J Appl Biotechnol Rep. 2025 September;12(3):1766-1775

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2025.548230.1915

Original Article

Molecular Impact of Social Isolation on Implantation Gene Expression in Uterine Tissue: A Rabbit Model for Stress-Induced Fertility Disorders

Alaa Kamil Abdulla [1*], **Hasanain Riyadh Al-Isaw** [2], **Qasim Zamil Bneed** [1]

[1] Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Diwaneyah, Iraq
[2] Department of Basic Medical Science, College of Nursing, University of Al-Qadisiyah, Al-Diwaneyah, Iraq

Corresponding Author: Alaa Kamil Abdulla, PhD, Associate Professor, Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Diwaneyah, Iraq. Tel: +9647807311256, E-mail: alaa.abdulla@qu.edu.iq

Received September 15, 2025; **Accepted** September 27, 2025; **Online Published** September 30, 2025

Abstract

Introduction: Psychosocial stress, particularly social isolation, is increasingly recognized as a potential factor influencing female infertility by disrupting hormonal balance and impairing the implant ability of the endometrium. Women who undergo major life transitions such as migration, displacement, or adaptation to new sociocultural environments show greater vulnerability to chronic stress, which can negatively impact reproductive function and contribute to delayed conception. To investigate the effect of social isolation on reproductive hormones and implantation-related gene expression in female rabbits, serving as a model for human psychosocial stress.

Materials and Methods: Eighty healthy female rabbits (6–8 months old) were randomly assigned into two groups: the control group (n = 40), which received standard housing and care, and the stress group (n = 40), which was in complete social isolation for 14 days before and 14 days after mating. On day 7 post-mating, blood collection for hormone level measurement (FSH, LH, estrogen, progesterone, and cortisol) was done using standard protocols of the ELISA technique. On the same day, a hysterectomy was performed on these two groups, and uterine tissues were collected for analysis of gene expression by real-time PCR analysis of three implantation-related genes including *LIF*, *HOXA10*, and *Integrin β3*.

Results: Social isolation significantly reduced FSH, LH, estrogen, and progesterone levels while increasing cortisol ($p<0.05$). Gene expression analysis revealed significant downregulation of *LIF* ($p = 0.000014$), *HOXA10* ($p = 0.0145$), and *Integrin β3* ($p = 0.0004$) in the stress group compared to controls, indicating impaired uterine receptivity for implantation.

Conclusions: It is concluded that chronic social isolation leads to hormonal imbalance and suppression of implantation-related gene expression, highlighting potential mechanisms for stress-related infertility and opening the door to clinical interventions targeting stress management to improve reproductive outcomes.

Keywords: Social Isolation, Psychosocial Stress, Fertility, Implantation Genes

Citation: Abdulla AK, Al-Isawi HR, Bneed QZ. Molecular Impact of Social Isolation on Implantation Gene Expression in Uterine Tissue: A Rabbit Model for Stress-Induced Fertility Disorders. J Appl Biotechnol Rep. 2025;12(3):1766-1775. doi:10.30491/jabr.2025.548230.1915

Introduction

Fertility is one of the vital factors in the continuity of living organisms and affects their ability to reproduce and achieve biological success.[1] The level of fertility is mainly related to the quality of eggs, hormonal balance, and environmental conditions that directly impact the interaction between genes and the environment.[2] Recent studies have begun to demonstrate the effects of psychological and social stress on human fertility, highlighting the importance of studying this phenomenon in animals as a model to understand its effects on human reproduction in the environment.[3] Some studies, such as those by Smith et al.[4] and Zhang et al.[5] have indicated that stress is not only a psychological burden but also a biological factor that disrupts the hypothalamic-pituitary-gonadal (HPG) axis and leads to changes in reproductive outcomes in both humans and animals.

Rabbits provide an appropriate model with which to study this effect since they share comparable reproductive physiology to that of a human, as well as a short life cycle and ease of controlling their experimental conditions.[6] Researchers have found that when rabbits are exposed to psychological or social stress, they secrete more cortisol which subsequently influences sex hormones and decreases overall fertility.[7]

Many studies have shown that psychological stress, whether social or environmental, significantly affects hormonal balance and reduces fertility rates.[8] Psychological stress activates the hypothalamic-pituitary-adrenal axis, leading to increased cortisol levels.[9] Cortisol, a hormone known for its inhibitory effect on ovulation, another study confirmed that increased cortisol levels lead to a disturbance in the secretion of sex hormones involved in ovulation, such as GnRH, FSH, and LH.[10] Furthermore, chronic stress and

social isolation have been reported to cause impaired ovarian follicle development and endometrial receptivity to embryos, which may cause decreased fertility and implantation failure.[11] It is worth noting that the importance of studying stress-induced fertility disorders extends beyond the laboratory setting, as modern societies record increasing rates of chronic stress and social isolation.[12] Epidemiological data indicate that approximately 30% of women of reproductive age experience significant psychological stress, with a documented association between high levels of stress and an increased incidence of infertility, particularly unexplained infertility.[13] This underscores the urgent need to decipher the molecular and hormonal mechanisms through which psychosocial factors influence reproductive success—an opportunity that this study aims to address using a controlled rabbit model. Rabbits are an ideal model for reproductive studies due to their short reproductive cycle and easy control of their environment. Research has shown that rabbits exhibit tangible responses to social stress through elevated cortisol levels and changes in their fertility.[6] Proper implantation requires certain genes, such as leukemia inhibitory factor (LIF),[14] Homeobox A10 (HOXA10),[15] and Integrin β3[16,17] which are essential for the early interaction between the fertilized egg and the uterine lining. In addition to hormonal disturbances, stress has been shown to modulate the expression of genes associated with implantation, suggesting that molecular pathways in the uterus are directly influenced by psychosocial stress.[18]

A favorable uterine environment is essential for successful implantation and is governed by the accurate timing and spatial distribution of many fertility-regulating genes. These include *LIF*, *HOXA10*, and *Integrin β3*, which play a critical role in the development of uterine receptivity and the coordination of embryo-endometrial interactions.[19] The *LIF* gene is a vital cytokine in the process of embryo implantation, mainly by regulating the activity of endometrial stromal cells and facilitating the differentiation process that enables receptive endometrium. There is a direct link between low expression of the transcription factor LIF and impaired implantation and infertility.[20]

