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Effects of rs929271 SNP in Leukemia Inhibitory Factor Gene on Recurrent Pregnancy Loss, Placental Location and Fetal Gender

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Abstract

Leukemia inhibitory factor (LIF) has an essential role in embryo implantation and placentation. The purpose of this study was to investigate the association of rs929271 SNP on LIF gene with recurrent pregnancy loss (RPL), placental location, and fetal gender in Iranian Azeri women. A total of 300 Azeri women (150 women with at least two recurrent miscarriages and 150 women with at least two healthy deliveries, as the control group) were genotyped for the 3'UTR region T>G SNP on LIF gene (rs929271) by real-time polymerase chain reaction (PCR) method. Placental location and fetal gender were determined by two-dimensional sonography. The analysis showed that female embryos were more likely to abort than males (OR = 1.806, CI = 1.275 to 2.557, P value = 0.00083). The highest odds percentage ratio (OPR) of abortion were in women with fundal (OPR = 1.817) and anterior locations (OPR = 1.483); and the lowest in lower-lying placental locations (OPR = 0.448) (P = 0.0284). In this study, the frequency of T and G alleles were 0.99 and 0.01, respectively, and only two TT and TG genotypes were identified. Women with TG genotype had significantly more recurrent pregnancy loss than TT genotypes (P = 0.0133). The women with TG genotype compared with the TT genotypes were more likely to have low lying (OPR = 2.614) and anterior (OPR = 1.641) placental position. Also, the lowest OPR (0.231) for placental position of TG versus the TT genotype was posterior location (P = 0.0464). The allele G caused more (about 1.9 folds) female embryos than T allele, yet the difference was not significant (P = 0.34). In general, there are indications that the effect of this polymorphism on RPL is repeated due to its effect on the placental location and fetal gender. However, to confirm these results, there is a need to repeat this study in populations with more frequent G allele as well as other polymorphisms on the LIF gene.

Keywords: Leukemia Inhibition Factor, Recurrent Pregnancy Loss, Polymorphism, rs929271, Placenta, Gender

1. Background

1.1. Placental Location

There are several definitions, by which researchers have used localization of the implantation and placental site. Anterior, posterior, lateral, fundal, low-lying positions are the most accepted definitions for placental location. Anterior and posterior are the most frequent positions (60 to 90%) reported in previous studies (1). Placental position and its probable effect on pregnancy outcome have been investigated by a few studies. These studies showed that placental position might have implications for poor pregnancy outcome, including preterm birth (2), small for gestational age (3), fetal malposition, mal-presentation, and the development of preeclampsia (3, 4). A study on 474 Saudi Arabia women showed that anterior placenta had an association with a larger risk of gestational diabetes mellitus, pregnancy-induced hypertension, and placental abruption, while posterior placenta had a significant relationship with preterm labour (2).

1.2. Sex Ratio

In the XY sex-determination system, such as humans, the father determines the sex of the child. However, maternal effects also determine, which sperm is more probable to reach conception. Also, sex-specific mortality of embryos affects the sex ratio at birth (5). Some researchers have showed a sex differential prenatal selection as very high male-to-female ratio for early in utero loss (6-9). More recent studies of aborted foetuses showed an increased male-to-female ratio in anatomically (10) and cytogenetically studies of preterm birth (11-16). Thus, prenatal selection may act differentially on males and females.

1.3. Recurrent Pregnancy Loss

Recurrent pregnancy loss (RPL) has been defined as the loss of two or more consecutive pregnancies before 20

weeks of gestation in the guidelines of the ASRM (American Society of Reproductive Medicine) (17). recurrent spontaneous miscarriage (RSM) is a loss of intrauterine pregnancy without the involvement of external factors and is divided to two groups of primary and secondary recurrent miscarriages. In the primary abortion, the child is not born alive, yet in the secondary abortion, one or more births occur, followed by abortions (18). Recurrent spontaneous miscarriage is known as a multifactorial syndrome and the cause of more than 50% of cases of recurrent miscarriage remains unexplained. Immunological refusal may account for a fraction of apparently unexplained pregnancy losses (19, 20).

