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The Synergistic Impact of IL-10 Gene Polymorphisms and Epidemiological Variables on Brucellosis-Induced Abortion in Ewes

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Abstract

The present study sought to ascertain the correlation between genetic polymorphisms of the IL-10 gene and abortion in aborted ewes, utilizing a sample size of 100 (75 aborted ewes and 25 pregnant ewes as a control group). The current study indicated that age may influence the risk of brucellosis; ewes were categorized into three age groups, with the 5-7 year age group exhibiting a considerable rise in brucellosis cases (35 out of 48 cases; 72.9%). The high incidence of brucellosis in the first age group (1-4 years) accounted for 17 out of 23 cases, representing 73.9%. In the age bracket of 8-9 years, 13 out of 48 cases (27.1%) exhibited a reduction in abortions. The findings of this study demonstrate a significant correlation between the gestational stage and the etiology of abortion. Conversely, abortion cases occurring in the first and second trimesters were ascribed to factors unrelated to Brucella melitensis. During that period, a direct sequencing method was employed for the analyzed PCR amplicons. Subsequently, the identified variations were positioned according to their locations within the corresponding genomic DNA sequences. The current results demonstrated the presence of the 117 C>G, 118 G>C polymorphisms, and C-del 256 of the IL-10 gene. The current findings indicate a non-significant connection (p value: 0.09204) between the 117C>G and 118G>C polymorphisms of the IL-10 gene polymorphisms and abortion in aborted ewes.

Keywords: Abortion, ewe, IL-10, polymorphism, brucellosis.

I. Introduction:

Brucellosis, also referred to as Malta fever, is a bacterial zoonotic illness first identified in the mid-19th century (Corbel et al., 2006; Godfroid et al., 2014). The disease is predominantly transmitted to humans via direct contact with tissues or secretions from infected animals, especially those linked to abortion, including deceased fetuses, placentas, and vaginal discharges (Corbel et al., 2006; Dadar et al., 2021), or through the consumption of unpasteurized dairy products and undercooked meat (Casalinuovo et al., 2016; Dadar et al., 2019). A study conducted by Laine et al. (2023) indicates. The global estimate of human brucellosis infections is roughly 2.1 million new cases annually, markedly exceeding prior estimates of 500,000 cases, so underscoring the actual global burden of the illness, particularly in low-income areas. Brucella bacteria induce inflammation in multiple tissues, such as reproductive organs, lymph nodes, and the spleen, resulting in edema and necrosis. In gestating animals, Brucella infection induces placental impairment, elevating the likelihood of abortion and adversely affecting both animal health and agricultural economy (Corbel et al., 2006; Dadar et al., 2021). The bacteria that cause brucellosis are classified under the Brucella genus, characterized as gram-negative rods having a distinctive capacity to endure and proliferate within host cells, especially mononuclear phagocytes. This capability enables them to circumvent the immunological response and endure within the host via a method termed stealth colonization, rendering early detection by the immune system challenging (Martirosyan et al., 2011).

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In animals, abortion is the primary clinical manifestation, with reproductive losses including fetal demise, poor progeny, infertility, and neonatal mortality representing significant economic repercussions of the condition. Notwithstanding the widespread occurrence of brucellosis, the molecular processes responsible for abortion remain little elucidated. Recent investigations, including one by Rossetti (2024), indicate that Brucella bacteria demonstrate a significant propensity to infect the uterus during the third trimester of pregnancy, utilizing both phagocytic cells and trophoblast cells to fulfill their infection cycle. Research indicates that genetic variables, including Single Nucleotide Polymorphisms (SNPs), influence immunological responses to Brucella infection, potentially affecting the immune system's capacity to fight the infection. Certain genetic variants may influence the intensity of infection and an individual's vulnerability to abortion upon exposure to Brucella, underscoring the necessity for additional research into the interplay between pathogen and host genetics (Dabagh et al., 2014).

Due to the crucial function of Interleukin-10 (IL-10) in regulating immunological responses and reducing inflammation, numerous studies have indicated a correlation between genetic polymorphisms (SNPs) in the IL-10 gene and vulnerability to various infectious diseases, such as brucellosis. These genetic differences may affect the expression levels of IL-10, potentially resulting in modifications to the immune response in infected animals (Oliveira et al., 2015; Ahmed et al., 2016). To comprehend the genetic determinants that may heighten vulnerability to abortion in animals afflicted with brucellosis and to ascertain how these determinants affect the infection's progression and its consequences. This study aims to investigate the association between IL-10 gene polymorphism and abortion in aborted ewes infected with B. Melitensis.

