocysts. Fetal trophoblast cells will penetrate and transition into the maternal decidua during the implantation operation. The trophoblasts somewhere at the implant site already developed cytotrophoblast and syncytiotrophoblast complexes through this time(Carter et al., 2015). Trophoblast cells progressively degrade the membranes of the maternal spiral arteries, transforming them from muscular vessels to flaccid sinusoidal sacs covered with endovascular trophoblast cells. The purpose of the

invasion would be to replace small, high-resistance vessels with large, low-resistance vessels in maternal spiral arteries, that help ensure a constant flow of blood between both the fetus and the mother (Knöfler et al., 2019).

2.7. Role of cytokines in Embryo Implantation

Cytokines are multi-functionalized glycoproteins with many physiological roles. Their acts are linked to a variety of bodily functions, particularly cellular proliferation and differentiation, as well as immune function. They are involved in important reproductive activities including ovulation and implantation, as well as healing and inflammatory-like pathways that happen throughout the menstrual cycle in the female endometrium (Bhurke et al., 2018). The cytokine family is characterized by polymorphism and diversity, with several molecules playing overlapping or even opposing positions. Numerous cytokines are thought to play a role in the implantation mechanism. The IL-6 family of cytokines is a group of cytokines that play an essential part in embryonic implantation. LIF, IL-6 , IL-11 , neurotrophic factor, oncostatin M, and cardiotrophin 1 are all members of this family of cytokines (Rose-John, 2018).

The mutual intracellular signaling by glycoprotein-130 (gp130) is an essential feature of this collection of cytokines. In addition, members of the IL-6 family are signal transducers and activators of transcription -3 (STAT3) activators (Culig & Puhr, 2012). In the course of implantation, the endometrial epithelium and stromal cells manufacture IL-6, which is a proinflammatory cytokine. The luminal epithelium periodically produces IL-6 with levels peaking within the implantation and menstruation periods. Reduced IL-6 mRNA output has been linked to frequent miscarriages in the latest research, likely due to the reduction in IL-6's functions in tissue regeneration, decidualization, and placenta/trophoblast growth (Vesce, 2021).

The cytokine IL-11 is a multipurpose cytokine that has anti-inflammatory effects that are formed by stromal and epithelial cells, and the highest peak of it through decidualization. The luminal and glandular epithelia activate the IL11

receptor, which is known as IL11R. In-vitro research indicates that IL11 plays a large part in human decidualization enhancement, likely via stromal cells.(Winship & Dimitriadis, 2018). Nevertheless, it appears that throughout trophoblast invasion, the embryo produces IL-11, allowing it to maintain control over the endometrium. In some situations of female infertility, a mutation in IL-11 or even its receptor could be the cause (Sabry et al., 2014). The importance of these observations, however, is still unknown.

IL-1 is a multipurpose cytokine that is often used in the implantation window. It causes the endometrium to produce more LIF, as well as leptin synthesis and its receptor. Moreover, IL-1 boosts the function of the integrin 3 subunit, binding molecules that aid in adhesion and apposition. IL-1 tends to play a role in decidualization as well. In addition to its roles as a cytokine modulator, leptin frequently induces the activity of the integrin 3 subunit, which it associates with IL-1 (Sabry et al., 2014).

The IGF/IGFBP mechanism is involved in coordination between the embryo and the endometrium as well. IGF2 is thought to promote implantation and invasion (by fostering aggression), while IGFBP1 is thought to reverse this activity by inhibiting IGF2 function (Gupta, 2015). The cytotrophoblast also produces osteopontin (OPN), which is regulated by progesterone, reinforcing the role of OPN in implantation (van Mourik et al., 2008).

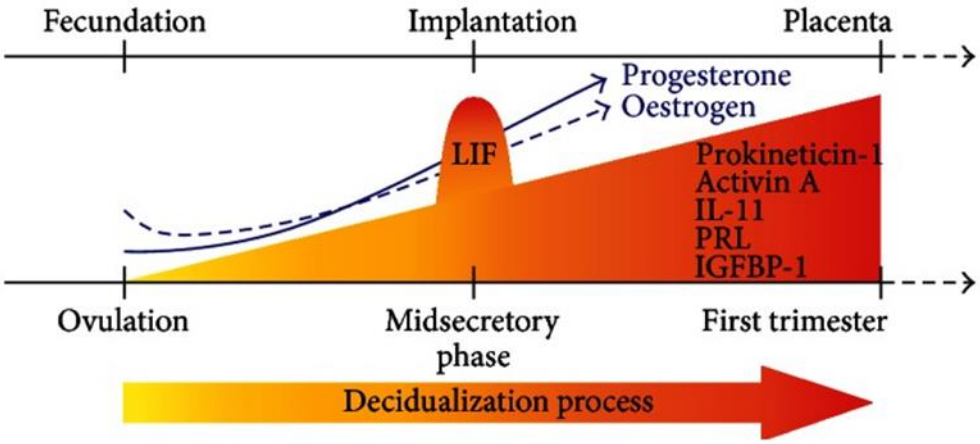

Figure 2.8. Temporal representation of the effects of the cytokine on (ovulation, implantation, and decidualization) (Salleh & Giribabu, 2014a).

Some other method for regulating aggression in the adhesion stage of implantation is to look at the sequence of events, such as the suppression of proinflammatory TNF by anti-inflammatory IL11 once TNF reaches its peak mostly in the secretory process (Hwang & Lee, 2018). Table (2.3) summarizes the most essential properties of cytokines and their effects.

Table 2.3: Cytokines Involved in Implantation: An Overview

Compound	Inflammatory effect	Timespan	Sources/location
LIF	Proinflammatory	Days 18–28	Endometrium
IL-6	Proinflammatory	Secretory	Endometrium and blastocyst
IL-11	Proinflammatory	Decidualization	Endometrium
IL-1	Proinflammatory	Secretory	Endometrium and blastocyst
IL-18	anti-inflammatory	Decidualization	Endometrium
Leptin	Proinflammatory	Throughout	Endometrium and blastocyst
TNF-α	Proinflammatory	Secretory	Endometrium
OPN	anti-inflammatory	Implantation window	Endometrium and blastocyst
IGF-2	Proinflammatory	Secretory	Endometrium
IGFBP	anti-inflammatory	Decidualization	Endometrium

LIF, Leukemia inhibitory factor, IGF-2, insulin-like growth factor-2; IGFBP, IGF binding protein; OPN, osteopontin; IL-(6-11-1-18) Interleukin.

