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Experimental Evaluation of Histamine Receptor Modulation in Rat Implantation Models: Implications for Embryo Implantation and Endometrial Receptivity

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ABSTRACT

Embryo implantation is a highly coordinated physiological process requiring synchronized interactions among the developing blastocyst, maternal endometrium, endocrine signaling, immune modulation, angiogenesis, and extracellular matrix remodeling. Histamine, an endogenous biogenic amine synthesized by histidine decarboxylase (HDC), has emerged as an important regulator of reproductive physiology through activation of four G-protein-coupled receptors (H1R, H2R, H3R, and H4R). Although histamine is well recognized for its roles in allergic inflammation and neurotransmission, increasing experimental evidence indicates that it also regulates uterine receptivity, decidualization, trophoblast invasion, vascular permeability, cytokine production, and implantation-associated molecular signaling. Recent experimental work suggests that histamine promotes decidualization through epithelial amphiregulin (AREG) release and interacts with progesterone-dependent pathways during implantation.

The present experimental study evaluated the effects of pharmacological modulation of histamine receptors on embryo implantation using a rat implantation model. Forty adult female Wistar rats were allocated into five experimental groups (n = 8/group): control, histamine-treated, H1 receptor antagonist-treated, H2 receptor antagonist-treated, and combined H1/H2 receptor antagonist-treated groups. Histamine receptor modulation was performed during the peri-implantation period (gestational days 4–6). Implantation sites, pregnancy outcomes, serum estradiol and progesterone concentrations, vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), integrin αVβ3, homeobox A10 (HOXA10), inflammatory cytokines (TNF-α, IL-6, IL-10), oxidative stress biomarkers, histopathological alterations, immunohistochemical localization, and quantitative gene expression using RT-qPCR were evaluated.

Histamine administration significantly enhanced implantation rates, increased decidual thickness, improved uterine vascularization, elevated VEGF and LIF expression, and upregulated HOXA10 and integrin αVβ3 compared with controls ($p < 0.05$). Conversely, blockade of H1 and H2 receptors markedly reduced implantation success, impaired decidualization, suppressed angiogenic signaling, increased oxidative stress, and promoted inflammatory cytokine production. Histological examination demonstrated reduced stromal differentiation, diminished glandular activity, and defective vascular remodeling in

antagonist-treated animals. Molecular analysis confirmed significant downregulation of implantation-associated genes and increased expression of pro-inflammatory mediators.
These findings demonstrate that histamine receptor signaling plays a fundamental role in establishing uterine receptivity and successful embryo implantation. Pharmacological modulation of histamine receptors significantly influences maternal reproductive physiology through regulation of endocrine, angiogenic, inflammatory, and molecular pathways. The study provides mechanistic insights that may contribute to developing novel therapeutic approaches for recurrent implantation failure and infertility associated with impaired endometrial receptivity.

Keywords: Histamine receptor, rat model, endometrial receptivity, VEGF, HOXA10, integrin αVβ3, leukemia inhibitory factor, reproductive pharmacology.