1.4. Leukemia Inhibitory Factor

The central point of the immunological regulation of pregnancy is changes that occur in the production of cytokines. In fact, non-regulation of the cytokine network can interfere with a natural pregnancy and lead to recurrent abortion. On the other hand, the role of these cytokines, including the inhibition of leukemia, has been proven in implantation and placentation and can play a significant role in recurrent abortions (21). Furthermore, LIF is a multi-functional pleiotropic cytokine member of the Interleukin-6 family. Its gene is located at 22q12.2, and has three exons, and its protein structure consists of four alpha spirals produced by various cells, such as osteoblasts, hepatocytes, fibroblasts, macrophages, monocytes, and T cells (22, 23). In addition, epithelial cells of endometrium express LIF, and their expression on the very first days post-fertilization creates a favourable environment for blastocyst implantation (24, 25). Furthermore, LIF has been presented to mediate several processes of embryo implantation ranging from blastocyst growth and development, uterine preparation for implantation, decidualization, uterine inflammatory responses towards the implanting embryos, embryo endometrial interaction, and trophoblast invasion (26). A higher frequency of mutations close to the initial codon of exon 1 and exon 3 was observed in infertile females and has been related to unexplained infertility and recurrent implantation failure after IVF and embryo transfer (27-30). One of the most important gene polymorphisms in the LIF gene is single nucleotide polymorphism (SNP) thymine (T)/guanine (G) located in the 3'UTR region (rs929271/c.1414T>G). The study of this polymorphism in the population of Brazilian women attempting IVF-ICSI showed that the G/G genotype in women was associated with an increase in implantation and pregnancy after IVF/ICSI (31). Another study by Oliveira et al. (22) on women under ICCSI showed that this polymorphism could be used as a sensitive biomarker to predict implantation efficacy and pregnancy outcomes. Kang et al. (25) showed that there is a significant relationship between G allele and infertility, especially in patients younger than 35 years old.

2. Objectives

The objective was to study the polymorphism of rs929271 on the LIF gene in Iranian Azeri women and its relationship with recurrent pregnancy loss, placental location, and fetal gender. This study was the first report of a genetic association with RPL-placental location-fetal gender complex.

3. Methods

3.1. Subjects

Overall, 150 Azeri women with at least two recurrent abortions, who referred to infertility centers of the Ardebil, Hamedan, Zanjan, West and East Azarbaijan provinces, and 150 Azeri women with at least two healthy deliveries were selected as the control group from the health centers of five Azerbaijan cities. They completed the consent form and ethics code was received. A questionnaire was completed and a blood sample was taken. The questionnaire included maternal age, maternal abortion/deliveries numbers, abortion/birth week, fetal/child sex in each delivery, placental location in each pregnancy, and kinship/non-kinship parents. Determining the gestational age, location of the placenta (anterior, posterior, lateral, fundal, and low-lying positions), and fetal sex using two-dimensional sonography in different weeks with intervals of two months (from 6 to 20 weeks of pregnancy) by a gynecologist and using the Accuvix V10 (Samsung Medison Co. Ltd., Korea) ultrasonographic apparatus was used. The fetal gender in the control group was assured by post-birth control. For women, who had abortions in the early weeks of pregnancy (under eight weeks of age), due to the inability to correctly identify the sex, fetal gender was not recorded for such cases.

3.2. Genotyping

To study the LIF T/G (rs929271) single nucleotide polymorphism, a sample of peripheral venous blood was collected from each participant in to an EDTA-containing tube. The DNA was extracted using the DynaBio TM DNA Blood kit (Takapo Biotech Co, Tehran, Iran). Real-time PCR amplification was used for genotyping. This study used two separate reactions for each sample by a Taqman SNP genotyping assay (Applied Biosystems). The PCR temperature and time conditions were as follows: 95°C for 15 minutes, 45 cycles at 95°C for 20 seconds, then

annealing and extension at 60°C for one minute. The samples were assayed in duplicates following the manufacturer's instructions for the chosen SNP. A validated TaqMan® SNP genotyping assay (rs929271) with FAM/VIC prob 5'[G/T]CAAGACAGAAAGGCACCCGG3', forward primer 5'GTGACTTGCTTTAGGGTGTC3' and reverse primer 5'CCAAGAACAGTGTGAACCAG3' was used. The PCR cycling reactions were performed on an ABI Step One System. Five samples of each genotype were sequenced in an automatic sequencer XL 3500 Genetic Analyzer (Applied Biosystems) to validate the genotyping results.