2.8 Leukemia inhibitory factor LIF

LIF (leukemia inhibitory factor) is a member of the interleukin-6 (IL-6) family of cytokines; IL-6 and IL-11 are also part of the group. LIF receptor alpha (LIFRa), LIF receptor beta (LIFRb), and glycoprotein gp130 are LIF's receptors (Rose-John, 2018). The LIF receptor is only found in the luminal epithelium and is only activated mostly within secretory and post-ovulatory stages of the oestrous cycle. Membrane-associated, diffusible, and truncated types of LIF have already been recognized as paracrine variables in embryo implantation (Raheem, 2018).The protein-encoding by the LIF gene is a pleiotropic cytokine that has many functions, containing 202 amino acids with a Molecular mass (22008) Da. It plays a significant role in hemopoietic differentiation in regular and myeloid cancer cells, neural cellular proliferation, mesenchymal to epithelial transformation during kidney formation, and immunity tolerance at the maternal-fetal interfaces.The genomic location of the LIF gene is (22q12.2) with exon count for 5, in addition to the top transcription factor binding sites in the LIF gene promoter: ATF-2,c-Jun, c-Myc, Max, STAT1, STAT1beta, STAT3 and STAT5A (*LIF Gene - GeneCards*, 2021) Figure (2.9).

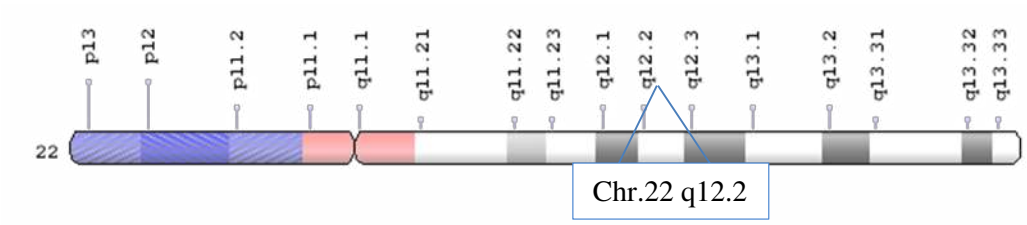

Figure 2.9. The genomic location of LIF gene (McLean K et al., 2019)

2.8.1 Role of Leukemia inhibitory factor LIF in embryo implantation

LIF, gp130, and STAT are indeed essential for embryo implantation, according to numerous studies. In LIF gene knockout within mice, blastocyst implant rejection has been registered (Chu et al., 2015).Rodents having either a gp130 mutation or removal of the STAT-binding region were infertile, showing where both

gp130 and STAT were essential for LIF activity control. During the implantation window, ovarian steroids are thought to make a significant contribution in controlling LIF, LIFR, and gp130 expressions in the uterus (Salleh & Giribabu, 2014b).

Concurrent administration of estrogen and progesterone upregulate LIF receptor mRNA expression in a female endometrial stromal cell line, according to in-vitro research. Human Chorionic gonadotrophins (hCG) have also been shown to increase LIF expression in females (Chu et al., 2015).LIF is related to the subsequent activities throughout implantation: endometrial transformation into the receptive state, embryo endometrial interaction, stromal decidualization, trophoblast invasion, blastocyst development and growth, and uterine leukocyte infiltration, according to evidence (Yoshinaga, 2018).Figure (2.10)

Numerous variations in protein expression were already identified in the uterine luminal epithelium through uterine receptivity, including accelerated synthesis of epithelial growth factor (EGF). Inside the endometrial epithelia, LIF influences the development of growth factors. Amphiregulin (Ar), heparin-binding epidermal growth factor (HB-EGF), and epiregulin (Ereg) have not been expressed at the blastocyst apposition site in LIF-deficient female.mice (Salleh & Giribabu, 2014b) Figure (2.10,a).

LIF performs a crucial role in decidualization. In LIF-deficient mice, stromal cells have been shown to fail to differentiate into primary decidual cells. LIF frequently increases STAT3 phosphorylation, which promotes estrogen and progesterone-induced decidualization, in addition to the necessity for the expression.of.cyclooxygenase-2.(COX-2)(Marquardt et al., 2019), Figure(2.10,b).

LIF has been shown to make a significant contribution in the first stages of pregnancy in the control of immune responses in the uterus. LIF influences the subpopulation of uterine leukocytes and attracts a particular group of leukocytes to the implant site. LIF mRNA is expressed in the decidual leukocytes themselves (Sharkey et al., 1999), Figure (2.10, c).

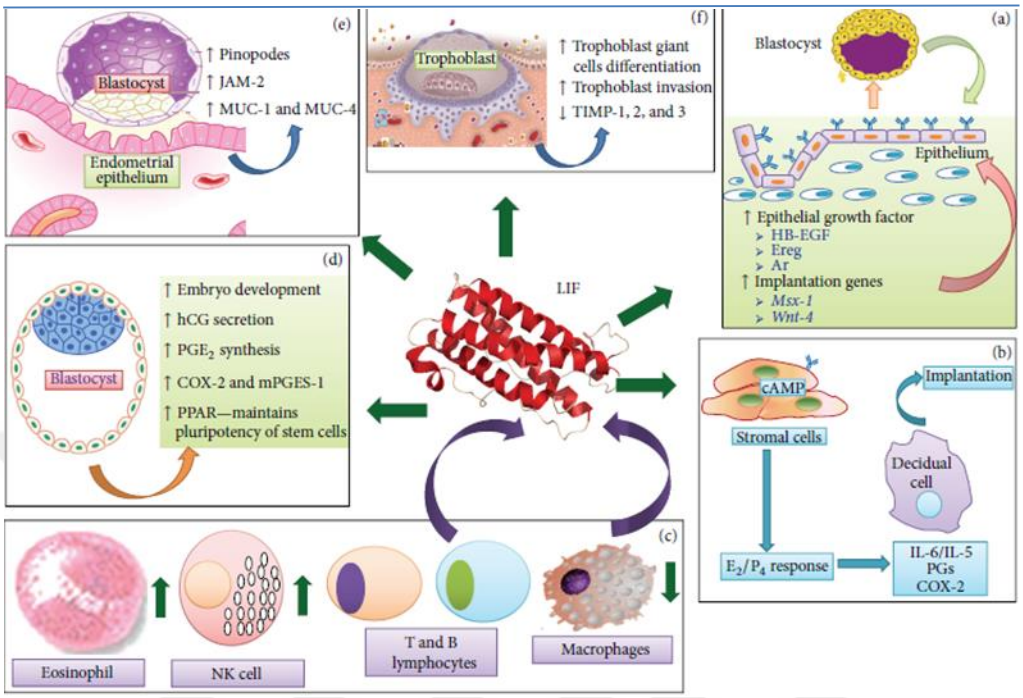

Figure 2.10. LIF's known functions in embryo implantation are summarized. **(a)** Throughout the receptive endometrium, LIF enhances the activity of EGF and implantation genes. **(b)** In the meantime, LIF promotes stromal decidualization via rising cytokine and prostaglandin output**. (c)** LIF plays a role in the recruiting of a particular type of leucocyte that takes part in the inflammation action during implantation **(d)** The embryo's growth and development are controlled by LIF, which is formed mostly by the endometrium and blastocyst. **(e)** Via pinopodes as well as adhesion receptors, LIF helps to improve embryo-endometrial connectivity**. (f)** Lastly, LIF promotes trophoblast differentiation and increases the capacity of trophoblasts to enter the uterine stroma. **LIF:** leukemia inhibitory factor, HB-**EGF**: heparin binding-epidermal growth factor, **Ereg**: epiregulin, **Ar**: amphiregulin, E2: estrogen, P4: progesterone, IL: interleukins, PGs: prostaglandins, COX: cyclo-oxygenase, NK: natural killer, PPAR: peroxisome proliferator-activated receptor, PGE2: prostaglandins E2, hCG: human chorionic gonadotrophin, MUC: mucin, JAM: junctional adhesion molecules (Salleh & Giribabu, 2014a).