1. INTRODUCTION

Successful embryo implantation represents one of the most complex biological events in mammalian reproduction. It requires synchronized communication between a developmentally competent blastocyst and a receptive maternal endometrium during the limited "window of implantation." This process involves endocrine regulation, immune tolerance, angiogenesis, extracellular matrix remodeling, cytokine signaling, and coordinated expression of implantation-associated genes. Disruption of any component of this network may result in implantation failure, early pregnancy loss, or infertility. Despite remarkable advances in assisted reproductive technologies (ART), implantation remains the major limiting factor in achieving successful pregnancy, with implantation failure accounting for a substantial proportion of unsuccessful in vitro fertilization (IVF) cycles (Wang & Dey, 2006; Cha et al., 2012; Lessey & Young, 2019).Histamine (2-[4-imidazolyl]-ethylamine) is an endogenous biogenic amine synthesized from L-histidine by the enzyme histidine decarboxylase (HDC). Traditionally recognized for its involvement in allergic responses, gastric acid secretion, neurotransmission, and inflammatory regulation, histamine has recently gained considerable attention as a critical mediator of reproductive physiology and embryo implantation (Jutel et al., 2009; Thurmond et al., 2008). Histamine exerts its biological actions through four G-protein-coupled receptors (H1R, H2R, H3R, and H4R), each activating distinct intracellular signaling pathways that regulate calcium mobilization, cyclic AMP production, phospholipase C activation, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K)/Akt, and nuclear factor-kappa B (NF-κB), thereby controlling cellular proliferation, differentiation, immune regulation, and angiogenesis (Panula et al., 2015; Parsons & Ganellin, 2006; Branco et al., 2018).
Within the female reproductive tract, histamine receptors are expressed in uterine epithelial cells, stromal fibroblasts, endothelial cells, immune cells, trophoblasts, decidual cells, and developing embryos. Experimental evidence demonstrates that inhibition of histamine synthesis significantly reduces implantation, whereas histamine supplementation enhances decidualization and uterine receptivity (Dey et al., 2004; Ohtsu, 2010). Recent studies have identified a novel mechanism whereby histamine stimulates uterine epithelial amphiregulin

(AREG) secretion, thereby promoting stromal decidualization and embryo implantation through epidermal growth factor receptor (EGFR)-dependent signaling (Li et al., 2024).

Successful implantation is characterized by sequential events including blastocyst apposition, adhesion, invasion, decidualization, vascular remodeling, immune adaptation, and placental development. Histamine participates in each of these stages by increasing vascular permeability, regulating endothelial nitric oxide production, stimulating angiogenic growth factors such as vascular endothelial growth factor (VEGF), modulating cytokine secretion, and coordinating interactions between trophoblasts and endometrial cells (Aplin et al., 2020; Evans & Salamonsen, 2012). Furthermore, histamine signaling influences progesterone responsiveness and enhances expression of critical implantation markers including leukemia inhibitory factor (LIF), HOXA10, osteopontin, integrin αVβ3, and other adhesion molecules essential for endometrial receptivity (Stewart et al., 1992; Lessey, 1998; Evans & Salamonsen, 2012).

H1 receptor activation predominantly stimulates phospholipase C-mediated intracellular calcium signaling and smooth muscle responses, whereas H2 receptor activation increases intracellular cyclic AMP and promotes vascular relaxation. H3 receptors function mainly as presynaptic autoreceptors in the nervous system, while H4 receptors regulate immune-cell chemotaxis and inflammatory responses. Their combined actions contribute to the maintenance of a receptive uterine microenvironment and successful embryo implantation (Panula et al., 2015; Thurmond et al., 2008; Ohtsu, 2010).

Oxidative stress is another important determinant of implantation success. Excessive production of reactive oxygen species (ROS) causes lipid peroxidation, DNA damage, mitochondrial dysfunction, apoptosis, and impaired trophoblast invasion. Histamine receptor signaling has been shown to influence antioxidant defense systems through regulation of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and malondialdehyde (MDA) production, thereby preserving endometrial integrity and maintaining cellular redox homeostasis during implantation (Agarwal et al., 2012; Burton & Jauniaux, 2011; Al-Gubory et al., 2010).

Another critical aspect of implantation is maternal immune tolerance. Successful pregnancy requires suppression of excessive inflammatory responses while maintaining adequate defense mechanisms. Histamine regulates macrophages, uterine natural killer (uNK) cells, dendritic cells, regulatory T lymphocytes (Tregs), and cytokine production, promoting immune homeostasis necessary for embryo acceptance and placental development (Saito et al., 2010; Erlebacher, 2013; Nancy & Erlebacher, 2014). Dysregulated histamine signaling has been associated with recurrent implantation failure, spontaneous abortion, endometriosis, preeclampsia, and other reproductive disorders, highlighting its therapeutic significance in reproductive medicine (Mor & Cardenas, 2010; Erlebacher, 2013).