3.3. Statistical Analysis

After genotyping the samples, genotypic and allelic frequencies were estimated and then Hardy-Weinberg equilibrium test was done by the SAS 9.4 software (2014). Estimation of odds ratio and comparing of the genotypic and allelic frequencies between the controls and the patients was performed by the FREQ procedure of the mentioned software. Also, other association analysis between genotypes, alleles, abortion, and fetal gender were done by the FREQ procedure of the SAS software.

4. Results

Table 1 shows that in general, the percentage of males in this sample was lower than females in both women, who spontaneously conceived and delivered (48.67% males versus 51.33% female) and women with RPL (34.43% males versus 65.57% female). However, the odds ratio of female embryos abortion is about 1.8 times that of males, and this difference was significant at the 5% level (OR = 1.806, CI = 1.275-2.557, P value = 0.00083).

Based on the results of Table 2, the highest chances of occurrence of abortion were in women with fundal placentas (OPR = 1.817) and the lowest in low-lying placentas (OPR = 0.448). Based on the chi square test, this difference in the occurrence of abortion for different placental locations was significant (P = 0.0284). Paired comparisons were performed to determine the difference between the paired odds ratios (Table 3) and significance of these comparisons was shown in the form of similar or non-similar (a-c) letters above individual odds percent ratios in Table 2. Similar letters showed non-significant differences and non-similar letters indicated a significant difference between odds ratios. The odds ratio for the abortion of fundal and the anterior placentas was highest and their difference was not significant (OR = 0.816, P = 0.5120), and vice versa; the lower placentas had the lowest chance of abortion yet their difference with lateral placentas was not significant (OR = 0.6680, P = 0.3149).

According to the results shown in Table 4, there was no GG genotype in the studied subjects, and only six women had the heterozygote genotype (TG), and the rest of the individuals had the TT genotype. The frequency of T and G alleles were 0.99 and 0.01, respectively, and the population was in a Hardy-Weinberg equilibrium (P = 0.98). The percentage of the TT genotype in women who spontaneously conceived and delivered and women with recurrent miscarriage was approximately equal (100% versus 96%), yet the TG genotype was observed only in women with recurrent abortions (Table 5) i.e. women with the TG genotype were significantly more likely to have RPL (P = 0.0133). Because of the null frequency of TG genotype in the control group, it wasn't possible to estimate the odds ratio of RPL in women with the TT genotype versus TG. Despite the low frequency of G allele in this population, this allele was significantly associated with increasing RPL (P = 0.0138).

The association of genotypes and alleles of this polymorphism with fetal gender was not significant (Table 6). Although the female to male ratio of embryos in women with TG genotype was about two time higher than the TT genotype, this difference was not statistically significant (OR = 1.903, 95% CI = 0.499 - 7.256, P = 0.3378) and the ratio estimated for the G allele was 1.89 folds increase in comparison with the T allele, yet the association of alleles with embryos sex ratio was not significant (P = 0.3403). The low frequency of G alleles in this sample may be a reason for this non-significant association.

The results in Table 7 showed that women with the TG genotype had lowest and highest odds percent ratio (OPR) in posterior and low lying positions, respectively.

In other words, if placenta locations of women with TG genotype were to be determined, it could be suggested that they were low-lying, anterior, fundal, lateral, and eventually posterior, respectively. Statistically, the odds ratio of the TT genotypes was significant for posterior placentas compared to the low-lying position (OR = 11.296, p = 0.0004) and anterior (OR = 7.093, P = 0.0037), while other positions were not statistically significant in this regard (Table 8).

5. Discussion

The odds ratio of abortion for female embryos in this study was significantly higher than that of males, which is in agreement with the results of previous studies that showed the female embryos may be more likely to be aborted during embryogenesis, implantation, and early embryonic development than males (32). According to the evolutionary theory, imprinted male genes of the placenta are slightly more prone to favour a male embryo than a female one, and it may explain the higher prevalence of fe-

Table 1. Relationship Between Fetal Sex and Abortion in the First Two Pregnancies of Iranian[a]

Gender	Control[b]	Case[c]	Odds	Odds Ratio (95% CI)	χ^2	P Value
Female	154 (51.33)	160 (65.57)	1.039	1.806 (1.275-2.557)	11.18	0.00083
Male	146 (48.67)	84 (34.43)	0.575			
Total	300 (100)	244 (100)				

Abbreviation: CI, Confidence interval.
[a] Values are expressed as No. (%).
[b] Normal women.
[c] Women with recurrent pregnancy loss.