LIF mRNA transcript has been found in mice, rabbits, and human blastocysts, which aids in the development of preimplantation embryos. It has also been confirmed to stimulate the expression of the COX-2 and microsomal PGE synthase-1 (mPGES-1) enzymes, which are involved in PGE synthesis, in the human trophoblast cell lines (Shaw et al., 2010) , Figure(2.10,d).

For the embryo endometrial interaction to begin, LIF is required Pinopodes were found to be absent in mice without LIF. Meanwhile, in female endometrium having uterodomes at various stages of maturation, both luminal and glandular epithelia displayed higher amounts of LIF and LIFR on luteal days 6 through 9(Quinn et al., 2007), Figure(2.10,e).

LIF is involved in trophoblast invasion and induces trophoblast giant cell differentiation through the JAK1-STAT3 pathway. Even so, extracellular signaling from soluble LIF triggers trophoblast invasion via STAT3 stimulation. LIF has also been shown to reduce integrinβ4 mRNA expression in trophoblast cells, which facilitates trophoblast invasion (Tapia et al., 2008), Figure(2.10,f).

2.9. Interleukin-11 (IL-11):

Interleukin-11 (IL-11), LIF, OSM, IL-6, CT-1, and ciliary neurotrophic factor (CNTF) are all members of the IL-6 family of cytokines. The human IL-11 gene, which has five exons and four introns, is found on the chromosome (19q13.42) and codes for a 23-kDa protein (Taub, 2004) Figure (2.11).

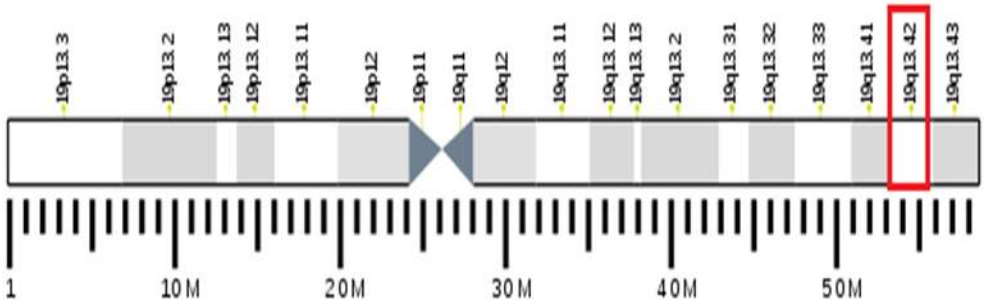

Figure 2.11 The genomic location of IL-11 gene (McKinley et al., 1992).

It stimulates hematopoiesis and has a wide range of biological interactions in different types of cells. In addition, it can induce B cell differentiation, stimulate osteoclast growth, disrupt adipocyte differentiation, and modulate extracellular matrix metabolites (Rose-John, 2018).IL-11 is thought to be essential for decidualization in the endometrium. Within cells, IL-11 has an impact by attaching to a plasma membrane receptor that is made up of two subunits: IL-11Rα and gp130. The IL-11R molecule, which is activated at elevated rates in fibroblasts as well as several stromal cells but not immune cells, provides signal selectivity, in contrast to IL6 receptors, which have been expressed at an elevated level in immune cells but low rates in stromal cells (Cardó-Vila et al., 2008).

2.9.1. The Role of Interleukin-11(IL-11) in embryo implantation

Uterus IL-11 is primarily produced by the luminal and glandular endometrial epithelium, as well as decidualized stromal cells, mostly during the receptive phase (whenever the uterus is receptive to an implanting blastocyst) in females(Cork et al., 2002).

When comparing the endometrium of females with unexplained/ congenital infertility or endometriosis infertility to those of the fertile controls, epithelial IL-11 and its receptor (IL11RA) are dysregulated (Dimitriadis et al., 2006). The sensitivity of females to ovarian stimulation previous to in vitro fertilization (IVF) is linked to IL11 concentrations in uterine flushings (obtained throughout receptivity). It is possible that decreased IL11 development, blame for the lower implantation and/or pregnancy rates seen in excessive ovarian responders during IVF (Makkar et al., 2006).According to these findings The uterine epithelium secretes IL-11 into the uterine lumen, which functions mostly on endometrial luminal epithelium and blastocyst to promote blastocyst binding and implantation. Thus, blocking the activity of uterine IL11 throughout the time of uterine receptivity can prevent the blastocyst from attaching to the luminal epithelium and preventing implantation, result in pregnancy inability (Wang et al., 2013). LIF and IL-11 are specifically

produced apically into the uterine lumen (both from endometrial glands as well as luminal epithelium) since they are found in pg-ng/mL concentrations from the mid secretory stage to up to 12 days after the LH surge Figure (2.12). Determining the course and fundamentals of LIF/IL11 secretion by endometrial epithelial cells during the cycle of receptivity will help us better recognize the cytokines' necessary activities at this time (Yap et al., 2011). LIF is produced in the Fallopian tubes with little difference during the menstrual cycle, while IL11 is found in Fallopian tube secretions at an unspecified time during the cycle.

As a result, these cytokines can be able to influence early embryo development even before they reach the uterine cavity (Winship & Dimitriadis, 2018). Primary female endometrial epithelial cells bind to fibronectin and collagen IV more effectively when LIF and IL-11 are activated, who were found on the surfaces of blastocysts and female first trimester trophoblasts. Respectively stromal and epithelial cells produce IL-11, whereas epithelial cell expression is higher than stromal cell expression (Yap et al., 2011).