The *HOXA10* gene is critical for the development and functioning of the uterus. It regulates the expression of numerous downstream target genes involved in dead cell clearance, immune regulation, and endometrial remodeling.[21] The *HOXA10* gene has been found to be downregulated or epigenetically silenced in conditions such as endometriosis and polycystic ovary syndrome (PCOS).[22] They are temporally expressed during implantation and are essential for successful embryo attachment.[23] Their impact on reproduction involves a regulatory network of these genes that determines the success of early pregnancy. Any dysfunction in this network can negatively impact uterine receptivity and lead to poor fertility or infertility.[24] Stress correlates with increased expression of HSP70 and BAX, key stress response and cell death (apoptosis) genes. This affects implantation by decreasing the efficiency of genes involved in egg differentiation and implantation in the uterus.[25]

The *Integrin β3* gene is located on chromosome 17 (17q21.32) and encodes a membrane protein belonging to the integrin family, which plays a pivotal role in cell adhesion and cell-to-extracellular matrix (ECM) communication. Alterations in the expression level of this gene have been linked to fertility disorders such as unexplained infertility, recurrent implantation failure, and early miscarriage. In the reproductive context, its expression in the endometrium during the "implantation window" is a key indicator of uterine receptivity, facilitating the attachment of early trophoblasts to the uterine epithelium, enabling successful implantation (https://www.ncbi.nlm.nih.gov/gene/3690).

This study hypothesizes that social stress leads to significant changes in gene expression in fertilized eggs of female rabbits, particularly in genes associated with implantation and early embryonic development. Accordingly, this study aims to investigate the molecular effects of social stress on female fertility using rabbits as an experimental model. It specifically focuses on determining the effect of social stress on gene expression in fertilized eggs at the beginning of pregnancy, with a focus on genes associated with implantation and early embryonic development, such as *LIF*, *HOXA10,* and *Integrin β3*. The study also seeks to compare stress-exposed groups with control groups to assess changes in implantation efficiency, as well as to evaluate the suitability of rabbits as a model for studying the effect of psychosocial stress on human fertility at the molecular level. Our review of previous studies reveals that few studies have systematically examined the impact of molecular psycho logical distress on certain genes important in preparing the rabbit uterus for embryo reception. Given their physiological similarity to humans in implantation biology, rabbits provide a valuable, albeit underutilized, model for elucidating how social stress affects altered reproductive outcomes.

Materials and Methods

Experiment Animals

An experimental study using an animal model (rabbits) was conducted, with exposure to social stress as the independent variable and its effect on hormonal changes and gene expression associated with early implantation as dependent variables. Eighty sexually mature female domestic rabbits aged 6 to 8 months, with an average weight of 2.5 to 3.5 kg, were used in the study. The animals were carefully selected for their health and underwent thorough vet checks to ensure they did not have any chronic diseases or congenital defects that could affect the study results. All animal procedures were conducted without surgical intervention or harm to the

animals. The study followed ethical guidelines for animal use and obtained approval from the Ethics Committee of the College of Biotechnology, University of Al-Qadisiyah, Iraq (https://bt.qu.edu.iq/?page_id=22686).

The rabbits were kept in individual metal cages (60 x 50 x 40 cm) with adequate ventilation to reduce undesired social interaction that could influence their psychological or behavioral condition. The cages were placed in an environ mentally controlled experimental room maintained at a constant temperature of 22 +/- 2 °C, with a consistent artificial lighting regime (12 hours light/12 hours dark) to mimic natural conditions. All the animals received high-quality, balanced nutrition in the form of concentrated pellets containing all necessary nutrients (proteins, fats, fiber, minerals, and vitamins) and had access to clean water through an automatic drinking system. The rearing environment and cages underwent regular cleaning to maintain hygiene and minimize the risk of infection or stress due to an unclean environment. The rabbits were randomly divided into two equal groups (n = 40 per group) as follows: the first group was a control group (CM): This group was not exposed to any stressful stimuli or stress and was kept under the standard conditions mentioned above throughout the experimental period. The second group was a stress group (SM): This group was subjected to a specific stress protocol based solely on social isolation, which served as the sole stressor in this model according to.[26] Each female rabbit was housed separately in a completely isolated cage, preventing direct visual, auditory, or olfactory contact with other rabbits, with minimal human interaction during routine