Table 2. Association Between Placenta Location and Abortion in Iranian Azeri Women[a]

Placental Position	Control[b]	Case[c]	Odds[d]	Odds Percent Ratio[e,f]	χ^2	P Value
Posterior	95 (44.60)	151 (37.66)	1.5895	0.844[B]	10.841	0.0284
Low lying	32 (15.02)	27 (6.73)	0.8438	0.448[C]		
Anterior	48 (22.54)	134 (33.42)	2.7917	1.483[A]		
Fundal	19 (8.92)	65 (16.21)	3.4211	1.817[A]		
Lateral	19 (8.92)	24 (5.99)	1.2632	0.672[BC]		
Total	213 (100)	401 (100)				

[a] Values are expressed as No. (%).
[b] Normal women.
[c] Women with recurrent pregnancy loss.
[d] (n case)/(n control).
[e] case (%)/control (%).
[f] Non similar letters (A - C) above odds percent ratios show significant differences between placental position odds ($P < 0.05$).

Table 3. Estimated Odds Ratio and the P Values (in Parentheses) for Pair Comparing of Abortions in Five-Placenta Locations in Iranian Azeri Women

	Posterior	Low Lying	Anterior	Fundal	Lateral
Posterior	1	0.530836 (0.0288)	1.756347 (0.0079)	2.152318 (0.0077)	0.794702 (0.4884)
Low Lying		1	0.3022 (< 0.0001)	0.2466 (< 0.0001)	0.6680 (0.3149)
Anterior			1	0.816 (0.5120)	2.2101 (0.0216)
Fundal				1	2.7083 (0.0120)
Lateral					1

Table 4. Genotypic and Allelic Frequencies in LIF-rs929271 Polymorphism in Iranian Azeri Women and Hardy-Wienberg Equilibrium Test in This Population

Genotypes[a]/Alleles	Number	Frequencies	χ^2	P Value
TT	294	0.98	0.031	0.98
TG	6	0.02		
GG	0	0		
Total	300	1		
T	594	0.99		
G	6	0.01		
Total	600	1		

[a] Only two genotypes TT and TG were seen in this population. The TG genotypic frequency in control group was 0, and for that reason, it is not possible to estimate odds ratio for comparison TT vs. TG genotypes and also allele T vs. G.

males in abortion. In other words, in the first trimester of pregnancy, miscarried females may be partially due to paternal imprinted genes (32).

The blood supply of the uterus is not uniformly distributed. The implantation site and placental location within the uterus are likely important determinants of placental blood flow, as measured by uterine artery Doppler velocimetry and therefore pregnancy success (2, 33). There are restricted reports on the association between placental location and abortion. Hill et al. (34) and Zia (2) have

Table 5. Association Between rs929271 LIF SNP and RPL in Iranian Azeri Women

Genotypes[a]/Allels	Control[b]	Case[b]	Odds	χ^2	P Value
TT	150 (100)	144 (96)	0.96		
TG	0	6 (4)	> 6 (infinity)	6.1224	0.0133
GG	0	0	0		
Total	150	150			
T	300 (100)	294 (98)	0.98		
G	0	6 (2)	> 6 (infinity)	6.0606	0.0138
Total	300	300			

[a] Only two genotypes TT and TG were seen in this population. The TG genotypic frequency in control group was 0, and for that reason, it is not possible to estimate odds ratio for comparison TT vs. TG genotypes and also allele T vs. G.
[b] Values are expressed as No. (%).

Table 6. Relationship Between rs929271 Polymorphism of LIF Gene and Fetal Gender in the First Two Pregnancies of Iranian Azeri Women

	Female	Male	Total
	Genotypes[a]		
TT[b]	311 (58.35)	222 (41.65)	533 (100)
TG[b]	8 (72.73)	3 (27.27)	11 (100)
Odds	0.0257	0.0135	
Odds ratio (%95 CI)	1.903 (0.499-7.256)		
χ2	0.9187		
P	0.3378		
	Allels		
T[b]	630 (58.5)	447 (41.5)	1077 (100)
G[b]	8 (72.73)	3 (27.27)	11 (100)
Odds	0.0127	0.0067	
Odds ratio (%95 CI)	1.892 (0.499-7.171)		
χ2	0.909		
P	0.3403		

[a] No GG genotype was seen in this study.
[b] Values are expressed as No. (%).