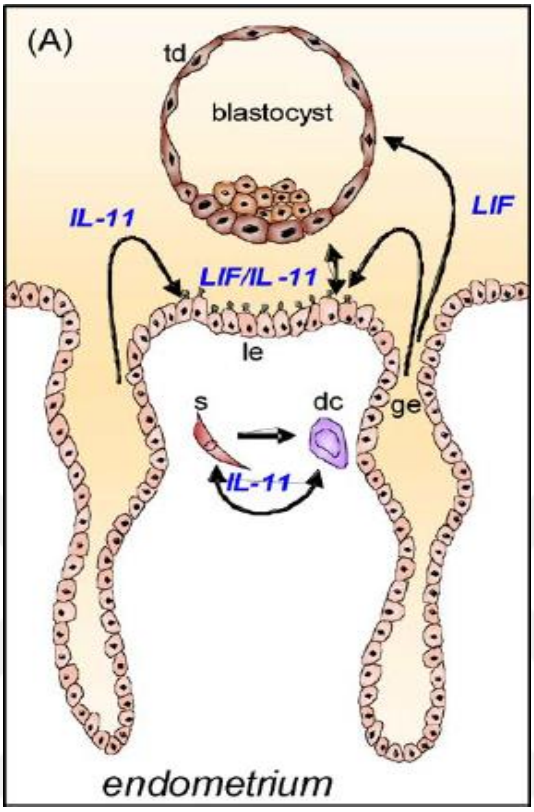

Figure 2.12. LIF/IL11 mediates implantation cross-talk among trophoblast and endometrium. (td) trophectoderm; endometrial luminal/glandular epithelium (le/ge); ecidual cells (dc); s-stroma (Paiva et al., 2009)

3. MATERIAL AND METHODS

3.1. Subjects

This perespective case study was carried out in Erciyes University hospitals, Medical faculty, in the Department of Medical Genetics between June 2019 to July 2021 for postgraduate studies. The study was approved by the Scientific Ethical Committee of the Medical Faculty, Çukurova University. 75 patient's women suffering from 2 to 7 years of primary infertility between 23 and 45 years of age were studied in Kayseri city from 2019 to 2021. The patient groups were divided into three categories depending on the cause of infertility. The first group contains 25 patients with PCOS; the second group 25 patients with TFI and the third group were also 25 patient UI additional to 25 control women group. To complete the study, all women recruited were not taking any medication or fertility drugs known to affect ovulation for at least 3 months before the study, and they are having irregular periods, no periods, or abnormal bleeding. All clinically unhealthy women who had a miscarriage experience, thyroid dysfunction, cardiovascular illness, endometriosis, hyperprolactinemia, anima, smoking or drinking any type of alcohol, were excluded from the study

In general, the subgroups of the patients and the controls were examined in terms of clinical characteristics such as age, body mass index (BMI), all study participants gave their informed consent.

Based on the updated 2003 Rotterdam ESHRE/ASRM consensus criteria and the elimination of associated illnesses, each patient with PCOS matched diagnostic criteria for PCOS. Oligomenorrhea was defined as oligo and/or anovulation i.e. less than eight cycles per year.

The diagnosis of the TFI was done by specialists based on a patient having two of three findings as (pelvic inflammation, tubal obstructions) with the exclusion of congenital adrenal hyperplasia, androgen-secreting tumors, and Cushing's syndrome. The control group consisted of 25 women free from signs and symptoms of PCOS, TFI dependent on the medical criteria. All were clinically healthy women

who had regular menstrual cycles (shorter than 30 days),

Venous blood samples were collected from an antecubital vein, between 08:00 a.m. and 4:00 p.m. The samples were immediately saved at (2◦C) for leukocyte extraction. A full physical examination was performed including an assessment of weight. BMI was calculated by the formula: weight (kg)/height (m^2). All subjects were asked to complete a general questionnaire and gave informed consent before the onset of the study.

3.2 Materials

3.2.1 Equipment:

Table3.1. Apparatus, equipment, chemicals, and kits.

S.No.	Name of Equipment and Instrument	Name of Chemicals and Kits
1	Centrifuge (Biorad,USA)	TriPure Isolation Reagent(Roche, Switzerland)
2	Deep Freezer -20°,-80°C)(Presto,India)	EvoScript Universal cDNA Master kit (Roche, Switzerland)
3	Thermal Cycler (Biobase,China)	Ethanol (Sigma,Germany)
4	NanoDrop™ 2000 (Thermo Fisher Scientific,USA)	Isopropanol (Sigma,Germany)
5	Vortex	Red cell lysis reagent (Thermo Fisher Scientific,USA)
6	ightCycler 480 II (Roche, Switzerland)	Light Cycler 480 Probes Master) (Roche,Switzerland)
7	ht Cycler 480 Multiwell Plate 96	Trizol (Thermo Fisher Scientific,USA)
		Distilled water (Sigma,India)

3.3. Methods

3.3.1. Questionnaire design

Before beginning the study an interview questionnaire has been done with close and open-ended questions and that was according to the review of the literature related to women suffering from infertility with PCOS, TFI, and UI. A face-to-face

meeting between the researcher and subjects was the method of filling the questionnaire. The questionnaire includes several areas of questions such as personal data (e.g. age, BMI), menstrual irregularities, ovulation problems, smoking, and other related important question.

3.3.2. Body mass index (BMI)

Body mass index (BMI) was measured, for each, by dividing the weight in kilograms by the height in squared meters (kg/m^2). The measurements were graded to determine their level of obesity. The females with a BMI of < 18.5 were considered as underweight, (18.5-24.9) was normal, (25-29.9) as overweight, and > 30 as obese.

3.3.3. Blood sampling

Venous blood collections (10 mL) were taken from a healthy as well as a patient volunteer who signed a written informed consent form. Each blood sample was saved in EDTA tubes and stored at the refrigerator temperature between (2-8 ^0C) for molecular studies.

3.3.4. Isolation leukocytes from Blood Samples

The leukocytes were extracted using Trizol reagent and red cell lysis reagent from (Thermo Fisher Scientific, USA) following the standard procedures as described in the protocol by the manufacturing.

Red cell lysis

16,5 g ammonium chloride (NH_4Cl)

2 g potassium bicarbonate ($KHCO_3$)

4 mL Ethylenediaminetetraacetic acid solution (EDTA)

1- All the (10 mL) blood samples were transferred from the EDTA tubes

to Falcon® tubes, and then red cell lysis was added as 3 times as the amount of blood.

2- After red cell lysis buffer has been added to the Falcon® tubes, the suspension was left at (4^0C) for 15-20 minutes to ensure complete lysis of red blood cells with minimal effects on leukocytes.

3- Centrifugation was done for 10 minutes at (600x*g*) to ensure the leukocytes has passed to the bottom of the tube.

4- The Falcon® tube containing the mixture has removed, and the liquid supernatant was discarded as hazardous waste.

5- Step 2 has been repeated, by adding red cell lysis buffer to the pellet as 2 times as the amount of blood and left the suspension was at (4^0C) for 15-20 minutes ensure from the complete washing step.

6- Another centrifugation was done for 10 minutes at (600x*g*).

7- The supernatant was discarded. The erythrocytes around the pellet were cleaned.

8- 1000 µL of Trizol reagent were added to keep the integrity of RNA.

9- The mixture of the samples was transferred from the Falcon® tubes, then sealed and saved at (-20^0C).

3.4. Genomic RNA isolation protocol

There are major techniques extensively used for genomic RNA extraction, Figure (3.1) explain (Trizol RNA extraction).