II. Materials and Methods

Collection of samples

Seventy-five blood samples were taken from the jugular vein of aborted ewes at a veterinary hospital in Thi-Qar province between October and November 2024, while twenty-five blood samples from pregnant ewes were collected as a control group. A total blood volume of 5 ml was obtained using sterile syringes and subsequently transferred into blood collection tubes containing EDTA. Rose Pinkal test (ID VET / Germany) utilized for the detection of B. Antibody to Melitensis in samples. Additionally, the B. Melitensis found via real-time PCR targeting the IS711 transposase gene.

Extraction of genomic DNA from blood samples

DNA was extracted from blood samples utilizing the gSYNC™ DNA Extraction Kit, in accordance with the manufacturer's instructions.

Polymerase Chain Reaction (PCR) identification of the IL-10 gene

The precise primer pairings for IL-10 are as follows: Forward: CACAGTTTGACCCGGGACTC; Reverse: ACCAGATGCAAAGCTGGAGAG (in this investigation).

The total capacity of the reaction tubes is 20µl, comprising 12.5µl of Master Mix, 1µl each of forward and reverse primers specific to this gene, 3µl of DNA template, with the remainder filled with nuclease-free water. The isolated DNA samples were electrophoresed by combining 5µl of DNA with loading dye and thereafter put into the designated wells, then subjected to an electric field (70V for 45-60 minutes). The thermocycling protocols for the IL-10 gene are presented in Table 1.

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Table 1: IL-10 Gene Program.

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5min	1
Denaturation	94	30 sec	30
Annealing	54	30 sec	
Extension	72	35 sec	
Final extension	72	5 min	1

Interpretation of sequencing data

The sequencing findings of the PCR products from various samples were edited, aligned, and examined alongside the corresponding sequences in the reference database with BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The identified differences in each sequenced sample were enumerated in PCR amplicons and their corresponding positions within the reference genome. Each identified variant within the examined gene was annotated using SnapGene Viewer version 4.0.4 (https://www.snapgene.com).

Analyzing the C>G 117, G>C 118 SNPs, and C-del 256

The identified SNP and any variations were submitted to the dbSNP database to verify their authenticity. Each specific SNP was emphasized based on its position in the reference genome. Subsequently, the identification of the prior SNP was conducted by examining its associated dbSNP position. The dbSNP location for the identified SNP was recorded.

Statistical analysis

The data from the current study were statistically analyzed using SPSS software version 26. The Hardy-Weinberg equilibrium for the three single nucleotide polymorphisms (SNPs) in both groups was assessed using the chi-square test.

III. Results and Discussion

The current study indicated that age may contribute to an elevated risk of brucellosis, with ewes categorized into three age groups. The group aged 5-7 years exhibited a notable rise in brucellosis incidence (35 out of 48 cases; 72.9%). Furthermore, there was a reduction in the frequency of abortions in the age range of 1-4 years, which exhibited the lowest incidence of brucellosis at 6 out of 23 cases (26%).

The high incidence of brucellosis in the first age group (1-4 years) accounted for 17 out of 23 cases, representing 73.9%. In the age group of 8-9 years, 13 out of 48 cases (27.1%) shown a decline in abortions, as indicated in Table 2.

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Table 2: Quantities and proportions of infections across age intervals for both brucellosis and other etiologies in abortions

Abortions				
Effect of age period		**Brucellosis**	**Another reason**	**Total**
1-4 years	Count	6	17	23
	% within age	26.1%	73.9%	100.0%
5-7 years	Count	35	13	48
	% within age	72.9%	27.1%	100.0%
8-9 years	Count	2	2	4
	% within age	50.0%	50.0%	100.0%
Total	Count	43	32	75
	% within age	57.3%	42.7%	100.0%

The results of this study reveal a robust correlation between the gestational stage and the etiology of abortion. The current data indicate that all abortion cases attributed to Brucella melitensis infection, as verified by RT-PCR method, transpired solely during the third trimester of gestation. Conversely, abortion cases occurring in the first and second trimesters were ascribed to factors unrelated to Brucella melitensis, as determined by RT-PCR diagnostics (/////). These findings correspond with those of Rossetti (2024), who revealed that Brucella bacteria display a significant tropism for placental tissues during the later stages of pregnancy. This is due to the proliferation and increased activity of trophoblast cells and receptors during this phase, which promotes placental colonization and elevates the risk of abortion.

The present study indicated that abortions linked to Brucella melitensis constituted 57.3% of all abortion cases, a figure comparable to the 56% reported by Al-Dabbagh (2014) in Mosul Province, and somewhat exceeding the 53.5% seen by Dhahir (2002) in Baghdad Province. Moreover, all abortion instances attributed to Brucella infection (43 cases) transpired during the third trimester, constituting 100% of the infections—exceeding the 68% reported by Al-Dabbagh for the same trimester (22 confirmed cases) and significantly surpassing the 19.9% documented by Dixon et al. (2007).