Table 7. Association of rs929271 LIF SNP and Placental Position in Iranian Azeri Women[a]

Placental Position	TT	TG	Odds[b]	OPR[c,d]	χ^2	P Value
Posterior	244 (41.15)	2 (9.52)	0.008	0.231^{B}		
Low Lying	54 (9.11)	5 (23.81)	0.093	2.614^{A}		
Anterior	172 (29.01)	10 (47.62)	0.058	1.641^{A}	9.1934	0.0464
Fundal	81 (13.66)	3 (14.29)	0.037	1.046^{AB}		
Lateral	42 (7.08)	1 (4.76)	0.024	0.672^{AB}		
Total	593 (100)	21 (100)				

[a] Values are expressed as No. (%).
[b] (n TG)/(n TT).
[c] TG (%)/TT (%).
[d] Similar letters (A, B) above odds percent show no significant differences between these placental position odds. Non similar letters show significant differences between them ($P < 0.05$).

described that placental location has no implications for spontaneous pregnancy loss. In contrast, this study found that fundal and anterior placenta were more significantly miscarried compared with posterior placenta in women

Table 8. Estimated Odds Ratio and P Values (in Parentheses) For Pair Comparing of Odds Ratio Differences of TT and TG Genotypes in Five-Placenta Locations in Iranian Azeri Women

	Posterior	Low Lying	Anterior	Fundal	Lateral
Posterior	1	11.296 (0.0004)	7.093 (0.0037)	4.518 (0.074)	2.905 (0.367)
Low lying		1	0.628 (0.410)	0.4 (0.2091)	0.263 (0.1925)
Anterior			1	0.637 (0.4989)	0.4095 (0.3861)
Fundal				1	0.6429 (0.7036)
Lateral					1

with recurrent pregnancy losses. Hadley et al. (2005) (4) supposed that the fundal location of the placenta is the weakest point of the membrane over the cervical Os. As the result of irregular uterine blood supply (33), the posterior wall of the pregnant uterus is longer (35) and rather thicker (36). Each of these factors may affect uterine blood supply, especially as the uterus expands to accommodate the pregnancy.

The G allele in rs929271 LIF gene SNP has been reported at a relatively high frequency in most populations. This frequency in the Caucasian women has been reported as 28.6% [(25) and Hu et al., 2011] and in Brazilian women, it is about 35% (22, 31, 37). However, no G allele was reported in the African American population (25), which is close to results of this study, due to the small amount of G allele in this study (1%). Since the polymorphism was observed only in two TT and TG genotypes in the current study, and the number of women with the TG genotype was very low (6 women), it may be concluded that this small number has been caused by marriages of parents from two different ethnicities in previous generations (2).

Oliveira et al. (22) studied the LIF rs929271 SNP in 411 Brazilian women. They did not find any significant difference among the three genotypes in RPL and pregnancy (22). Results of Paskulin et al. (37) demonstrated no association between LIF polymorphism (rs929271) and endometriosis-related infertility or IVF failure patients. The importance of LIF variants in human fertility was investigated by Kang et al. (25) and an association of LIF (rs929271) gene with human fertility was observed in this study, especially in patients under the age of 35 years old.

This study observed that women with TG genotypes had significantly more recurrent pregnancy loss rate than TT genotypes. This is in contrast with the results of previous studies, caused by lower frequency of G Allele in the Iranian Azeri women population. Also, the current findings showed that LIF SNP had no association with sex of embryos/offspring. This is similar with the findings of Ucisik-Akkaya et al. (38) that examined LIF SNP rs929271 for sex-specific prenatal loss. The association was with wild-type homozygosity for the LIF SNP rs929271, which yielded an odds ratio of 0.71 (P = 0.10).

The 3′ UTR is a versatile region that is enriched by regulatory elements and is vital for correct spatial, temporal expression of genes, or both. It has been claimed that the LIF SNP (rs929271), located in the 3′UTR region of the LIF gene, reduces the stability of the mRNA (39).