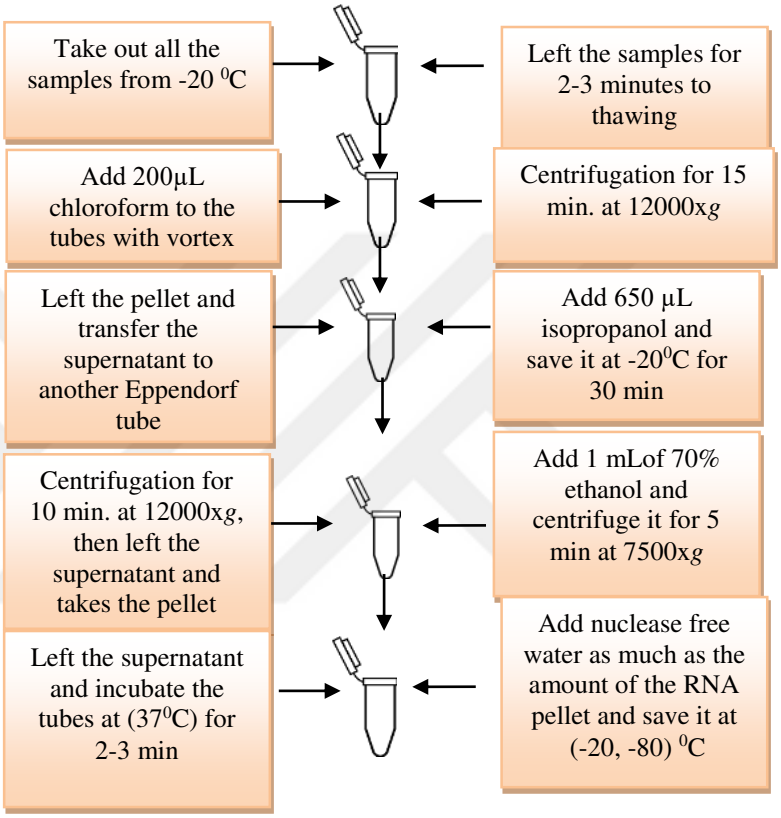

Figure 3.1. Genomic RNA isolation protocol

3.4.1. Estimation of the RNA concentration

The RNA concentration of the samples was estimated by using NanoDrop™ 2000 instrument (Thermo Fisher Scientific,USA) by putting 1µL of the extracted RNA in the machine, the software program will measure the concentration of RNA in (ng/µL) and the purity obtained by noticing the ratio of optical density (O.D) 260/280 nm to detect the contamination of samples. The accepted 260/280 ratio for pure RNA was between (1.9 – 2).

3.4.2. Dilution of RNA concentration

To proceed in the molecular analysis for cDNA synthesis and qPCR amplification, the high concentration quantity of RNA has been diluted to ensure from having reproducible results. The highest concentration was 2600 ng/µL, and the lowest one was 300 ng/µL.

3.5. Complementary DNA (cDNA) Analysis

By using the kit of (EvoScript Universal) System for general laboratory use from (Roch) company, complementary DNA (cDNA) was synthesized using reverse transcriptase (RT).

3.5.1. (cDNA) Analysis Protocol

- For each patient, RNA concentrations were diluted to be 300 ng/µL and RNA amounts were determined individually.
- The determined amounts were transferred to reaction tubes.
- The products in the kit (except RT) were left to dissolve on the cold block. It was vortexed and centrifuged briefly.
- When RT was used, care was taken by putting it in the cold block.
- Reaction buffer, 5x conc. were appear cloudy after initial thawing, a small Vortex was done to the tube that containing the reaction buffer several times and centrifuged briefly for several seconds.
- In each Eppendorf tubes, 4 µL from the reaction buffer were added.
- 9 µL PCR Grade water were added to the Eppendorf tubes as a second step.
- 5 µL diluted RNAs were added to Eppendorf tubes for each patient and control.
- The total volume of the reagent (18 µL) was mixed very well and centrifuged briefly to collect the sample at the bottom of the Eppendorf

tube.

- For at least 5 minutes the tubes were incubated on ice to let the primers anneal to RNA.
- 2µL of RT, 10x conc. were added to the final volume as the final step. To be the total volume (20 µL) for each sample.
- Reaction tubes prepared for cDNA synthesis were kept in a thermal cycler according to the time and temperatures indicated in Table 3.1.
- The cDNA products obtained were stored at −20 ºC.

Table 3.1. Temperature and time cycling in the thermal cycler for cDNA synthesis.

Temperature	Time per minute
+42°C	15 minutes
+85°C	5 minutes
+65°C	15 minutes
+4°C	unlimited hold time

3.6. Quantitative Real-Time PCR (qRT–PCR):

In this study, the expression level of LIF, IL-11 genes were examined by qRT-PCR and the expression analyzes were performed with LightCycler 480 II (Roche Diagnostics Ltd., Switzerland) device about 35 cycle in a different range of temperature. Analyzes were made with LightCycler 480 Software (release 1.5.0 SP4), as well as the assay was performed by using specific primers were provided by (Light Cycler 480 Probes Master) (Roch) for each marker (LIF, IL-11) (Table 3.2). Lyophilized primers were dissolved in a DNase/RNase-free water to give a final concentration of 100 pmol/µL and kept as stock in -20ºC as recommended by the design provider according to National Center for Biotechnology Information (NCBI).

Table 3.2. Primer Sequences

Gene	Primer Sequences
Leukemia inhibitory factor LIF	F: 5-TCTCTCCTGGCGGACACG-3
	R: 5-AATCCAGGTTGTGGTCCCC-3
Interleukin 11 IL-11	F: 5-TGGTTCTGCACTGGAAACATG-3
	R: 5-GTAATAGAGAATAAAGAGGGCATTGG-3

Amplification was performed on ice in aseptic conditions using 200 µL tight cap Eppendorf tubes. To achieve homogeneity of reagents and reduce the risk of contamination a master mix for all samples was prepared containing all the components of the reaction except cDNA, mixed gently with sterile distilled water (PCR grade water) to achieve the appropriate volume. In each PCR experiment, a negative control reaction including all of the reaction's components was set up, but template cDNA so that any contaminating cDNA present in the reaction would be detected.

3.6.1 The Protocol of qPCR Amplifications:

Each patient was studied in duplicate, and two negative controls and calibrators were used in each study. The products were prepared and loaded on the device according to the protocol stated below:

- The obtained cDNAs were diluted by adding 20 µL of distilled water to 5 µL of concentrated cDNA to be the total volume of 25µL.
- A separate mixture was prepared for each gene as indicated in Table 3.3. Beta-actin was used as the housekeeping gene.
- Into each well on the PCR plate 15µL of the mixture were dispensed.
- Diluted cDNA was added as 5 µL into the PCR plate.
- The plate was covered with sealing tape.
- The plate was briefly centrifuged with perfect spin and loaded on the Roche Light Cycler 480 II device.