Concerning first-trimester abortions, the present study documented a rate of 19%, in contrast to 28% reported by Al-Dabbagh (2014) and merely 3.7% by Dixon et al. (2007). The abortion rate in the second trimester of this study was 13%, roughly resembling Dixon's 11.5%, but Al-Dabbagh reported a higher rate of 27%.

These results corroborate the findings of Carson (2018), who underscored the pivotal role of bacteria as key causal agents of abortion. Veterinary diagnostic findings in the UK (2011–2018) indicated that bacterial illnesses were the predominant infectious etiology of abortion. Clune et al. (2020) also stated that 81% of documented abortion cases in Australia from 2000 to 2018 were attributable to infectious pathogens.

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Research has demonstrated that endotoxins released by gram-negative bacteria, including Escherichia coli and Salmonella spp. May induce the synthesis of prostaglandins, resulting in abortion (Schlafer et al., 1994; Youngquist and Threlfall, 2006). Non-infectious variables, including the provision of moldy hay or substandard silage to pregnant ewes, can elevate the chance of abortion (Radostits and Gay, 2017). Additional microbial etiologies of abortion encompass Campylobacter spp., Listeria spp., Coxiella burnetii, and Chlamydia abortus, alongside viruses like Border disease virus, Bluetongue virus, and Schmallenberg virus, as well as parasites such as Toxoplasma gondii and Neospora caninum (Njaa, 2011).

Besides infectious causes, non-infectious factors—such as deficiencies in vitamin E or selenium, trauma, toxemia, stress, overcrowding, and consumption of toxic plants—have a relatively minor impact, though they may be significant in specific instances (Youngquist and Threlfall, 2006; Pugh and Baird, 2012).

The current findings suggested that the infection was caused by B. Melitensis is associated with gestational age, categorizing pregnancy into three stages. In the third trimester of pregnancy, 43 out of 43 cases (100%) resulted from infection with B. Melitensis; conversely, the first and second trimesters recorded (19/32) and (1/3) instances (100%) respectively. Furthermore, no instances of abortion attributed to other causes have been documented during the third trimester of pregnancy in ewes. Refer to table (3).

Table 3: The severity of Brucella infection during the final third of gestation in aborted ewes compared to the preceding two thirds of gestation.

Sort		Reason		Total
The third effect		Brucella	Another reason	
First third	Count	0	19	19
	% within third	0.0%	100.0%	100.0%
Second trimester	Count	0	13	13
	% within third	0.0%	100.0%	100.0%
Third trimester	Count	43	0	43
	% within third	100.0%	0.0%	100.0%
Total	Count	43	32	75
	% within age	57.3%	42.7%	100.0%

Arrange

Table (2) illustrates a substantial association between age and the probability of abortion associated with Brucella infection. The peak infection rate occurred in ewes aged 5–7 years, representing 72.9% of bacterial abortion instances and 48% of total abortion cases. This is likely because to a heightened incidence of pregnancies and recurrent exposure to infectious agents during this reproductive zenith, along with the potential cumulative deterioration of the immune response.

Despite younger sheep (1–4 years) having a more vigorous immune system, their less exposure to parturition and infectious secretions diminishes their infection risk (26.1%). In older sheep (8–9 years), the infection rate was equally divided between bacterial and non-bacterial etiologies (50%), potentially due to age-related modifications in the immune system.

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These findings correspond with those of Jamil et al. (2020), who found that mid-life animals have greater susceptibility to Brucella infection compared to younger or older counterparts. This conclusion is corroborated by the research of Megersa et al. (2011) and Chimana et al. (2010), both of which emphasized the influence of age on seropositivity in brucellosis diagnostic assessments.

Molecular investigation

The current findings indicate that the DNA taken from both the aborted ewe and the control group (100/100; 100%) contained the IL-10 gene.

IL-10 gene sequences were examined in 100 samples, comprising 75 samples from aborted ewes and 25 control samples. The sequencing reactions confirmed the precise identity of the amplified genomic segment following the execution of NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This engine demonstrated over 99% sequence homology between the amplified samples and the designated reference target sequences, which largely encompass the IL-10 gene. The comparison of the observed DNA changes in the tested samples with the retrieved DNA sequences (GenBank acc. NC_056065.1) elucidated the exact placements and further features of the retrieved PCR amplicons (Fig. 1).