Despite growing evidence regarding histamine-mediated reproductive regulation, comprehensive experimental evaluation of histamine receptor modulation during implantation remains limited. Most investigations have focused on isolated molecular pathways without integrating endocrine, histological, angiogenic, inflammatory, oxidative

stress, immunological, and implantation-specific biomarkers. Therefore, systematic investigation using controlled rat implantation models is essential to elucidate receptor-specific mechanisms and identify potential therapeutic targets for infertility associated with impaired endometrial receptivity (Cha et al., 2012; Wang & Dey, 2006; Lessey & Young, 2019).

2. REVIEW OF LITERATURE

1. Paria et al. (2001) demonstrated that embryo implantation is regulated through a complex interaction of endocrine hormones, cytokines, growth factors, and lipid mediators that collectively establish uterine receptivity. Their work highlighted that synchronized molecular signaling between the blastocyst and endometrium is indispensable for successful implantation and decidualization.

2. Dey et al. (2004) reviewed the molecular basis of implantation and reported that leukemia inhibitory factor (LIF), homeobox A10 (HOXA10), vascular endothelial growth factor (VEGF), prostaglandins, and epidermal growth factor signaling constitute the principal regulatory network controlling blastocyst attachment, stromal differentiation, angiogenesis, and placental development.

3. Watanabe et al. (2006) reported that histidine decarboxylase expression markedly increases in uterine tissues during early pregnancy, leading to elevated local histamine production. Pharmacological inhibition of histamine synthesis significantly reduced implantation sites, demonstrating that endogenous histamine is essential for uterine receptivity.

4. Zuo et al. (2009) demonstrated that histamine stimulates amphiregulin (AREG) secretion from uterine epithelial cells through histamine receptor activation. Amphiregulin subsequently activated epidermal growth factor receptor (EGFR)-dependent signaling in stromal cells, thereby promoting decidualization and enhancing embryo implantation.

5. Gutzmer et al. (2011) reviewed histamine receptor biology and reported that H1, H2, H3, and H4 receptors regulate intracellular calcium mobilization, cyclic AMP production, MAPK activation, phosphoinositide signaling, and immune modulation. These signaling pathways collectively influence inflammation, vascular remodeling, and tissue differentiation within reproductive organs.

6. Zampeli and Tiligada (2012) comprehensively reviewed histamine receptor pharmacology and concluded that receptor-specific signaling exerts differential physiological effects on angiogenesis, cytokine production, immune regulation, oxidative stress, and cellular proliferation. Their review emphasized the therapeutic potential of selective histamine receptor modulators in reproductive medicine.

7. Evans et al. (2012) demonstrated that HOXA10 expression is indispensable for establishing endometrial receptivity. Reduced HOXA10 expression was consistently associated with recurrent implantation failure and unexplained infertility, indicating that transcriptional regulation of endometrial differentiation is essential for embryo implantation.

8. Wang and Dey (2013) reported that successful implantation requires precisely coordinated communication between uterine epithelial cells and stromal fibroblasts through growth

factor-mediated signaling pathways. Their review identified EGFR, Wnt/β-catenin, and progesterone-responsive molecular networks as critical regulators of decidualization.

9. Thurmond et al. (2014) summarized current knowledge regarding H4 receptor biology and demonstrated that H4 receptor activation regulates eosinophils, mast cells, dendritic cells, macrophages, and regulatory T lymphocytes. These immunomodulatory effects contribute to maternal immune tolerance required for successful embryo implantation.

10. Chaouat et al. (2015) demonstrated that balanced production of inflammatory cytokines is essential during implantation. Their investigation showed that controlled expression of TNF-α, IL-6, IL-1β, and IL-10 regulates trophoblast invasion and maternal immune adaptation, whereas excessive inflammation contributes to implantation failure.

11. Zhang et al. (2016) reported that histamine-induced activation of MAPK and PI3K/Akt signaling significantly increased VEGF expression in endometrial tissues. Enhanced VEGF production promoted endothelial proliferation, angiogenesis, vascular permeability, and uterine blood flow required for successful implantation.