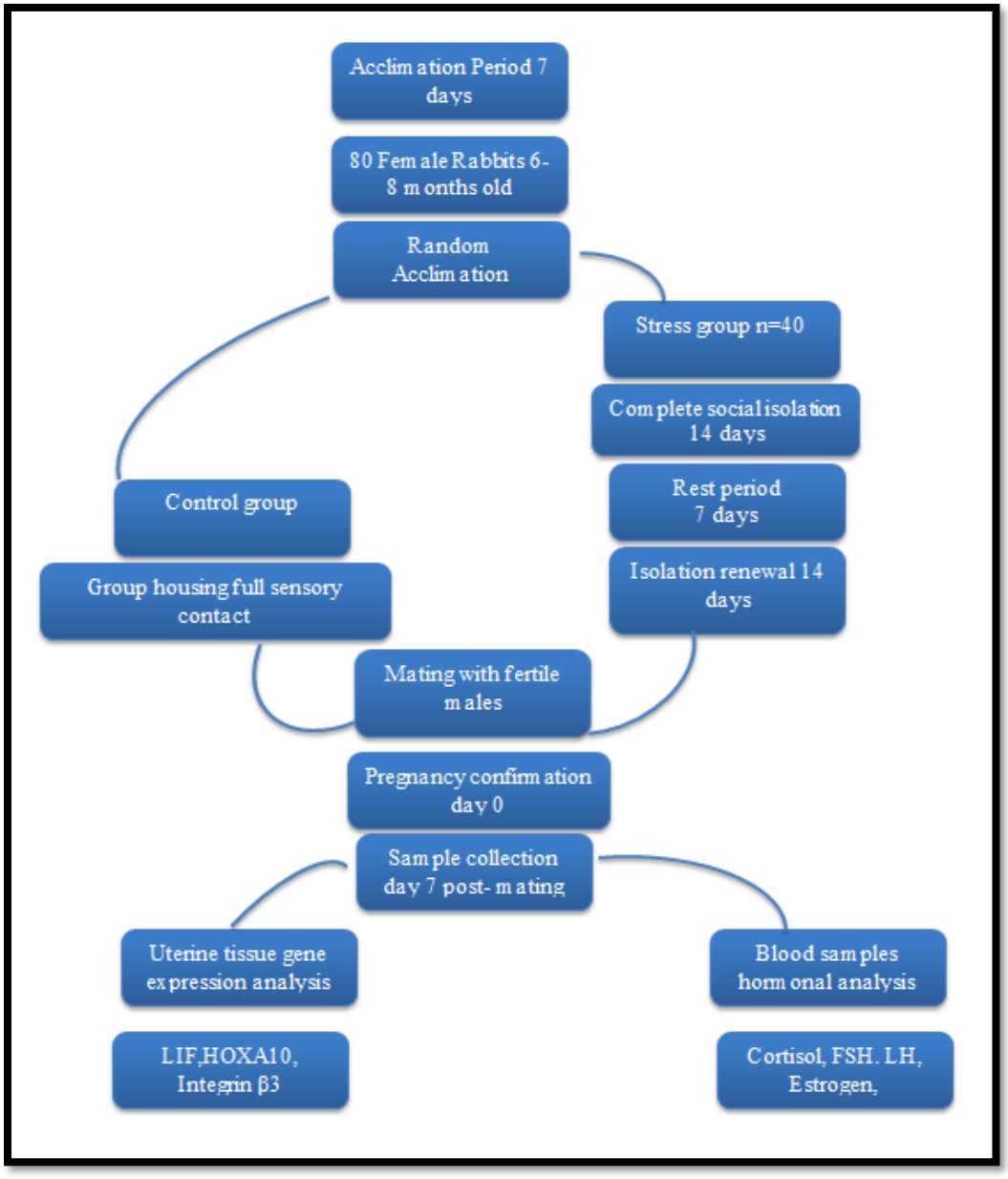

Figure 1. Study Design for Investigating the Impact of Chronic Social Isolation on Reproductive Gene Expression.

care periods. A 14-day social isolation regime was implemented, and the animals were returned to the natural control group enclosure for one week to adhere to research ethics. The quarantine regime was then reinstated for another 14 days, after which male rabbits were introduced, and attempts were made to facilitate natural mating. After approaching the males for three or four consecutive days, each female was mated once with a fertile male, and mating was confirmed by direct observation of insemination. Day 0 of pregnancy was considered the day of mating. The males were then removed, and a 7-day period was allowed, which is the sensitive time period for embryo implantation in the uterus.[27] Social isolation was chosen because it is a common and recognized form of chronic stress in psychophysio logical studies, due to its proven effects on hormonal and immune systems and gene expression in organisms, particularly in mammals. This design aimed to simulate the psychological stresses of social separation that females might experience in contemporary environments and assess the impact of this type of stress on implantation genes and reproductive health (Figure 1).

Sample Collection

On day 7 post-mating, which is the known onset of embryo implantation in rabbits, animals from both groups were anesthetized using a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg) via intramuscular injection to ensure painless interventions.[28] After achieving the depth of anesthesia, the uterus was carefully excised, and endometrial tissue was separated under sterile conditions to avoid contamination and ensure sample quality, according to international protocols for the care and welfare of animals in research.[29] Blood samples were also drawn from the ear vein of both groups for hormone analysis using the ELISA assay. Uterine tissue was cryopreserved in liquid nitrogen (-196 °C) and stored at -80 °C until molecular analysis and gene expression studies of implantation genes, following recom mended procedures to ensure the reliability of the results.

ELISA Assay

The blood samples were collected from the ear vein of rabbits on the morning of the seventh day after mating, coinciding with the time of implantation. The samples were collected in anticoagulant-free tubes, and the serum was separated by centrifugation at 3,000 rpm for 15 minutes. The serum was then stored at -20 °C until analysis using commercial ELISA kits designed. The enzyme linked immunosorbent assay (ELISA) technique was applied to advanced hormonal analysis to determine the physiological impact of exposure to social stress on hormonal balance in female rabbits in the two study groups. ELISA is a precise and faithful instrument to quantitatively estimate the level of hormones in biological samples.[30] We used the Elisys Uno®, Fully Automated ELISA Analyzer, HUMAN Co, Germany, with rabbit ELISA kits (My BioSource Co., Alaska, USA) as the per manufacturers recommendations. In this study, it was used to measure changes in the concentration of a group of key hormones associated with the stress and reproductive axes, including: Follicle-stimulating hormone (FSH) for its role in follicle growth and stimulating ovarian activity,[31] luteinizing hormone (LH) as an indicator of gonadal function,[32] estrogen (Estradiol E2) for its role in preparing the endometrium for implantation,[33] progesterone for its support of early pregnancy maintenance and regulation of the uterine environment,[34] and the cortisol as a primary indicator of the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the stress response.[35]

Gene Expression

The uterine tissue samples were used for total RNA extraction using an RNA extraction kit (Qiagen RNeasy Mini Kit) and then stored at -20 °C until needed. The reagents were used according to the manufacturer's directions. RNA quality and purity were assessed with a NanoDrop spectro photometer (NanoDrop OPTIMA®, JAPAN), and only samples with an absorbance ratio between 1.8 and 2.0 were selected. The extracted RNA was converted into complementary DNA (cDNA) using a universal RT-PCR Kit (M-MLV, free Taq polymerase, Solarbio®, China). All primers were supplied by Macrogen company (Macrogen®, Inc., South Korea), The lyophilized primers were reconstituted in DNase/RNase-free water at 100 pmol/µl as a stock solution, then diluted to a working solution of 10 µM by mixing 10 pmol/µl in 90 µl of deionized water, yielding a final concentration of 10 µM. The expression levels of target genes were analyzed using quantitative real-time polymerase chain reaction (qRT-PCR) with the Real-Time PCR Accurate 96-x4/x6 system (Accurate Biosystem®, China). To confirm the expression of test genes, SYBR Green I dye (SYBR Green I, Thermo Fisher Sci.®, USA) was used as the fluorescent DNA-binding marker in quantitative real-time PCR. The mRNA levels of the reference gene *GAPDH* were amplified and used to normalize the mRNA levels of the target genes. The National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) was used to design the required primer sequences through the GenBank database. Each primer pair was further analyzed for possible dimer formation using the PCR Primer Stats tool available in the sequence manipulation suite (http://www.bioinformatics.org/sms2/pcr_primer_stats.html) (Table 1). We applied the ΔCT method with a reference gene to calculate the relative expression levels of *LIF*, *HOXA10* and *Integrin β3* mRNA using the formula: (Ratio (reference/ target) = $2^{CT(reference) - CT(target)}$).[36] The previously described primer sets, product sizes, and annealing temperatures were used. The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 minutes (one cycle),