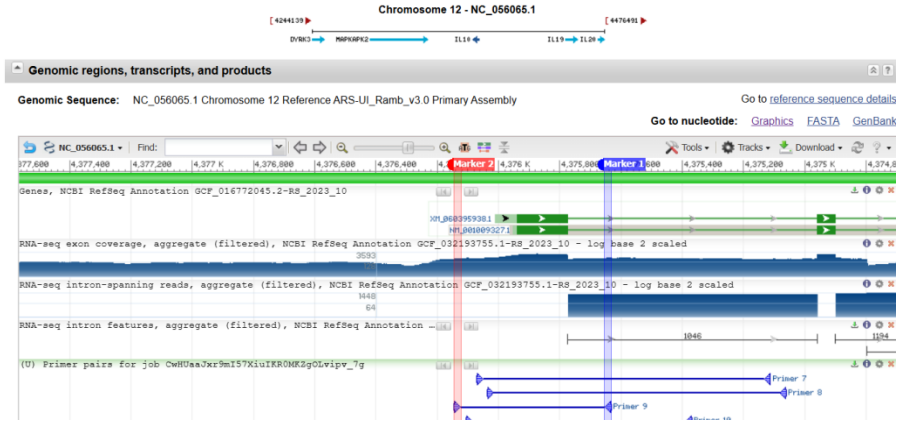

Figure. The precise location of the isolated 513 bp amplicon that partially encompasses a segment of the IL-10 gene on chromosome 12 (GenBank accession number NC_056065.1).

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The alignment results of the 513bp samples indicated nucleic acid variation in both cases and controls when compared to the appropriate reference sequences. Fig.2..

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

Ref
CCGAAGGCAGCTCGGACGTCCCGCAGCATGTGGGGCAGGCTGGCTGGGAAGTGGGTACAGCTGCTGT
CAGACAGGGTGCTGGCATCTCGGCTGGCTGCCA

S1 .....GC.....

S2 .....GC.....

S3 .....GC.....

S4 .....GC.....

S5 .....GC.....

S6 .....GC.....

S7 .....GC.....

S8 .....GC.....

S72 .....GC.....

S73 .....GC.....

S74 .....GC.....

S75 .....GC.....

C1 .....GC.....

C2 .....GC.....

C3 .....GC.....

C6 .....GC.....

C7 .....GC.....

C8 .....GC.....

210 220 230 240 250 260 270 280 290 300

....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

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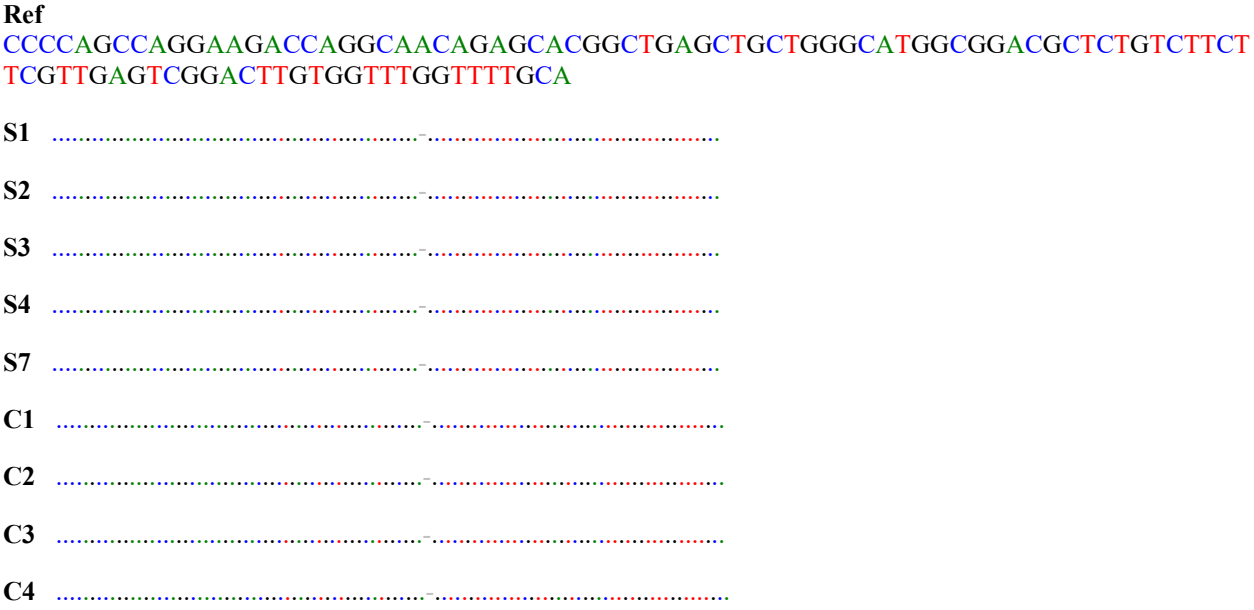

Fig. 2. DNA sequences alignment of some samples with their corresponding reference sequences of the 513bp PCR fragments of the analyzed *IL-10* gene. The letter "ref." refers to the NCBI referring sequence, and the letters (S) refer to the samples, and (C) refer to the control

The identified polymorphic locus demonstrated predominance in the examined samples. Consequently, it was essential to investigate additional specifics of this variant in its respective locations within the genomic DNA sequences of the IL-10 gene recorded in the dbSNP database. To identify the genomic positions of the targeted variant in relation to its recorded SNP database of the sequenced 513bp fragments, the relevant position of the IL-10 gene was obtained from the dbSNP server (https://www.ncbi.nlm.nih.gov/projects/SNP/). Additionally, to elucidate the specific characteristics of the found SNP, graphical representations were provided regarding the IL-10 dbSNP database on chromosome 12 utilizing the GenBank accession number. Negative. NC_056065.1

The sequencing analysis of the IL-10 gene in both aborted and control ewes, for the identification of variations, coupled with its comprehensive annotations by Mega 11, was recorded and presented according to the corresponding locus in the amplified 513 bp PCR fragments, as illustrated in the Fig. 3.