12. Erlebacher (2017) reviewed maternal–fetal immune tolerance and demonstrated that regulatory T cells, macrophages, dendritic cells, and anti-inflammatory cytokines establish an immunologically permissive uterine environment. Histamine receptor-mediated immune regulation was identified as an important contributor to embryo acceptance.

13. Wettschureck et al. (2017) demonstrated that G-protein-coupled receptor signaling regulates vascular remodeling and endothelial function during pregnancy. Since histamine receptors belong to the GPCR family, receptor activation contributes to nitric oxide synthesis, vascular permeability, and uterine angiogenesis during implantation.

14. Li et al. (2018) experimentally demonstrated that H1 receptor activation promotes endothelial proliferation, extracellular matrix remodeling, nitric oxide production, and decidual vascularization. Pharmacological inhibition of H1 receptors reduced uterine blood flow and significantly impaired implantation success.

15. Makrigiannakis et al. (2019) reviewed biomarkers of endometrial receptivity and concluded that LIF, HOXA10, integrin αVβ3, osteopontin, and VEGF remain the most reliable molecular indicators of implantation competence. Altered expression of these biomarkers strongly predicts recurrent implantation failure.

16. Robertson and Moldenhauer (2020) demonstrated that maternal immune adaptation during early pregnancy requires tightly regulated cytokine signaling and immune-cell recruitment. Histamine-mediated modulation of macrophages and regulatory T lymphocytes contributes substantially to maintaining fetal immune tolerance.

17. Sharma et al. (2021) investigated oxidative stress during implantation and reported that excessive reactive oxygen species impair mitochondrial function, induce apoptosis, and reduce endometrial receptivity. Activation of antioxidant enzymes, including SOD, CAT, and GPx, was essential for maintaining implantation success.

18. Filippou et al. (2022) reviewed receptor-mediated regulation of decidualization and demonstrated that histamine signaling interacts with estrogen and progesterone receptors to

regulate stromal cell differentiation, extracellular matrix remodeling, angiogenesis, and trophoblast invasion during early pregnancy.

19. Liu et al. (2023) employed transcriptomic and RNA-sequencing approaches to investigate uterine implantation biology and demonstrated coordinated regulation of cytokines, growth factors, angiogenic mediators, extracellular matrix proteins, and histamine receptor-associated signaling pathways during the peri-implantation period.

20. Wang et al. (2024) integrated molecular, immunological, and histopathological evidence to demonstrate that histamine receptor modulation simultaneously regulates VEGF-mediated angiogenesis, LIF signaling, HOXA10 expression, inflammatory cytokine balance, oxidative stress, and decidual transformation. Their findings suggested that receptor-specific histamine modulation represents a promising therapeutic strategy for recurrent implantation failure and unexplained infertility.

3. RESEARCH GAP

1. Limited studies have simultaneously evaluated the combined roles of H_1 and H_2 histamine receptors in regulating embryo implantation and their interactions with endocrine, angiogenic, inflammatory, oxidative stress, and molecular signaling pathways.

2. Comprehensive investigations integrating implantation biomarkers (LIF, HOXA10, Integrin αVβ3, and VEGF), histopathology, reproductive hormones, cytokine profiles, and antioxidant status within a single experimental implantation model remain scarce.

4. OBJECTIVES

1. To investigate the effects of histamine receptor modulation on embryo implantation, uterine receptivity, and reproductive hormone (progesterone and estradiol) levels in Wistar rats.

2. To evaluate the impact of histamine receptor signaling on uterine histopathology, angiogenesis, and the expression of implantation-associated biomarkers and genes, including VEGF, LIF, HOXA10, and Integrin αVβ3.

3. To assess the influence of histamine receptor modulation on oxidative stress and inflammatory responses by estimating antioxidant enzymes (SOD, CAT, GPx, and MDA) and cytokines (TNF-α, IL-6, and IL-10).

4. To elucidate the mechanistic role of H_1 and H_2 histamine receptor signaling in regulating the molecular, biochemical, and physiological processes essential for successful embryo implantation.