Table 1. Primer Sequences used for Semi-Quantitative RT-PCR

Gene	Primer Sequence (5' – 3')	NCBI Gene ID	Amplicon Size (pb)	Annealing Temp (°C)
LIF	F: GACCTATCCTCGTCTTGCGG R:ACTTTACCTGGATGGCAGGC	100358914	107	53.4
HOXA10	F: CTCCTCCTGAGTAGGCCCTT R:CCTGATGGAGGATTCGGAGC	100343397	161	54.5
Integrin β3	F: CCATAGCAGGGCACAAGGAA R: GAGAGAATGGGTTGTGGGGG	100339049	99	53.5
GAPDH	F: TCCCGTTGATGACCAGCTTC R: GTATGATTCCACCCACGGCA	100349551	71	52.5

followed by denaturation at 95 °C for 40 seconds, annealing at 57 °C for 40 seconds, and extension at 72°C for 1 minute. This sequence was repeated for 35 cycles, and finally a hold step at 4 °C for one cycle was performed.[37]

Statistical Analysis

A comprehensive statistical analysis was performed using IBM SPSS Statistics (version 23.0). All quantitative data were expressed as mean ± standard deviation. Normal distribution of data was verified using the Shapiro-Wilk test, and homogeneity of variance was examined using Levene's test. Results were compared between the control group (CM) and the social stress group (SM) using an independent-samples t-test for parametric data. For multiple comparisons, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. Effect size was calculated using Cohen's d for t-tests and partial eta-squared (η^2) for ANOVA analysis. A *p*-value of less than 0.05 was considered statistically significant for all analyses.[38]

Results

Hormonal Analysis

Data were statistically analyzed using a t-test in SPSS version 23. The results (Table 2 and Figure 2) showed a significant decrease ($p<0.001$) in follicle-stimulating hormone (FSH) levels in the stress group (SM) (0.711 ± 0.039) compared to the control group (CM) (1.075 ± 0.066), representing a relative decrease of 34%. Luteinizing hormone (LH) levels also decreased significantly ($p<0.001$) in the stress group (0.812 ± 0.030) compared to the control group (1.705 ± 0.101), representing a relative decrease of 52%. Estrogen concentrations decreased significantly ($p<0.001$)

Table 2. Comparative Analysis of Serum Hormone Profiles Following Social Stress Exposure

Hormone	Control Group (CM) (n = 40)	Stress Group (SM) (n = 40)	*p*-value
FSH (mIU/ml)	1.075 ± 0.066	0.711 ± 0.039	<0.001
LH (mIU/ml)	1.705 ± 0.101	0.812 ± 0.030	<0.001
Estradiol (ng/ml)	0.153 ± 0.010	0.098 ± 0.007	<0.001
Progesterone (ng/ml)	6.875 ± 0.369	3.843 ± 0.233	<0.001
Cortisol (µg/dl)	3.424 ± 0.231	6.726 ± 0.525	<0.001

FSH; Follicle-Stimulating Hormone, LH; Luteinizing Hormone.

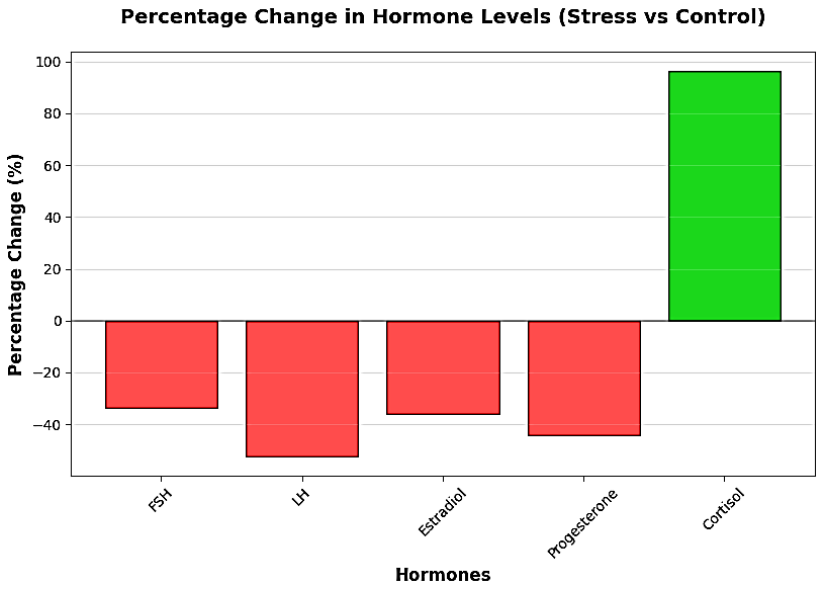

Figure 2. Percentage Change in Hormone Levels (%) Between Stress and Control Groups. The graph shows the percentage change in hormone levels between the group of rabbits exposed to social stress and the control group, indicating the direction of change (decreased reproductive hormones and increased cortisol).

from 152.915 ± 9.94 in the control group to 98.05 ± 7.41 in the stress group, a 36% decrease. Progesterone levels decreased significantly (p<0.001) from 6.875 ± 0.369 to 3.843 ± 0.233, a 44% decrease. Cortisol levels, on the other hand, increased significantly (p<0.001) from 3.424 ± 0.231 in the control group to 6.726 ± 0.525 in the stress group, a 96% increase. These significant relative changes illustrate the profound impact of social stress on reproductive hormonal balance.