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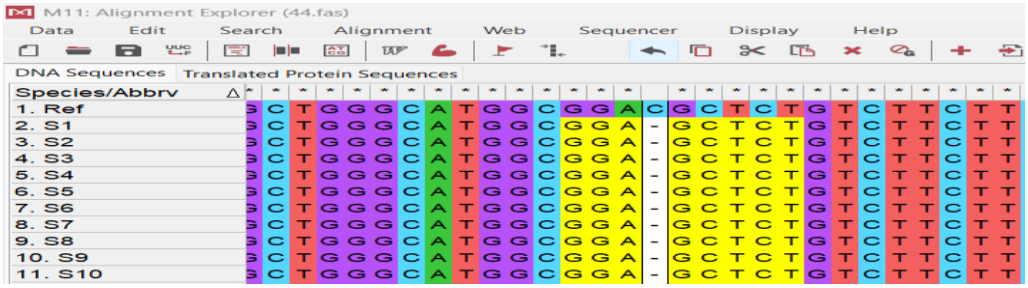
A



B

Figure. 3: The identified single nucleotide polymorphism (SNP) within the Mega 11 of the targeted 513 base pair amplicons of the IL10 gene. A: 117 C>G SNP and 118 G>C SNP; B: deletion of C at position 256.

The Hardy-Weinberg equilibrium (HWE) is a principle asserting that genetic variation within a population will stay stable throughout generations in the absence of disruptive influences. To compute Hardy-Weinberg equilibrium (HWE), the genotype and allele frequencies of the IL10 117 C>G SNP and 118 G>C SNP were analyzed. The findings of this study align with HWE, yielding a non-significant result (P= 0.9204) for both the C117G SNP and the G118C SNP.

The identified polymorphisms in the examined samples included a substitution of C nucleotide with G nucleotide at position 117 of the amplified PCR fragments (117C>G) and a substitution of G nucleotide with C nucleotide at position 118 (117G>C or G118C).

There exist three polymorphic variants of the 117C>G SNP: CC, CG, and GG. The homozygous CC genotype was absent in both cases and controls; the GG genotype was present in the majority of case samples (71/75) and all controls (25/25), whereas the heterozygous CG genotype was identified solely in the cases (4/75), as shown in Table 4.

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Table 4: Genotype frequency of 117C>G IL10 in aborted ewes and controls

IL-10 gene	**Aborted case**	**Percentage (%)**	**Control**	**%**	**OD**	**95% CI**	**P.value**
CC	0	0%	0	**0%**	0.3377	0.0065 to 17.4645	0.5897
CG	4	5.3%	0	**0%**	3.2098	0.1669 to 61.7299	0.4395
GG	71	94.7%	25	100 **%**	0.3115	0.0162 to 5.9916	0.4395

Likewise, there exist three polymorphic variants of the 118G>C SNP: GG, GC, and CC.

The CC and GC patterns were absent in both cases and controls (0%); the GG status was identified in all samples, with cases (75/75; 100%) and controls (25/25; 100%), as shown in Table 5.

Table 5: Genotype frequency of 118G>C IL10 in aborted ewes and controls

IL10 gene	**Aborted case**	**%**	**Control**	**%**	**OD**	**95% CI**	**P. value**
GG	75	100%	25	100 **%**	2.9608	0.0573 to 153.0977	0.5897
GC	0	0%	0	**0%**	0.3377	0.0065 to 17.4645	0.5897
CC	0	0%	0	**0%**			

The allele frequency of the IL10, 117C>G SNP in aborted ewes and controls exhibited a statistically non-significant connection between IL-10 gene polymorphisms 117C>G SNP and abortion in sheep (p-value = 0.7657), as illustrated in Table 6.

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Table 6 : Allele Frequency for IL10 Gene 117C>G

IL10 gene	Allele frequency	Abortion		control		**OD**	**95% CI**	**P.value**
		No	%	No	%			
117C>G	C	4	2.67%	0	0%	1.5666	0.0818 to 29.9877	0.7657
	G	146	97.33%	25	100%			

The allele frequency of the IL10, 118G>C SNP among aborted ewes and controls exhibited a statistically non-significant association between IL-10 gene polymorphisms 118G>C SNP and abortion in sheep (p-value = 0.3775), as illustrated in Table 7.