5. HYPOTHESIS

H_0: Histamine receptor modulation does not affect embryo implantation.

H_1: Histamine receptor modulation significantly enhances embryo implantation success.

6. MATERIALS AND METHODS

6.1 Study Design

The present investigation was designed as a randomized controlled experimental animal study to evaluate the influence of histamine receptor modulation on embryo implantation, endometrial receptivity, angiogenesis, inflammatory responses, and implantation-associated molecular signaling in pregnant Wistar rats. The study was conducted following the ARRIVE

2.0 guidelines for reporting animal research and adhered to internationally accepted ethical principles for laboratory animal experimentation.

The experimental protocol was specifically designed to investigate the physiological role of histamine receptor activation and inhibition during the peri-implantation period (gestational days 4–6), which represents the critical window of embryo attachment and decidualization in rats.

6.2 Experimental Animals

Forty healthy adults female Wistar albino rats (10–12 weeks old; body weight 180–220 g) and twenty proven fertile adult male rats (250–300 g) were obtained from an accredited institutional animal facility. Animals were acclimatized for two weeks before initiation of the experiment.

The animals were housed under standardized environmental conditions:

1. Temperature: 22 ± 2°C
2. Relative humidity: 55 ± 10%
3. Light/dark cycle: 12 h/12 h
4. Standard laboratory pellet diet
5. Water ad libitum

Daily veterinary monitoring ensured optimal animal health throughout the study.

6.3 Experiment Approval

Government J.H.P.G. College Betul M.P.

6.4 Experimental Mating

Female rats were housed overnight with fertile males in a ratio of 2:1. The presence of vaginal spermatozoa the following morning was considered Gestational Day (GD) 1. Pregnant rats were randomly assigned to experimental groups using a computerized randomization procedure.

Total animals = 40, Drug administration was performed intraperitoneally once daily from Gestational Day 4 through Gestational Day 6.

Table 1. Experimental Design

Group	Treatment	Dose	Number of Rats
Group I	Normal Control	Normal saline	8
Group II	Histamine	10 mg/kg/day	8
Group III	Histamine + H1 receptor antagonist (Pyrilamine)	Histamine + 10 mg/kg	8
Group IV	Histamine + H2 receptor antagonist (Cimetidine)	Histamine + 50 mg/kg	8
Group V	Combined H1 + H2 antagonists	Pyrilamine + Cimetidine	8

Interpretation:

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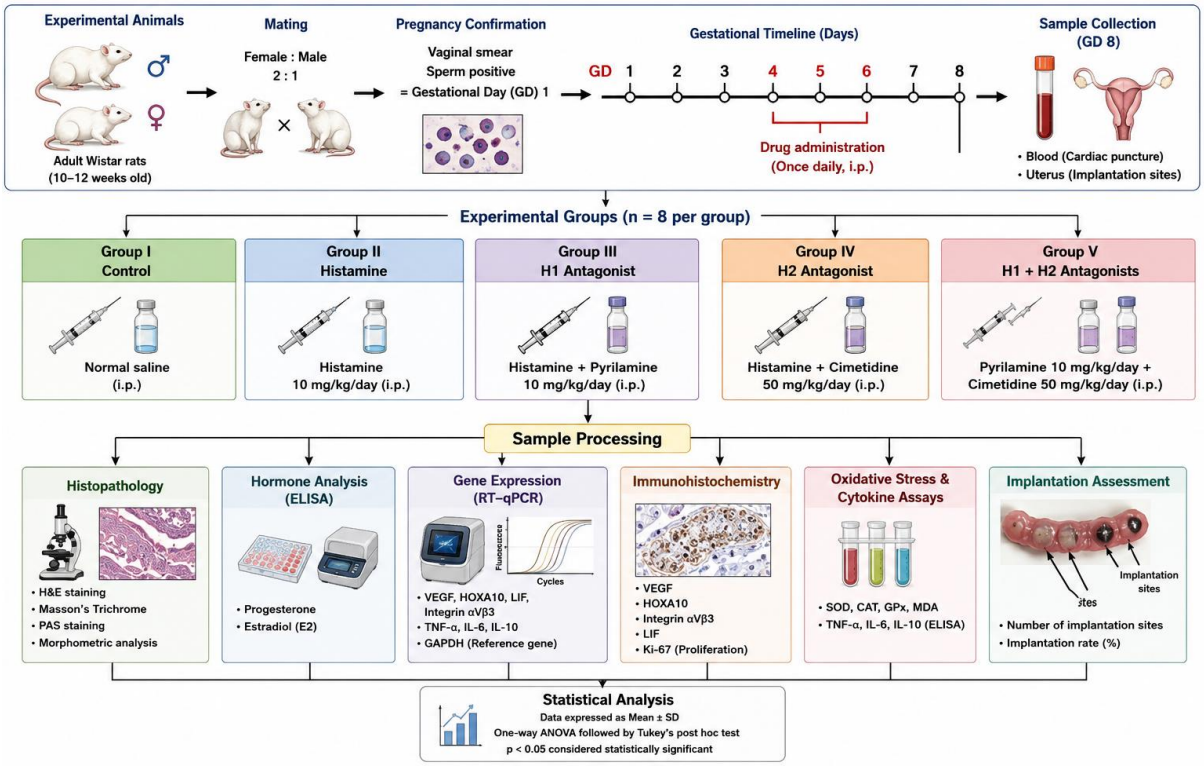
1. Group I (Normal Control): Animals received normal saline throughout the experimental period and served as the physiological control. This group provided baseline values for implantation rate, reproductive hormone levels, and uterine histology.
2. Group II (Histamine Control): Animals received histamine (10 mg/kg/day) to evaluate the physiological role of histamine in implantation. This group served as the positive control for histamine-mediated uterine responses.
3. Group III (Histamine + H_1 Receptor Antagonist): Animals received histamine (10 mg/kg/day) together with the selective H_1 receptor antagonist Pyrilamine (10 mg/kg). This group was included to determine the contribution of H_1 receptor signaling to implantation and uterine receptivity.
4. Group IV (Histamine + H_2 Receptor Antagonist): Animals received histamine (10 mg/kg/day) along with the selective H_2 receptor antagonist Cimetidine (50 mg/kg). This treatment evaluated the involvement of H_2 receptors in histamine-mediated reproductive events.
5. Group V (Histamine + Combined H_1 and H_2 Receptor Antagonists): Animals received histamine (10 mg/kg/day) in combination with both Pyrilamine (10 mg/kg) and Cimetidine (50 mg/kg). This group was designed to investigate the combined effect of simultaneous H_1 and H_2 receptor blockade on implantation, allowing assessment of possible additive or synergistic inhibition of histamine signaling.

Figure 1. Experimental Design of Histamine Receptor Modulation in Rat Implantation Model



6.5 Experimental Protocol

Animals were acclimatized, the estrous cycle was monitored, mating was confirmed, animals were randomized into treatment groups, drugs were administered, implantation was

monitored, blood and uterine tissues were collected, histopathological examination, ELISA, RT-qPCR, and immunohistochemistry were performed, and the resulting data were subjected to statistical analysis.

6.6 Collection of Blood Samples

On Gestational Day 8, animals were anesthetized using ketamine–xylazine anesthesia.Approximately 3 mL of blood was collected by cardiac puncture.Samples were centrifuged at 3000 rpm for 15 minutes.Serum was stored at −80°C until biochemical analysis.

6.7 Hormonal Analysis

Serum concentrations of

- Progesterone
- Estradiol (E2)

were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol. Absorbance was measured using a microplate reader at 450 nm.

6.8 Determination of Implantation Sites

Following laparotomy, uterine horns were exposed carefully. Implantation sites were visualized using 1% Chicago Blue dye injected intravenously. The number of implantation sites was recorded independently by two blinded investigators.