Gene Expression

The study results (Tables 3 and 4 and Figure 3) revealed statistically significant differences between the control group (CM) and the social stress group (SM) in the gene expression levels of genes associated with embryo implantation in the uterine lining, namely: LIF, HOXA10, and Integrin β3. Data were analyzed using a t-test, and probability values indicated that all differences were statistically significant at a 5% significance level. The mean gene expression of the *LIF* gene showed a significant decrease in the stress group (1.98) compared to the control group,[3,29] with a probability value (p = 0.000014), indicating a highly statistically significant difference, representing a relative decrease of 39.8%. Gene expression of the *HOXA10* gene also decreased significantly in the stress group (1.49) compared to the control group (1.888), with a probability value (p = 0.0145), indicating a statistically significant difference at the 5% level, representing a relative decrease of 21.1%. Gene expression of the *Integrin β3* gene decreased significantly from 2.51 in the control group to 1.34 in the stress group, with a probability value (p = 0.0004), a highly statistically significant difference, representing a significant relative decrease of 46.6%. These results indicate that social stress negatively affects the gene expression of genes important in preparing the uterine lining for embryo reception, which may contribute to reduced uterine readiness for embryo implantation in early pregnancy. These data support the hypothesis that chronic stress affects the molecular environment of the uterus by inhibiting genes regulating fertility.

Table 3. Expression Levels of Implantation-related Genes (*LIF, HOXA10, Integrin β3*) in Control and Stressed Rabbits Measured by RT-qPCR

Gene	Ct		ΔCt		$2^{-\Delta Ct}$	
	CM	SM	CM	SM	CM	SM
LIF	12.0055	12.578	-1.653	-0.925	3.291	1.9762
HOXA10	12.814	12.9565	-0.845	-0.546	1.888355	1.4930
Integrin β3	12.439	13.144	-1.22	-0.359	2.511707	1.3413

Ct Values are presented as relative gene expression ($2^{-\Delta Ct}$) compared to the control group.

Table 4. Relative Expression Levels of Implantation-related Target Genes in Control (CM) and Stress (SM) Model Rabbit Groups

Genes \ Groups	CM (n = 40)	SM (n = 40)	*p*-value
LIF	3.29	1.98	0.000014
HOXA10	1.89	1.49	0.0145
Integrin β3	2.51	1.34	0.0004

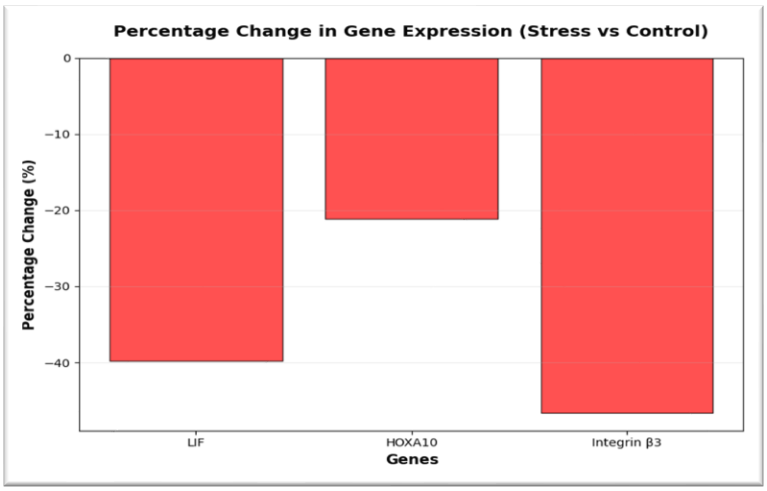

Figure 3. Comparative Analysis of Implantation Gene Expression Between Stress and Control Groups. The graph shows the changes in gene expression of three key genes responsible for the embryo implantation process (*LIF, HOXA10, Integrin* β3) between the socially stressed rabbit group and the control group, with the percentages of significant decrease in gene expression indicated.

Discussion

Observations on delayed pregnancy indicate that a significant proportion of women suffer from infertility without apparent medical causes,[39] often associated with psychological disorders

and exposure to a range of social pressures. Given the reluctance of this group to participate in direct medical studies, rabbits were used as an experimental model to study the impact of psychological and social stress on fertility. Rabbits were exposed to stressful conditions, including specific periods of social isolation, in accordance with ethical research standards. Hormonal changes and gene expression of genes associated with implantation and early pregnancy were assessed, and compared to a control group (not exposed to stress) to determine the effect of stress on reproductive outcomes. The study hypothesized that psycho social stress negatively affects the gene expression of genes responsible for implantation, reducing the likelihood of successful pregnancy. This opens the door for future research on the relationship between social stress and human reproductive health.[40] This study specifically investigated the effects of social stress on gene expression in fertilized rabbit oocytes during early pregnancy. Although physiological responses to social stress are common in mammals, this is the first report describing hormonal adaptations and genetic changes in response to such stressors and their impact on the hypothalamic-pituitary-reproductive (HPR) axis and fertility.[41] The findings indicate that daily stress affects reproductive physiology not only before ovulation but also during early post-ovulatory periods, enhancing the maternal psychological influence on reproductive function. The study focused on hormonal dynamics and gene expression changes immediately before and after ovulation.[42]

In this study, hormonal analyses showed a significant decrease in follicle-stimulating hormone (FSH) levels in rabbits exposed to stress, reflecting suppression of the hypothalamic-pituitary-ovarian (HPO) axis, impaired follicular development, and decreased reproductive activity. A significant decrease in luteinizing hormone (LH) levels was also observed, indicating a defect in ovulation induction, consistent with Brecchia.[43] Elevated corticosterone levels resulting from acute stress inhibit the HPO axis, thereby reducing LH and estradiol production and negatively impacting fertility and insemination outcomes without necessarily affecting ovulation, as demonstrated in the study of Yang.[44]

The results (Table2) showed that psychosocial stress rapidly suppresses the activity of the hypothalamic-gonadal (HPG) axis by suppressing GnRH-releasing cells and increasing the activity of GnRH-inhibiting cells, leading to decreased luteinizing hormone (LH) secretion. Estrogen concentrations were also significantly lower in the stress group, which may impair endometrial formation and reduce implantation. Furthermore, decreased progesterone levels were observed, reflecting impaired corpus luteum function and impaired early pregnancy establishment. These obser vations are consistent with previous findings in animals, where stress exposure elicited similar behavioral and physiological responses.[45] The uterus comprises three major compartments: epithelium, stroma, and myometrium each regulated by ovarian estrogens and progesterone. In murine models, estradiol stimulates the uterine epithelium during days 0.5–1.5 post coitum (p.c.), while progesterone stimulation of the stroma occurs around day 2.5 p.c., ensuring sequential hormonal priming of the uterine stroma for implantation.[46] Stress activates the release of catecholamine's and glucocorticoids through the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis,[47] elevating cortisol levels, which inhibit the reproductive axis and disrupt reproductive hormones.[48] Cortisol regulates behavior, metabolism, endocrine function, and immune responses, with high levels observed in animals under stress.[35] The significant increase in cortisol levels in isolated rabbits confirms that social isolation exacerbates stress responses, which is reflected in anxiety patterns and affects reproductive physiological functions. This is consistent with the results of many studies, such as Elsayed et al. and Comin et al.[49,50]