Table 7 : Allele Frequency for IL10 Gene 118G>C

IL10 gene	Allele freq.	Abortion		control		**OD**	**95% CI**	**P.value**
		No	%	No	%			
118C>G	G	150	100%	25	100%	5.9020	0.1145 to 304.2020	0.3775

The current findings of Hardy-Weinberg equilibrium for the IL10 C>G 117 SNP in the control group indicate a non-significant relationship between the expected and observed genotypes (P value: 0.9204), as illustrated in Table 8.

Table (8) : The Hardy-Weinberg Equilibrium for the analyzed groups for the IL10 117C>G polymorphism.

IL10 gene	**All group**		**Abortion**		**Control**	
	Observed	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**
CC	0	0.05	0	0.04	0	0
CG	4	3.89	4	3.92	0	0
GG	96	96.04	71	96.04	25	25
HWE	0.0015		0.0027		0	
p-value	0.8383		0.8124		0.9204	

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The current results of the Hardy-Weinberg equilibrium for the IL10 gene, G>C 118 SNP among controls indicate a non-significant relationship between the predicted and observed genotypes (P value: 0.9204), as presented in Table 9.

Table (9) : The Hardy-Weinberg Equilibrium for the examined groups in the IL10 118G>C polymorphism.

IL10 gene	All group		abortion		Control	
	Observed	Expected	Observed	Expected	Observed	Expected
GG	100	100	75	75	25	75
GC	0	0	0	0	0	0
CC	0	0	0	0	0	0
HWE	0		0		0	
p-value	0.9204		0.9204		0.9204	

To clarify the locations of the targeted SNPs in relation to their recorded SNP database within the sequenced 513bp fragment of the IL-10 gene in aborted and control ewes, the relevant positions of the IL-10 gene were obtained from the dbSNP server (https://www.ncbi.nlm.nih.gov/projects/SNP/). A graphical representation was conducted to ascertain the characteristics of these SNPs in relation to the IL-10 dbSNP database on chromosome 12 (GenBank acc. NC_056065.1). By examining all fourteen identified SNPs in the dbSNP database.

There are few research targeting various genes, such as the IL-10 gene, to elucidate the relationship between gene polymorphisms and abortion in animals, as well as the resultant losses in animal production and infections caused by several abortifacient drugs.

Pregnancy induces significant alterations in immune system modulation (Tangri et al. 1994). Infection negates the pregnancy-induced suppression of pro-inflammatory cytokine production and may directly cause the abortions linked to these species. Genetic diversity in the genes encoding these cytokines may account for differences in susceptibility to infection by these pathogens and consequent abortion among individuals (Gazzinelli et al. 1993).

The recent findings of DNA sequencing indicate no significant connection between the 117C>G and 118G>C SNPs of the IL-10 gene and abortion in aborted ewes. Host genetic variation is recognized to influence susceptibility to certain infections. Nonetheless, despite the agricultural and economic significance of domestic sheep, there has been limited research on the influence of genetic diversity in regulating immunological reactivity to ovine diseases and the risk of abortion (Darlay et al., 2011). To yet, polymorphisms have been identified in fewer than 5% of ovine genes. The majority of these polymorphisms were identified while exploring methods to enhance ovine productivity, including the finding of variable tandem repeat numbers (VNTRs) in the MUC1 gene that may influence milk characteristics (Rosero et al. 2007).

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The allele frequencies of the IL-10 117 C>G SNP and 118 G>C SNP in aborted ewes and controls exhibited a statistically non-significant connection (p-values = 0.7657 and 0.3775, respectively) concerning abortion. Polymorphisms have been reported in minor genes associated with the immunological response, including IL2 (Luhken et al. 2005) and TLR4 (Zhou et al. 2007). Marcos-Carcavilla et al. (2007) discovered 30 polymorphic sites in the IL1B gene and an additional 3 in IL1RN. Polymorphism in ovine DRB1, an MHC class II gene, is well understood, with a highly polymorphic segment of exon 2 sequenced and characterized across numerous breeds (Konnai et al. 2003a,b).

The polymorphisms and genotyping assays we have developed may be utilized in future studies of the genetic basis of susceptibility to ovine diseases (Darlay et al., 2011).

The latest findings revealed a significant prevalence of the G allele for both the 117C>G polymorphism (97.33%) and the 118G>C SNP (100%) in the analyzed samples.

The anti-inflammatory cytokine IL-10 suppresses the synthesis of natural killer cell cytokines IL-1 and TNF by macrophages, in addition to IFN-γ and IL-2 by Th1 lymphocytes (Martinez-Espinosa et al., 2021; Wang et al., 2021). IL-10 is produced by various T-cells and possesses anti-inflammatory properties that protect uterine tissues from the aggressive effects of inflammatory cells and mediators by interacting with regulatory CD8+ T-suppressor cells (Iyer and Cheng, 2012).