- Roche LightCycler 480 software system was opened on the computer and the study was initiated according to the cycle in the program specified in Table 3.4.

Table 3.3.The reaction mixture for expression analysis (for 1 sample).

Reagents	Amount to be added to each well (µL)
Probe Master mix (LightCycler 480 Master)	10
PCR grade dH_2O	4
Probe (LIF,IL-11,beta actin)	1
cDNA	5
Final Volume	**20**

Table 3.4. Temperature and times programs for expression in LightCycler 480 II software

Initial denaturation	Temp : 94°C	Time: 5 min
No. of cycles = 35 cycles		
Denaturation	Temp : 94°C	Time:35 Sec
Annealing	Temp : 60°C	Time: 30 Sec
Extension	Temp : 72°C	Time: 30 Sec
.		
Final extension	Temp : 72°C	Time : 10 min

3.7. Statistical Analysis

GraphPad Prism was used to statistically analyze the data. The suitability of the data for normal distribution was tested using the Shapiro-Wilk test, the comparison of the groups analyzed using the nonparametric test, Kruskal Wallis, and a *post hoc*, and Dunn's tests.

4. RESULT AND DISCUSSION

4.1. Result

In this study; LIF gene, an interleukin 6 class cytokine, and IL-11 gene expressions evaluated in patients diagnosed with primary type of infertility as a result of several causes were investigated.

For this purpose, the patient's group of the study was composed of 75 individuals, and the control group was composed of 25 individuals. Differences in mRNA expressions of genes between patient and healthy control groups were compared. Individuals diagnosed with infertility for multiple reasons as a result of gynecological examinations.

The age limit of the patient group was 32-45 years, while the age limit of control group was between 23-43 years old. The mean value was 32.87 ± 7.41, 33.98 ± 8.11, 32.80 ± 7.10 for POCS, TFI, and und UI groups sequentially.

In addition, the findings of this current study indicated that BMI were significantly higher in the infertile patients compared with the control group.

There was no meaningful association between the remaining diagnostic indicators regarding waist/hip ratio and infertility duration Table (4.1).

Table 4.1 Parameters Indices of normal Controls and infertility Subjects women .

Parameters	Characteristics (n=100)				P-value
	Controls (n=25)	PCOS (N=25	TFI (n=25)	UI (n=25)	
BMI (kg/m2)	20.73 ± 2.36	32.73 ± 2.88	31.73 ± 2.36	31.88 ± 2.99	< 0.05
Waist/hip ratio (WHR)	0.80 ± 0.07	0.82 ± 0.06	0.80 ± 0.04	0.81 ± 0.06	0.25
Age (years)	28.45 ± 6.14	32.87 ± 7.41	33.98 ± 8.11	32.80 ± 7.10	< 0.05
Infertility duration (years)	-	3.78 ± 1.22	2.80 ± 1.01	4.09 ± 2.11	-

In terms of genetic analysis, the research data revealed that LIF gene expression level in the infertile patient groups was considerably lower than that in the control group 0.230 ± 0.029, 0.190 ± 0.022, 0.138 ± 0.021 for POCS, TFI, UI groups sequentially and mean average (0.230 ± 0.043) for control healthy group, demonstrating a highly significant association (Table 4.2).

Table 4.2. Mean, standard deviation, and P values of study groups for LIF gene

Study group (n=75)	Average and Standard Deviation	Control (n=25)	P value
PCOS	0.230 ± 0.029	0.230 ± 0.043	*< 0.0001
Tubal factor	0.190 ± 0.022		*< 0.001
Unexplained	0.138 ± 0.021		*< 0.001

Consecutively the expression gene level of IL-11 was remarkably higher in the healthy control group 0.223 ± 0.04 than in the UI group 0.190 ± 0.09 with a significant result P value < 0.05, the second group TFI also showed a highly notable result P value < 0.0001 compared to the control group (0.167 ± 0.026, 0.223 ± 0.04) sequentially. There was no significant meaning concerning PCOS group 0.239 ± 0.08 P value =0.19.

Table 4.3. Mean, standard deviation, and P values of study groups for IL-11 gene

Studying group (n=75)	Average and Standard Deviation	Control (n=25)	P value
PCOS	0.239 ± 0.08	0.223 ± 0.04	0.19
TFI	0.167 ± 0.026		*< 0.0001
UI	0.190 ± 0.09		*< 0.05

4.2. Discussion

A variety of factors can effect on the implantation process in women at any stage. Infertility in women is induced by several of the reasons, for instance, ovulation disorders, e.g., PCOS, fallopian tube dysfunction, e.g., TFI, and occasionally, the reason for infertility is rarely discovered, e.g., UI.

Within this study the results showed the following: The findings of this research showed that increased BMI had an effect with infertility patient groups

PCOS, TFI, and UI, indicating that a high level of BMI affects the reproductive functions through dysregulation of several pathways, e.g., gonadotropin secretion is affected because of the increased peripheral aromatization of androgens to estrogens. The insulin resistance and hyperinsulinemia in obese women leads to hyperandrogenemia. The sex hormone-binding globulin (SHBG), GH, and IGFBP are decreased and leptin levels are increased.

From the other side, the result shows a significant relationship between the older age infertility group and control. These observations suggest that LIF and IL-11 expression demonstrated a significant reverse relationship with maternal age, due to exhibiting reduced autophagy activity reach the blastocyst stage at a lower rate, which led to reduced expression levels of growth factors or cytokines in the pre-implantation embryo (Kawai K.,2018). However, this result was not considering as a direct reason for the low fertilization rate associated with advanced maternal age, and it should be emphasized that future research should always account for this aspect, and if a more diverse population is included, a study design that stratifies participants by age should be used.

In this clinical trial, the etiology of infertility was examined by assessing mRNA expressions LIF, an important factor in the embryo implantation process in women who had primary infertility with PCOS. Our data revealed that LIF levels in PCOS patients were considerably lower than in the control group.

Our outcomes were equivalent to a study by (Hussein, 2018). These researchers looked at LIF expression in women with PCOS with primary and secondary infertility who were undergoing (IVF) and found that females with PCOS had considerably lower LIF mRNA expression than the control group. This gene is supposed to be a receptivity indicator, and it is modified expressions may aid in the identification of females who have experienced implantation failure.

These results demonstrate that LIF performs a critical position in implantation regulation so that when the gene ceases to respond, the blastocysts are unable to implant and do not mature into clinical gestation.

Secondarily, compared to the control fertile healthy group, LIF expression level was significantly lower in the with TFI. The present result shows that the occurrence of the blockage in the fallopian tubes leads to a decrease in the LIF gene expression in the luminal epithelium of the Fallopian tube the process of the pre-implantation embryo happens, suggesting that the paracrine interactions involving LIF between the developing embryo and the Fallopian tube in addition to autocrine interactions occurring within the developing embryo and the Fallopian tube have been failed.