At the molecular level, the epigenome is highly susceptible to environmental factors during embryogenesis, which is characterized by rapid cell division and regulatory remodeling of epigenetic marks.[51] RT-qPCR analyses revealed significant downregulation of three implantation-related genes (*LIF*, *HOXA10*, and *integrin β3*) in stressed rabbits compared to controls, demonstrating the negative impact of social stress on the molecular environment required for successful implantation. In Tables 3 & 4 and Figures 2 & 3, we showed that LIF is critical for early embryo implantation and endometrial differentiation,[31] and its marked reduction suggests that stress disrupts essential molecular signaling during implantation. Integrin β3, a key adhesion molecule in the endometrium facilitating embryo attachment, was significantly downregulated, confirming compromised cellular adhesion under stress.[52] The *HOXA10* gene regulates endometrial development and cellular differentiation, essential for preparing the uterus for implantation. Downregulation of *HOXA10* expression in stressed rabbits indicates impaired endometrial remodeling, consistent with other studies, which highlighting its crucial role in establishing a uterine environment conducive to implantation.[53]

Integrin β3 is a biomarker of a receptive uterus during the implantation window. In the context of reproduction, it facilitates the attachment of early trophoblasts to the uterine epithelium, essential for successful implantation and early pregnancy. Disturbed expression of this gene has been associated with unexplained infertility, recurrent implantation failure, and early miscarriage, making it a pivotal gene in the study of fertility and reproductive biology.[54]

The study results showed decreased expression of the *Integrin β3* gene in the endometrium of rabbits exposed to psychosocial stress, suggesting a negative effect of stress on uterine readiness for implantation. This decrease may reflect

a reduced ability of trophoblasts to adhere to the uterine epithelium, thus increasing the likelihood of implantation failure or poor early pregnancy success. These results are consistent with previous studies.[55] From a molecular perspective, the decrease in Integrin β3 likely disrupts interactions with extracellular matrix proteins such as osteopontin and fibronectin, which play a pivotal role in trophoblast anchoring. These findings suggest that psycho logical stress not only affects the hormonal environment but also extends to the level of genes associated with implantation, illustrating a potential mechanism for the impaired fertility caused by social stress.[56]

The results of this study demonstrate that psychosocial stress significantly affects hormonal regulation, uterine receptivity, and the expression of implantation-related genes, providing an understanding of the molecular mechanisms linking environmental stressors and impaired fertility. Although based on a rabbit model, the results of this study suggest potential implications for human fertility. Unexplained infertility is a major clinical challenge, and evidence suggests that psychosocial stress significantly contributes to its development. The hormonal changes (decreased FSH, LH, estrogen, and progesterone) and decreased gene expression of implantation genes (*LIF*, *HOXA10*, and *Integrin β3*) observed under social isolation may represent similar mechanisms in women. In humans, hyper-activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to elevated cortisol, which impairs ovulation, reduces endometrial receptivity, and increases the risk of early pregnancy loss. These findings support the hypothesis that social stress affects fertility via hormonal and molecular mechanisms[57,58] and highlight the need for further clinical studies to investigate this relationship and develop preventive and therapeutic strategies targeting stress management to improve reproductive health.

Based on the results of our study, we suggest future research to investigate the epigenetic mechanisms of the observed effects and to investigate non-invasive biomarkers to detect the impact of stress on fertility. We also recommend developing targeted therapeutic interventions to reverse stress-induced molecular changes and studying the transgenerational effects of psychological stress on fertility, in addition to investigating the interaction between stress and other environmental factors.

Additionally, as limitations of the study, the use of an animal model may not fully reflect the complexity of the human stress response. The focus on specific implantation genes to the exclusion of other molecular pathways is another limitation. The lack of a recovery phase to assess the reversibility of effects and the reliance on physiological measures without complementary behavioral assessments are also limitations. This calls for cautious interpretation of the results and opens up avenues for future research.

Conclusion

This study shows that chronic social stress significantly reduces key reproductive hormones (follicle-stimulating hormone, luteinizing hormone, estrogen, and progesterone) while increasing cortisol levels. It also causes a notable decline in the expression of genes involved in implantation (*LIF*, *HOXA10*, and *Integrin β3*), offering a mechanistic explanation for endometrial dysfunction and stress-related infertility. These results highlight the importance of incorporating psychological assessment and stress management into fertility treatments and pave the way for future research aimed at developing targeted interventions, reversing molecular changes, and validating these mechanisms in human studies. The findings confirm that psychosocial stress leads to hormonal and genetic imbalances that contribute to infertility. This underscores the need for further research to create innovative diagnostic and treatment strategies, as well as integrating psychological care into comprehensive fertility programs.

Authors' Contributions

AKA and HRAI were responsible for sample collection; AKA, QZB, and HRAI were responsible for gene expression analyses and data validation of the study; HRAI participated in the preparation of the original draft and worked on review writing and editing. All authors approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

1. Emokpae MA, Brown SI. Effects of lifestyle factors on fertility: practical recommendations for modification. Reprod Fertil. 2021;2(1):R13-R26. doi:10.1530/raf-20-0046
2. Jain M, Singh M. Environmental toxins and infertility. StatPearls [Internet], StatPearls Publishing. 2023.
3. Negro-Vilar A. Stress and other environmental factors affecting fertility in men and women: overview. Environ Health Perspect. 1993;101(suppl 2):59-64. doi:10.2307/3431377
4. Smith MD, Wesselbaum D. Global evidence on the prevalence of and risk factors associated with stress. J Affect Disord. 2025;374:179-83. doi:10.1016/j.jad.2025.01.053
5. Zhang C, Shi L, Tian T, Zhou Z, Peng X, Shen Y, et al. Associations between academic stress and depressive symptoms mediated by anxiety symptoms and hopelessness among Chinese college students. Psychol Res Behav Manag. 2022:547-56. doi:10.2147/PRBM.S353778
6. Foote RH, Carney EW. The rabbit as a model for reproductive and developmental toxicity studies. Reprod Toxicol. 2000;14(6):477-93. doi:10.1016/S0890-6238(00)00101-5
7. Feng Y, Fan H, Liang X, Wang X, Gao G, Gun S. Environmental enrichment changes rabbits' behavior, serum hormone level and further affects cecal