In conclusion, the current results revealed a potential novel genetic biomarker for predicting placenta localization and recurrent pregnancy loss. The results demonstrate an approximate two-fold increased chance of female embryos miscarried compared to males. The embryos implanted in fundal and anterior positions had about a two-fold increased chance of abortion compared to the posterior position. Women with the LIF (rs929271) GT genotype had significantly more recurrent pregnancy loss and more anterior position for their placenta location. However, it had no significant effect on sex of the embryo/offspring. It may be concluded that part of the effects of allele G caused more recurrent pregnancy loss by its effect on implantation and therefore placenta location in the anterior position. Because of the low frequency of allele G in this population, further studies exploring the association of the SNP and also different genes involving implantation and their polymorphisms are needed and will help clarify the influence of genetic variants on the placental localization and gender of embryos/offspring in recurrent pregnancy loss.

Footnotes

Authors' Contribution: The idea and designing of experiment and also statistical analysis and manuscript preparation was performed by Yousef Mehmannavaz and genotyping, collecting of data was made by Mohammad Javad Heydarzadeh.

Conflict of Interests: There is no conflict of interests.

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References

1. Benirschke K, Kaufmann P, Baergen R. *Pathology of the human placenta*. New York: Springer; 2006.
2. Zia S. Placental location and pregnancy outcome. *J Turk Ger Gynecol Assoc*. 2013;**14**(4):190–3. doi: 10.5152/jtgga.2013.92609. [PubMed: 24592104]. [PubMed Central: PMC3935544].
3. Magann EF, Doherty DA, Turner K, Lanneau GJ, Morrison JC, Newnham JP. Second trimester placental location as a predictor of an adverse pregnancy outcome. *J Perinatol*. 2007;**27**(1):9–14. doi: 10.1038/sj.jp.7211621. [PubMed: 17080095].
4. Hadley CB, Main DM, Gabbe SG. Risk factors for preterm premature rupture of the fetal membranes. *Am J Perinatol*. 1990;**7**(4):374–9. doi: 10.1055/s-2007-999527. [PubMed: 2222633].
5. Krackow S. Potential mechanisms for sex ratio adjustment in mammals and birds. *Biol Rev Camb Philos Soc*. 1995;**70**(2):225–41. doi: 10.1111/j.1469-185X.1995.tb01066.x. [PubMed: 7605846].
6. Shettles LB. After office hours conception and birth sex ratios: A review. *Obstet Gynecol*. 1961;**18**(1):122–30.
7. Stevenson AC, Bobrow M. Determinants of sex proportions in man, with consideration of the evidence concerning a contribution from x-linked mutations to intrauterine death. *J Med Genet*. 1967;**4**(3):190–221. doi: 10.1136/jmg.4.3.190.
8. Lee S, Takano K. Sex ratio in human embryos obtained from induced abortion: Histological examination of the gonad in 1,452 cases. *Am J Obstet Gynecol*. 1970;**108**(8):1294–7. doi: 10.1016/0002-9378(70)90111-0. [PubMed: 5482865].
9. Kellokumpu-Lehtinen P, Pelliniemi LJ. Sex ratio of human conceptuses. *Obstet Gynecol*. 1984;**64**(2):220–2. [PubMed: 6738954].
10. Byrne J, Warburton D. Male excess among anatomically normal fetuses in spontaneous abortions. *Am J Med Genet*. 1987;**26**(3):605–11. doi: 10.1002/ajmg.1320260315. [PubMed: 3565477].
11. McGregor JA, Leff M, Orleans M, Baron A. Fetal gender differences in preterm birth: Findings in a North American cohort. *Am J Perinatol*. 1992;**9**(1):43–8. doi: 10.1055/s-2007-994668. [PubMed: 1550632].