According to (Li, 2020) research, the intensity of LIF gene expression in the embryonic culture medium could be used as a non-invasive supplementary biomarker for clinical pregnancy prediction in infertility females diagnosed with tubal problems, which are undergoing a single blastocyst transfer process.

LIF gene expression is also detected in a manner of significantly low level in women with unexplained infertility. Otherwise, the fertile control group showed higher levels of LIF expression. This result suggests that infertile women's LIF gene expression was dysregulated in both the proliferative and secretory phases, leading to defect in the endometrial LIF activity which can be the main cause of unexplained infertility and recurrent implantation failures. According to the findings, there was no statistical difference between infertility PCOS females and the control fertile group regarding the IL-11 gene expressions. While this correlation was discovered and it is linked to Adipocyte Proliferation by (Zhuang et al., 2019) study, indicating that stimulated adipocytes from adipose tissue, can promote cell proliferation by activating AKT/STAT3 signaling ,leading to increase level of IL-11 .

This discrepancy may be related to different inclusion criteria as well as the impossibility to rule out all factors that influence IL-11 gene expression levels.

The results of the present study revealed a highly significant difference in levels of IL-11 gene expression, which was higher in the control group then in the second group (infertility females with tubal factor). IL-11 plays a role in mediating the dialogue between the endometrium and the implanting blastocyst, which

regulates the embryo implantation process. IL-11 is reported to be destruction by tubal epithelial cells in response to *Chlamydia trachomatis* infection, the main cause of tubal factor infertility which causes extensive destruction of the ciliated cells. This effect confirms the direct role of IL-11 in the pathogenicity of tubal factor infertility women and that is what is demonstrated by our result.

There was a low statistically significant relationship between levels of IL-11 gene expression of the third group subjects (patients with unexplained infertility) and the gene expression of the control group. Hypothesized that IL-11 could be dysregulated in the glandular epithelium which leads to preventing the facilitation of its secretory. In addition, IL-11 present in the uterine lumen could not act on the endometrial uterine epithelium to facilitate attachment or adhesion of the blastocyst, leading to the formation main cause for unexplained infertility. Previous study by (Karpovich N., et al 2005) demonstrate the same result with infertility patients after noticed that levels of prolactin, IGFBP-1, and IL-11 secretion markedly reduced compared with fertile women during mediated decidualization.

5. CONCLUSION

LIF and IL-11 are undeniably necessary in mammals, particularly humans. Importantly, our findings show that low levels of LIF and IL-11 gene expression are linked to a variety of primary infertility conditions, including PCOS, tubal factor, and unexplained infertility since they play a fundamental role in embryo implantation.

We note that this was merely a preliminary study so that more LIF and IL-11 gene low expression infertile women are required for some further research. A thorough understanding of the complicated regulatory process could lead to new targeted therapies in the mechanics of the female genital system. Mutations in the IL-11 and LIF genes may have a role in failed pregnancies. Alternatively, the link between T helper cell cytokine regulation and reproductive results in infertile women could affect IL-11 and LIF expression. All of these suggest that it could play a role in infertility in these women.

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BIOGRAPHY

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APPENDICES

Additional File 1

GENETİK ÇALIŞMALAR İÇİN BGOF ÖRNEĞİ

Sayın Gönüllü**,**

İnfertilite kadınlarda hastalarda genetik (kalıtımsal) nedenlerini bulmak üzere yeni bir araştırma yapmaktayız. Araştırmanın adı "İnfertil kadın hastalarda İL-11 ve Leukımia inhibitory factor LIF sitokinlerinin gen ekspresyonlarının değerlendirilmesidir. Bu araştırmaya katılmak üzere davet edilmiş bulunmaktasınız. Araştırmaya katılacak gönüllü sayısı 100 olarak öngörülmektedir. Bu araştırmaya katılıp katılmamakta serbestsiniz. Çalışmaya katılım, gönüllülük esasına dayalıdır. Bu çalışmada yer almayı kabul etmeden önce çalışmanın ne amaçla yapılmak istendiğini anlamanız ve kararınızı bu bilgilendirme sonrası **özgür iradenizle** vermeniz gerekmektedir. Size özel hazırlanmış bu bilgilendirmeyi lütfen dikkatlice okuyunuz, sorularınıza açık yanıtlar isteyiniz. Çalışma hakkında bilgi sahibi olduktan sonra; araştırmaya katılmak isterseniz formu imzalayınız.

Araştırmaya davet edilmenizin nedeni; tekrarlayan gebelik kayıplarınızın olmasıdır. Kendinizde bir şikâyet olmasa bile katılımınız araştırmanın başarısı için önemLidir. Böyle bir analiz, ilgili genetik hastalığın nedeninin öğrenilmesinde yararlı olacaktır. Şu anda bu çalışmanın size veya çocuğunuza hemen bir fayda olarak dönüp dönmeyeceğini bilmiyoruz. Ancak ilgili hastalığın temelinde yatan nedenlerin öğrenilmesi; ileride ilgili hastalıktan etkilenmiş bireylere fayda sağlayacaktır. Bu çalışmaya katılmanız için sizden herhangi bir ücret istenmeyecektir. Çalışmaya katıldığınız için size ek bir ödeme de yapılmayacaktır.

Bu çalışma için, kolunuzdan alacağımız 10 mL kan örneğinden genetik materyal (RNA) elde edilecektir. Başka bir müdahale yapılmayacaktır.. Bu nedenle, bu araştırma, kan alınırken oluşabilecek riskler (*iğne batmasına bağlı acı; düşük bir ihtimal de olsa, iğne batması sonrasında kanamanın uzaması veya enfeksiyon riski; az bir ihtimalle çok kısa süreli baş dönmesi)* dışında ek bir risk içermez. Zaten, olası bir soruna karşı gerekli tedbirler tarafımızdan alınacaktır.