microbiota. PeerJ. 2022;10:e13068. doi:10.7717/peerj.13068
8. Palomba S, Daolio J, Romeo S, Battaglia FA, Marci R, La Sala GB. Lifestyle and fertility: the influence of stress and quality of life on female fertility. Reprod Biol Endocrinol. 2018;16(1): 113. doi:10.1186/s12958-018-0434-y
9. Mbiydzenyuy NE, Qulu LA. Stress, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis, and aggression. Metab Brain Dis. 2024;39(8):1613-36. doi:10.1186/s12958-018-0434-y
10. Karunyam BV, Abdul Karim AK, Naina Mohamed I, Ugusman A, Mohamed WM, Faizal AM, et al. Infertility and cortisol: a systematic review. Front Endocrinol. 2023;14:1147306. doi:10.3389/fendo.2023.1147306
11. Liu Z, Zhang Z, Xie P. Global research trends in endometrial receptivity from 2000 to 2024: bibliometric analysis. Front Med. 2024;11:1465893. doi:10.3389/fmed.2024.1465893
12. Zhu H, Shi L, Wang R, Cui L, Wang J, Tang M, et al. Global research trends on infertility and psychology from the past two decades: a bibliometric and visualized study. Front Endocrinol. 2022;13:889845. doi:10.3389/fendo.2022.889845
13. Simionescu G, Doroftei B, Maftei R, Obreja BE, Anton E, Grab D, Ilea C, Anton C. The complex relationship between infertility and psychological distress. Exp Ther Med. 2021;21(4):306. doi:10.3892/etm.2021.9737
14. Salleh N, Giribabu N. Leukemia inhibitory factor: roles in embryo implantation and in nonhormonal contraception. Sci World J. 2014;2014(1):201514. doi:10.1155/2014/201514
15. Daftary GS, Troy PJ, Bagot CN, Young SL, Taylor HS. Direct regulation of β3-integrin subunit gene expression by HOXA10 in endometrial cells. Mol Endocrinol. 2002;16(3):571-9. doi:10.1210/mend.16.3.0792
16. Chen G, Xin A, Liu Y, Shi C, Chen J, Tang X, et al. Integrins β1 and β3 are biomarkers of uterine condition for embryo transfer. J Transl Med. 2016;14:1-10. doi:10.1186/s12967-016-1052-0
17. Dharmaraj N, Gendler SJ, Carson DD. Expression of human MUC1 during early pregnancy in the human MUC1 transgenic mouse model. Biol Reprod. 2009;81(6):1182-8. doi:10.1095/biolreprod.109.079418
18. González-Hernández AI, Scalschi L, Vicedo B, Marcos-Barbero EL, Morcuende R, Camaces G. Putrescine: a key metabolite involved in plant development, tolerance and resistance responses to stress. Int J Mol Sci. 2022;23(6):2971. doi:10.3390/ijms23062971
19. Kara M, Ozcan SS, Aran T, Kara O, Yilmaz N. Evaluation of endometrial receptivity by measuring HOXA-10, HOXA-11, and leukemia inhibitory factor expression in patients with polycystic ovary syndrome. Gynecol Minim Invasive Ther. 2019;8(3):118-22. doi:10.4103/gmit.gmit_112_18
20. Terakawa J, Nakamura S, Ohtomo M, Uehara S, Kawata Y, Takarabe S, et al. LIFR-Mediated ERBB2 Signaling Is Essential for Successful Embryo Implantation in Mice. Biomolecules. 2025;15(5):698. doi:10.3390/biom15050698
21. Du H, Taylor HS. The role of Hox genes in female reproductive tract development, adult function, and fertility. Cold Spring Harb Perspect Med. 2016;6(1): a023002. doi:10.1101/cshperspect.a023002
22. Saftić Martinović L, Mladenić T, Lovrić D, Ostojić S, Dević Pavlić S. Decoding the Epigenetics of Infertility: Mechanisms, Environmental Influences, and Therapeutic Strategies. Epigenomes. 2024;8(3):34. doi:10.3390/epigenomes8030034
23. Kang YJ, Forbes K, Carver J, Aplin JD. The role of the osteopontin–integrin αvβ3 interaction at implantation: functional analysis using three different in vitro models. Hum Reprod. 2014;29(4):739-49. doi:10.1093/humrep/det433
24. Bonavina G, Taylor HS. Endometriosis-associated infertility: From pathophysiology to tailored treatment. Front Endocrinol. 2022;13:1020827. doi:10.3389/fendo.2022.1020827
25. Stamperna K, Giannoulis T, Dovolou E, Kalemkeridou M, Nanas I, Dadouli K, et al. Heat shock protein 70 improves in vitro embryo yield and quality from heat stressed bovine oocytes. Animals. 2021;11(6):1794. doi:10.3390/ani11061794
26. Watanabe S, Al Omran A, Shao AS, Liang J. Social isolation model: a noninvasive rodent model of stress and anxiety. J Vis Exp. 2022;189:e64567. doi:10.3791/64567
27. Nishimura M. Timing of implantation in New Zealand White rabbits. Congenit Anom. 2001;41(3):198-203. doi:10.1111/j.1741-4520.2001.tb00833.x
28. Lipman NS, Marini RP, Erdman SE. A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. Lab Anim Sci. 1990;40(4):395-8. doi:10.4314/sokjvs.v12i3.4
29. Institute of Laboratory Animal Resources (US). Committee on Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. 1986;86. doi:10.17226/25801
30. Aydin S, Emre E, Ugur K, Aydin MA, Sahin İ, Cinar V, et al. An overview of ELISA: A review and update on best laboratory practices for quantifying peptides and proteins in biological fluids. J Int Med Res. 2025;53(2): 03000605251315913. doi:10.1177/03000605251315913
31. Kuo SW, Ke FC, Chang GD, Lee MT, Hwang JJ. Potential role of follicle-stimulating hormone (FSH) and transforming growth factor (TGFβ1) in the regulation of ovarian angiogenesis. J Cell Physiol. 2011;226(6):1608-19. doi:10.1002/jcp.22491
32. Rao CV. Multiple novel roles of luteinizing hormone. Fertil Steril. 2001;76(6):1097-1100. doi:10.1016/s0015-0282(01)02863-1
33. Bourdon M, Maignien C, Ouazana M, Kefelian F, Marcellin L, Patrat C, et al. Estradiol and reproductive outcomes in ART: when too much of a good thing hurts. Reproductive BioMed Online. 2025:105131. doi:10.1016/s0015-0282(01)02863-1
34. Cable JK, Grider MH. Physiology, progesterone. StatPearls Publishing, Treasure Island (FL). 2023.
35. Thau L, Gandhi J, Sharma S. Physiology, cortisol. StatPearls [Internet], StatPearls Publishing. 2023. doi:10.1152/ajpgi.1985.248.1.g73
36. Lorenz TC. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. J Vis Exp. 2012;(63):3998. doi:10.3791/3998-v
37. Hosseini S, Ivanov D, Dolgui A. Review of quantitative methods for supply chain resilience analysis. Transp Res Part E Logist Transp Rev. 2019;125:285-307. doi:10.1016/j.tre.2019.03.001
38. Test OS. Independent-Samples T Test... An IBM® SPSS® Companion to Political Analysis. 2025.
39. Simionescu G, Doroftei B, Maftei R, Obreja BE, Anton E, Grab D, et al. The complex relationship between infertility and psychological distress. Exp Ther Med. 2021;21(4):306. doi:10.17918/etd-269
40. Siddiqui SA, Adli DN, Nugraha WS, Yudhistira B, Lavrentev FV, Shityakov S, et al. Social, ethical, environmental, economic and technological aspects of rabbit meat production-A critical review. Heliyon. 2024;10(8):e29635. doi:10.1016/j.heliyon.2024.e29635