12. Smith GC. Sex, birth weight, and the risk of stillbirth in Scotland, 1980-1996. *Am J Epidemiol*. 2000;**151**(6):614–9. doi: 10.1093/oxfordjournals.aje.a010249. [PubMed: 10733043].
13. Ingemarsson I. Gender aspects of preterm birth. *BJOG*. 2003;**110 Suppl 20**:34–8. doi: 10.1016/S1470-0328(03)00022-3. [PubMed: 12763109].
14. Zeitlin J, Ancel PY, Larroque B, Kaminski M, Epipage Study . Fetal sex and indicated very preterm birth: Results of the EPIPAGE study. *Am J Obstet Gynecol*. 2004;**190**(5):1322–5. doi: 10.1016/j.ajog.2003.10.703. [PubMed: 15167836].
15. Vatten LJ, Skjaerven R. Offspring sex and pregnancy outcome by length of gestation. *Early Hum Dev*. 2004;**76**(1):47–54. doi: 10.1016/j.earlhumdev.2003.10.006. [PubMed: 14729162].
16. Jongbloet PH. Fetal sex and very preterm birth. *Am J Obstet Gynecol*. 2005;**193**(1):302. doi: 10.1016/j.ajog.2005.01.079. [PubMed: 16021094].
17. Oguzulgen IK, Ekim N, Erkekol FO, Altinok B, Akar N. Is tissue-plasminogen activator gene polymorphism a risk factor for venous thromboembolism in every population? *J Thromb Thrombolysis*. 2005;**19**(1):61–3. doi: 10.1007/s11239-005-0941-5. [PubMed: 15976969].
18. Sierra S, Stephenson M. Genetics of recurrent pregnancy loss. *Semin Reprod Med*. 2006;**24**(1):17–24. doi: 10.1055/s-2006-931797. [PubMed: 16418974].
19. Lissauer D, Piper K, Goodyear O, Kilby MD, Moss PA. Fetal-specific CD8+ cytotoxic T cell responses develop during normal human pregnancy and exhibit broad functional capacity. *J Immunol*. 2012;**189**(2):1072–80. doi: 10.4049/jimmunol.1200544. [PubMed: 22685312].
20. Li J, Liu L, Liu B, Saravelos S, Li T. Recurrent miscarriage and birth sex ratio. *Eur J Obstet Gynecol Reprod Biol*. 2014;**176**:55–9. doi: 10.1016/j.ejogrb.2014.02.031. [PubMed: 24666800].
21. Orsi NM, Tribe RM. Cytokine networks and the regulation of uterine function in pregnancy and parturition. *J Neuroendocrinol*. 2008;**20**(4):462–9. doi: 10.1111/j.1365-2826.2008.01668.x. [PubMed: 18266939].
22. Oliveira JB, Vagnini LD, Petersen CG, Renzi A, Oliveira-Pelegrin GR, Mauri AL, et al. Association between leukaemia inhibitory factor gene polymorphism and pregnancy outcomes after assisted reproduction techniques. *Reprod Biomed Online*. 2016;**32**(1):66–78. doi: 10.1016/j.rbmo.2015.09.018. [PubMed: 26615902].
23. Mathieu ME, Saucourt C, Mournetas V, Gauthereau X, Theze N, Praloran V, et al. LIF-dependent signaling: new pieces in the Lego. *Stem Cell Rev*. 2012;**8**(1):1–15. doi: 10.1007/s12015-011-9261-7. [PubMed: 21537995]. [PubMed Central: PMC3285761].
24. Jacovas VC, Rovaris DL, Perez O, de Azevedo S, Macedo GS, Sandoval JR, et al. Genetic variations in the TP53 pathway in native Americans strongly suggest adaptation to the high altitudes of the andes. *PLoS One*. 2015;**10**(9). e0137823. doi: 10.1371/journal.pone.0137823. [PubMed: 26382048]. [PubMed Central: PMC4575214].
25. Kang HJ, Feng Z, Sun Y, Atwal G, Murphy ME, Rebbeck TR, et al. Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans. *Proc Natl Acad Sci U S A*. 2009;**106**(24):9761–6. doi: 10.1073/pnas.0904280106. [PubMed: 19470478]. [PubMed Central: PMC2700980].
26. Salleh N, Giribabu N. Leukemia inhibitory factor: Roles in embryo implantation and in nonhormonal contraception. *ScientificWorldJournal*. 2014;**2014**:201514. doi: 10.1155/2014/201514. [PubMed: 25152902]. [PubMed Central: PMC4131495].