Diğer taraftan, genetik bilginin kullanılmasına bağlı sosyal, ekonomik ve psikolojik sorunlar ortaya çıkabilir. Size ait genetik bilginin gizli kalması için, elimizden geleni yapacağız. Ancak hemen belirtmemiz gerekir ki; yaptığımız testler sizin veya ailenizin bir ferdinin yıllar içerisinde bu genetik hastalıktan etkilenebileceğini ortaya çıkarabilir. Böyle bir hastalığa sahip olduğunuzu öğrenmeniz sizi psikolojik yönden etkileyebilir. Sizin anormal bir gen taşıdığınızı saptadığımızda bulgularımızı herhangi bir ücret talep etmeden size bildireceğiz. Ancak böyle bir bilgiyi öğrenmeyi reddetmek, her zaman hakkınızdır. Yine hemen belirtmeliyiz ki; bu bilgiyi sizin

dışınızda biriyle paylaşmamız sadece sizin izninizle olacaktır. Genetik testlerin önemLi bir riski de, test sonucunda anne ya da

babanın biyolojik kimLiğinin saptanmasıdır. Böyle bir durumda gizlilik ilkesine bağlı kalınacaktır. Lütfen aşağıdaki kutucuklardan size uygun olanı işaretleyiniz:

 Bu çalışmada elde edilecek kendimLe ilgili bilgileri öğrenmek istiyorum.
İmza:
 Bu çalışmada elde edilecek kendimLe ilgili bilgileri öğrenmek istemiyorum.
İmza:

Bu çalışmaya katılmayı reddedebilirsiniz; katılmak tamamen isteğe bağlıdır ve reddettiğiniz takdirde size uygulanan tedavide herhangi bir değişiklik olmayacaktır. Çalışmanın herhangi bir aşamasında olurunuzu çekme hakkına da sahipsiniz. Eğer örneğinizin imha edilmesine karar verirseniz, bu isteğinizden önce üretilmiş her türlü veri ve yapılmış analiz ortadan kaldırılmayacak, ama daha fazla analiz yapılmayacaktır.

Sizden alınan örneklerin kullanımı, bu olur formunda tanımLanan araştırma ile sınırlıdır. Ancak, bu araştırma sırasında alınan örneklerin bir kısmı, benzer araştırmalarda kullanılmak üzere saklanabilir. Lütfen aşağıdaki seçeneklerden size en uygun olanını işaretleyiniz:

 Kan ve RNA örneklerimin sadece bu çalışmada kullanılması, çalışma sonunda kalan örneklerin uygun
şekilde yok edilmesini istiyorum. İleride yapılması planlanan çalışmalar için izin vermiyorum.
İmza:
 Kan ve DNA örneklerimin, hastalığımLa ilgili olan/olmayan, ileride yapılması planlanan tüm araştırmalarda kullanılmasına izin veriyorum.
İmza:
Bu örneklerin, başka test/amaçlar için kullanılması gerektiğinde; önce Etik Kurul onayı alınacak; onaylanan yeni araştırma için, sizden yeni bir BGOF imzalamanız istenecektir.

Gönüllünün Beyanı:

Bu araştırma ile ilgili yukarıdaki bilgiler, aşağıda adı belirtilen hekim tarafından bana aktarıldı. Bu bilgilendirmelerden sonra, bu araştırmaya "gönüllü" olarak davet edildim. Bana yapılan tüm açıklamaları ayrıntılarıyla anlamış bulunmaktayım. Bu araştırmaya katılırsam, hekimLe aramda kalması gereken bana ait bilgilerin gizliliğine büyük bir özen ve saygı ile yaklaşılacağına inanıyorum. Araştırma sonuçlarının eğitim ve bilimsel amaçlarla kullanımı sırasında kişisel bilgilerimin ihtimamLa korunacağı konusunda bana yeterli güven verildi.

Projenin yürütülmesi sırasında herhangi bir sebep göstermeden araştırmadan çekilebilirim. Ancak araştırmacıları zor durumda bırakmamak için araştırmadan çekileceğimi önceden bildirmemim uygun olacağının bilincindeyim. Ayrıca tıbbi durumuma herhangi bir zarar verilmemesi koşuluyla araştırmacı tarafından araştırma dışı da tutulabilirim. Araştırma için yapılacak harcamalarla ilgili herhangi bir parasal sorumLuluk altına girmiyorum. Bana da bir ödeme yapılmayacaktır.

İster doğrudan, ister dolaylı olsun araştırmanın uygulanmasından kaynaklanabilecek herhangi bir sağlık sorunumun ortaya çıkması halinde; her türlü tıbbi müdahalenin yapılacağı konusunda gerekli güvence de verildi (tıbbi müdahalelerle ilgili olarak, parasal bir yük altına girmeyeceğim). Araştırma sırasında bir sağlık sorunu ile karşılaştığımda ya da araştırmayla ilgili herhangi bir konuda bilgi edinebilmek için, günün 24 saatinde (Zahraa ALZAİDİ)'ye, (05392206099)'dan ulaşabileceğimi biliyorum.

Bu araştırmaya katılmak zorunda değilim. Araştırmaya katılmam konusunda zorlayıcı bir davranışla karşılaşmadım. Katılmayı reddedersem, bu durumun tıbbi bakımıma ve hekimLe olan ilişkime herhangi bir zarar getirmeyeceğini de biliyorum. Kendi başıma belli bir düşünme süresi sonunda, söz konusu bu araştırmaya, hiçbir baskı ve zorlama olmaksızın, özgür irademLe (kendi rızamLa) katılmayı kabul ediyorum. Bu formun imzalı ve tarihli bir kopyası bana verildi.

- Gönüllünün Adı / Soyadı / İmzası / Tarih:

- Açıklamaları Yapan Hekimin Adı / Soyadı / İmzası / Tarih:

- Gerekiyorsa Olur İşlemine Tanık Olan Kişinin Adı / Soyadı / İmzası / Tarih:
- Gerekiyorsa Yasal Temsilcinin Adı / Soyadı / İmzası / Tarih:

SorumLu Araştırmacı: Prof. Dr. Çetin SAATÇİ

Additional File 2

VERİ TOPLAMA FORMU

A - Genel Bilgi Tarih .../…/…

1- Dosya numarası : _____
2- Hastanın adı : _____
3- Hastanın yaşı : _____
4- Ağırlık _____ , boy _____ = (BMI) _____

B- SAĞLIK ÖYKÜSÜ

1- Sigara kullanma alışkanlığınız var mı? EVET HAYIR
2- ALKOL içer misiniz? EVET HAYIR
3- Daha önce geçirilmiş kadın hastalığınız var mı?
Evet (……..)
Hayır
4- Herhangi bir hastalığınız oldu mu ya da hiç yaşadınız mı?

- şeker hastalığı
- Kistik fibroz
- kan basıncı
- kalp hastalığı
- kanser
- Yumurtalık kisti
- Anemi

C - MENSTURASYON ÖYKÜSÜ

5- Adet düzensizliği yaşıyor musunuz? EVET HAYIR
6- Kaç günde bir adet olursunuz? (_____)
7- Adet düzensizliğine yönelik tedavi gördünüz mü? EVET HAYIR

D- İNFERTİLİTE ÖYKÜSÜ

8- İnfertilite sorunu kimden kaynaklanıyor? Kadın Erkek
9- infertilitenin nedeni ?
1- PCOS 2- Yaş Faktörü 3- tubal faktörü 4- Endometriozis
5- Açıklanamayan sebepler

10- Daha önce gebelik geçirdiniz mi? EVET (Kendiliğinden)
HAYIR
(Tedaviyle)
11- Daha önceki gebeliğiniz nasıl sonuçlandı? 1- Düşük 2-Doğum

12- Tedavi özgeçmişi :

- Daha önce hiç tedavi almamış