The current results indicate the deletion of the C nucleotide at position C-256 in the examined samples.

A comprehensive understanding of the genes, underlying mutations, and interactions with other variables that confer resistance is essential for developing disease-resistant cattle or eradicating illnesses (Pal and Chakravarty, 2020; Salim and Abdulkareem, 2019).

IV. **Conclusion** :

This study determined that there is no association between IL-10 gene variation and abortion in aborted ewes.

V. **References**

Al-Dabagh, I., Jasim, B., & Jarjees, M., (2014). Seroprevalence of antibodies to toxoplasmosis, brucellosis and chlamydiosis in abortive sheep in Nineveh governorate, Iraq. *Iraqi Journal of Veterinary Sciences*, *28*(1), 21-25. doi: 10.33899/ijvs.2014.89467

Ahmed, W., Zheng, K., Liu, Z. F. (2016). Establishment of chronic infection: Brucella's stealth strategy. *Frontiers in Cellular and Infection Microbiology*, 6, 30.

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 313-328

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Corbel MJ; Food and Agriculture Organization of the United Nations;World Health Organization; World Organisation for Animal Health.,(2006). *Brucellosis in humans and animals*. Geneva: World Health Organization.; [updated 2006; cited WHO/CDS/EPR/2006.7]. Availablefrom: https://apps.who.int/iris/handle/10665/43597

Carson A .,(2018). Abortion in sheep: an update. Vet Rec 183:528–529.

Casalinuovo F, Ciambrone L, Cacia A, Rippa P.,(2016). Contamination of bovine, sheep and goat meat with brucella Spp. *Ital J Food Saf*.;**5**(3):5913. [PubMed ID: 27853716]. [PubMed Central ID:PMC5090120]. https://doi.org/10.4081/ijfs.2016.5913.

Chimana, H.M.; Muma, J.B.; Samui, K.L.,(2010). ORIGINAL RESEARCH A Comparative Study of the Seroprevalence of Brucellosis in Commercial and Small-Scale Mixed Dairy—Beef Cattle Enterprises of Lusaka Province and Chibombo District, Zambia. Trop.Anim. Health Prod. **2010**, 42, 1541–1545. [CrossRef]

Clune T, Beetson S, Besier S, Knowles G, Paskin R, Rawlin G, et al., (2021) . Ovine abortion and stillbirth investigations in Australia. Aust Vet J. 99:72–8.

doi: 10.1111/avj.13040 .

Dadar M, Shahali Y, Fakhri Y.,(2021). Brucellosis in Iranian livestock: A metaepidemiological study. *Microb Pathog*.;**155**:104921. [PubMed ID:33930414]. https://doi.org/10.1016/j.micpath.2021.104921.

Dadar M, Shahali Y, Whatmore AM.,(2019). Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention. *Int J Food Microbiol*.;**292**:39–47.[PubMed ID:30572264].https://doi.org/10.1016/j.ijfoodmicro.2018 .12.009.

Dhahir SH.,(2002). Incidence of Brucellosis in animals and man. Iraqi J Vet Med.;26(2):140-144.

Dixon AB, Knights M, Winkler JL, Marsh DJ, Pate JL et al.,(2007). Patterns of late embryonic and fetal mortality and association with several factors in sheep. Journal of Animal Science;85 (5): 1274-1284. doi: 10.2527/jas.2006-129.

Godfroid J, DeBolle X, Roop RM, O'Callaghan D, Tsolis RM, Baldwin C, et al.,(2014). The quest for a true one health perspective of brucellosis. *Rev Sci Tech*.;**33**(2):521–38. [PubMed ID: 25707181].https://doi.org/10.20506/rst.33.2.2290.

Jamil, T.; Kasi, K.K.; Melzer, F.; Saqib, M.; Ullah, Q.; Khan, M.R.; Dadar, M.; Tayyab, M.H.; Schwarz, S.; Neubauer, H.,(2020). Revisiting Brucellosis in Small Ruminants of Western Border Areas in Pakistan. *Pathogens* , *9*, 929. https://doi.org/10.3390/pathogens9110929.

Laine CG, Johnson VE, Scott HM, Arenas-Gamboa AM. Global Estimate of Human Brucellosis Incidence. Emerg Infect Dis., (2023). Sep;29(9):1789-1797. doi: 10.3201/ei d2909.230052. PMID: 37610167; PMCID: PMC10461652.

Martirosyan A, Moreno E, Gorvel J-P., (2011). An evolutionary strategy for a stealthy intracellular Brucella pathogen. Immunological Reviews 240: 211–234.