27. Giess R, Tanasescu I, Steck T, Sendtner M. Leukaemia inhibitory factor gene mutations in infertile women. *Mol Hum Reprod*. 1999;**5**(6):581–6. doi: 10.1093/molehr/5.6.581. [PubMed: 10341007].
28. Kralickova M, Sima R, Vanecek T, Sima P, Rokyta Z, Ulcova-Gallova Z, et al. Leukemia inhibitory factor gene mutations in the population of infertile women are not restricted to nulligravid patients. *Eur J Obstet Gynecol Reprod Biol*. 2006;**127**(2):231–5. doi: 10.1016/j.ejogrb.2006.02.008. [PubMed: 16545901].
29. Novotny Z, Krizan J, Sima R, Sima P, Uher P, Zech N, et al. Leukaemia inhibitory factor (LIF) gene mutations in women diagnosed with unexplained infertility and endometriosis have a negative impact on the IVF outcome. A pilot study. *Folia Biol (Praha)*. 2009;**55**(3):92–7. [PubMed: 19545488].
30. Steck T, Giess R, Suetterlin MW, Bolland M, Wiest S, Poehls UG, et al. Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer. *Eur J Obstet Gynecol Reprod Biol*. 2004;**112**(1):69–73. doi: 10.1016/S0301-2115(03)00315-4. [PubMed: 14687743].
31. Vagnini LD, Nascimento AM, Canas Mdo C, Renzi A, Oliveira-Pelegrin GR, Petersen CG, et al. The relationship between vascular endothelial growth factor 1154G/A polymorphism and recurrent implantation failure. *Med Princ Pract*. 2015;**24**(6):533–7. doi: 10.1159/000437370. [PubMed: 26305668]. [PubMed Central: PMC5588281].
32. Del Fabro A, Driul L, Anis O, Londero AP, Bertozzi S, Bortotto L, et al. Fetal gender ratio in recurrent miscarriages. *Int J Womens Health*. 2011;**3**:213–7. doi: 10.2147/IJWH.S20557. [PubMed: 21845066]. [PubMed Central: PMC3150206].
33. Kalanithi LE, Illuzzi JL, Nossov VB, Frisbaek Y, Abdel-Razeq S, Copel JA, et al. Intrauterine growth restriction and placental location. *J Ultrasound Med*. 2007;**26**(11):1481–9. doi: 10.7863/jum.2007.26.11.1481. [PubMed: 17957042].
34. Hill JA, Reindollar RH, McDonough PG. Ultrasonic placental localization in relation to spontaneous abortion after mid-trimester amniocentesis. *Prenat Diagn*. 1982;**2**(4):289–95. doi: 10.1002/pd.1970020408. [PubMed: 7156025].
35. Andersen KV, Munck O, Larsen JF, Kjeldsen H. Placenta flow index in posterior wall placentas measured with 99mtechnetium-labelled human serum albumin. *Clin Physiol*. 1983;**3**(6):577–80. doi: 10.1111/j.1475-097X.1983.tb00867.x. [PubMed: 6686814].
36. Degani S, Leibovitz Z, Shapiro I, Gonen R, Ohel G. Myometrial thickness in pregnancy: Longitudinal sonographic study. *J Ultrasound Med*.

1998;**17**(10):661–5. doi: 10.7863/jum.1998.17.10.661. [PubMed: 9771611].
37. Paskulin DD, Cunha-Filho JS, Paskulin LD, Souza CA, Ashton-Prolla P. ESR1 rs9340799 is associated with endometriosis-related infertility and in vitro fertilization failure. *Dis Markers*. 2013;**35**(6):907–13. doi: 10.1155/2013/796290. [PubMed: 24427778]. [PubMed Central: PMC3880708].
38. Ucisik-Akkaya E, Davis CF, Do TN, Morrison BA, Stemmer SM, Amadio WJ, et al. Examination of genetic polymorphisms in newborns for signatures of sex-specific prenatal selection. *Mol Hum Reprod*. 2010;**16**(10):770–7. doi: 10.1093/molehr/gaq047. [PubMed: 20587610].
39. Fraga LR, Dutra CG, Boquett JA, Vianna FS, Goncalves RO, Paskulin DD, et al. p53 signaling pathway polymorphisms associated to recurrent pregnancy loss. *Mol Biol Rep*. 2014;**41**(3):1871–7. doi: 10.1007/s11033-014-3036-6. [PubMed: 24435975].