41. Toufexis D, Rivarola MA, Lara H, Viau V. Stress and the reproductive axis. J Neuroendocrinol. 2014;26(9):573-86. doi:10.1111/jne.12179
42. Hu Y, Wang W, Ma W, Wang W, Ren W, Wang S, et al. Impact of psychological stress on ovarian function: Insights, mechanisms and intervention strategies. Int J Mol Med. 2025;55(2):1-29. doi:10.3892/ijmm.2024.5475
43. Brecchia G, Bonanno A, Galeati G, Federici C, Maranesi M, Gobbetti A, et al. Hormonal and metabolic adaptation to fasting: effects on the hypothalamic–pituitary–ovarian axis and reproductive performance of rabbit does. Domest Anim Endocrinol. 2006;31(2):105-122. doi:10.1016/j.domaniend.2005.09.006
44. Yang JA, Song CI, Hughes JK, Kreisman MJ, Parra RA, Haisenleder DJ, et al. Acute psychosocial stress inhibits LH pulsatility and Kiss1 neuronal activation in female mice. Endocrinology. 2017;158(11):3716-23. doi:10.1210/en.2017-00301
45. Deiss V, Temple D, Ligout S, Racine C, Bouix J, Terlouw C, et al. Can emotional reactivity predict stress responses at slaughter in sheep?. Appl Anim Behav Sci. 2009;119(3-4):193-202. doi:10.1016/j.applanim.2009.03.018
46. Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science. 2002;296(5576):2185-8. doi:10.1126/science.1071601
47. Hefnawy A, Helal MAY, Sabek A, Shousha S. Clinical, behavioral and biochemical alterations due to shearing stress in Ossimi sheep. J Vet Med Sci. 2018;80(8):1281-6. doi:10.1292/jvms.18-0150
48. Di Natale MR, Soch A, Ziko I, De Luca SN, Spencer SJ, Sominsky L. Chronic predator stress in female mice reduces primordial follicle numbers: implications for the role of ghrelin. J Endocrinol. 2019;241(3):201-9. doi:10.1530/joe-19-0109
49. Elsayed M, Soliman F, Elghalid O, El-Sabrout K. Using different cage enrichments to improve rabbits' performance, behavior, and welfare. Animals. 2024;14(15):2271. doi:10.3390/ani14152271
50. Comin A, Zufferli V, Peric T, Canavese F, Barbetta D, Prandi A. Hair cortisol levels determined at different body sites in the New Zealand White rabbit. World Rabbit Sci. 2012;20(3):149-50. doi:10.4995/wrs.2012.1106
51. Akhatova A, Jones C, Coward K, Yeste M. How do lifestyle and environmental factors influence the sperm epigenome? Effects on sperm fertilising ability, embryo development, and offspring health. Clin Epigenetics. 2025;17(1):7. doi:10.1186/s13148-025-01815-1
52. Salleh N, Giribabu N. Leukemia inhibitory factor: roles in embryo implantation and in nonhormonal contraception. Sci world J. 2014;2014(1):201514. doi:10.1155/2014/201514
53. Li Q, Tang Y, Chen Y, Li B, Wang H, Liu S, et al. Melatonin Regulates the Expression of VEGF and HOXA10 in Bovine Endometrial Epithelial Cells through the SIRT1/PI3K/AKT Pathway. Animals. 2024;14(19):2771. doi:10.3390/ani14192771
54. Hameed ZC, AL-Masaoodi RA, Jabbar MH, Abbas ZA, AL-Wazni ZH. Study of αvß3 integrin gene expression in the endometrium of women with unexplained recurrent Spontaneous abortion. J Biosci Appl Res. 2024;10(5):63-7. doi:10.21608/jbaar.2024.395692
55. Tei C, Maruyama T, Kuji N, Miyazaki T, Mikami M, Yoshimura Y. Reduced expression of αvβ3 integrin in the endometrium of unexplained infertility patients with recurrent IVF-ET failures: improvement by danazol treatment. J Assist Reprod Genet. 2003;20(1):13-20. doi:10.1023/a:1021254620888
56. Labat-Robert J. Cell–matrix interactions, the role of fibronectin and integrins. A survey. Pathologie Biologie. 2012;60(1):15-9. doi:10.1016/j.patbio.2011.10.003
57. Wen L, Li R, Wang J, Yi J. The reproductive stress hypothesis. Reproduction. 2019;158(6):R209-18. doi:10.1530/REP-18-0592
58. Chand D, Lovejoy DA. Stress and reproduction: controversies and challenges. Gen Comp Endocrinol. 2011;171(3):253-7. doi:10.1016/j.ygcen.2011.02.022