Megersa, B.; Biffa, D.; Niguse, F.; Rufael, T.; Asmare, K.; Skjerve, E.,(2011). Cattle Brucellosis in Traditional Livestock Husbandry Practice in Southern and Eastern Ethiopia, and Its Zoonotic Implication. Acta Vet. Scand., 53, 1–8. [CrossRef]

Njaa BL .,(2011). Kirkbride's diagnosis of abortion and neonatal loss in animals. John Wiley & Sons.

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ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 313-328

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Oliveira, S. C., Carvalho, N. B., & Teixeira, H. C. (2015). Innate immunity and Brucella abortus: Yin and Yang of a bacterium. *Frontiers in Cellular and Infection Microbiology*, 5, 109.

Pugh DG, Baird NN., (2012). Sheep & Goat medicine-E-book. Elsevier, Health Sciences.

Radostits O, Gay C., (2017). A textbook of the diseases of cattle, horses, sheep, pigs and goats. Saunders Elsevier, Edinburgh.

Rossetti, C.A.,(2024). Patogénesis molecular del aborto por Brucella. *Rev. Vet.*; 35(2): 114-125.

Salim, A. H., & Abdulkareem, A. A. (2019). CAST/MspI gene polymorphism and its impact on growth performance and carcass traits of Shami goats breed in Iraq. In Journal of Physics: Conference Series, 1294 (9):09201. IOP Publishing.

Schlafer D, Yuh B, Foley G, Elssaser T, Sadowsky D, Nathanielsz P.,(1994). Effect of *Salmonella* Endotoxin Administered to the Pregnant Sheep at 133–142 Days Gestation on Fetal Oxygenation,Maternal and Fetal Adrenocorticotropic Hormone and Cortisol,and Maternal Plasma Tufnor Necrosis Factor α Concentrations.Biol Reprod 50:1297–1302.

Youngquist RS, Threlfall WR., (2006). Current therapy in large animal Theriogenology-E-Book. Elsevier Health Sciences, Amsterdam.

Darlay, R. J. ; McCarthy, A. J.; Illot, N. E. et al., (2011). Novel polymorphisms in ovine immune response genes and their association with abortion. Animal Genetics, 42, 535–543

Rosero R., Bianchi L., Cauvin E., Maione S., Sartore S., Soglia D. & Sacchi P. (2007) Analysis of the sheep MUC1 gene: structure of the repetitive region and polymorphism. Journal of Dairy Science 90, 1024–8.

Luhken G., Stamm I., Menge C. & Erhardt G. (2005) Functional analysis of a single nucleotide polymorphism in a potential binding site for GATA transcription factors in the ovine interleukin 2 gene. Veterinary Immunology and immunopathology 2005, 1–2.

Zhou H., Hickford J.G., Fang Q. & Lin Y.S. (2007) Allelic variation of the ovine Toll-like receptor 4 gene. Developmental and Comparative Immunology 31, 105–8.

Marcos-Carcavilla A., Calvo J.H., Gonzalez C. et al. (2007) IL-1 family members as candidate genes modulating scrapie susceptibility in sheep: localization, partial characterization and expression. Mammalian Genome 18, 53–63.

Konnai S., Nagaoka Y., Takeshima S., Onuma M. & Aida Y. (2003a) Sequences and diversity of 17 new Ovar-DRB1 alleles from three breeds of sheep. European Journal of Immunogenetics 30, 275–82.

Konnai S., Nagaoka Y., Takeshima S., Onuma M. & Aida Y. (2003b) Technical Note: DNA typing for Ovine MHC DRB1 using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Journal of Dairy Science 86, 3362–5.

Tangri S., Wegmann T.G., Lin H. & Raghupsthy R. (1994) Maternal anti-placental reactivity in natural, immunolgically-mediated fetal resorptions. Journal of Immunology 152, 4903–11.

Gazzinelli R.T., Denkers E.Y. & Sher A. (1993) Host resistance to T. gondii: model for studying the selective induction of cell-mediated immunity by intracellular parasites. Infectious Agents and Disease 2, 139–49.

Martinez-Espinosa, I.; Serrato, J.A.; Ortiz-Quintero, B. (2021) Role of IL-10-producing natural killer cells in the regulatory mechanisms of inflammation during systemic infection. Biomolecules, 21, 4. [CrossRef]

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 313-328

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Wang, Z.; Guan, D.; Huo, J.; Biswas, S.K.; Huang, Y.; Yang, Y.; Xu, S.; Lam, K.P. (2021) IL-10 enhances human natural killer cell effector functions via metabolic reprogramming regulated by mTORC1 signaling. Front. Immunol., 23, 619195.

Iyer, S.S.; and Cheng, G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit. Rev. Immunol. 2012, 32, 23–63.

Pal, A.; Chakravarty, A.K. Disease resistance for different livestock species. In Genetics and Breeding for Disease Resistance of Livestock; Academic Press: Cambridge, MA, USA, 2020; pp. 271–296.