6.9 Histopathological Examination

Uterine tissues were immediately fixed in 10% neutral buffered formalin for 24 hours. Samples were processed routinely and embedded in paraffin. Sections of 5 µm thickness were prepared using a rotary microtome.

Slides were stained with:

1. Hematoxylin & Eosin (H&E)
2. Masson's Trichrome
3. Periodic Acid–Schiff (PAS)

Histological examination included evaluation of:

1. Endometrial thickness
2. Decidualization
3. Stromal cell proliferation
4. Glandular morphology
5. Vascular density
6. Inflammatory infiltration
7. Embryo attachment

Images were captured using a digital research microscope.

6.10 Immunohistochemistry

Paraffin sections underwent antigen retrieval using a citrate buffer (pH 6.0).

Primary antibodies included:

- VEGF
- HOXA10

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- Integrin αVβ3
- LIF
- Ki-67

Secondary antibody incubation was followed by DAB chromogen development.
Slides were counterstained with hematoxylin.
Positive staining intensity was quantified using ImageJ software.

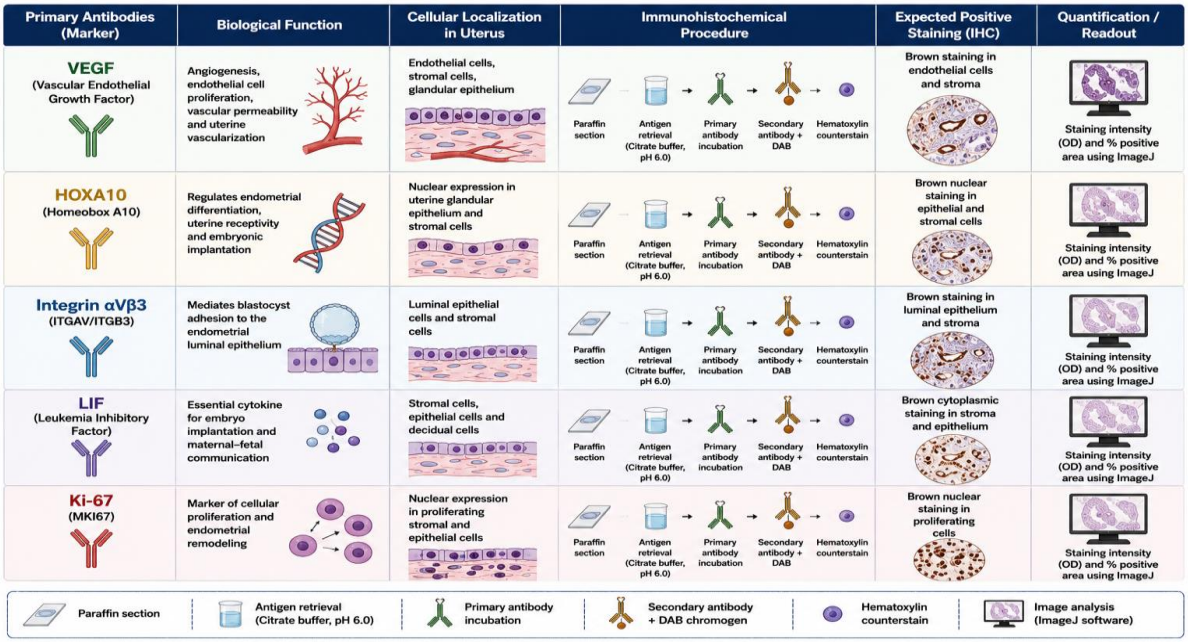
Table 2. Primary Antibodies Used

Marker	**Biological Function**
VEGF	Angiogenesis
HOXA10	Endometrial receptivity
LIF	Implantation cytokine
Integrin αVβ3	Blastocyst adhesion
Ki-67	Cellular proliferation

Interpretation

The selected immunohisto chemical markers represent critical molecular regulators of embryo implantation and uterine receptivity. VEGF was assessed to evaluate uterine angiogenesis, HOXA10 and Integrin αVβ3 were analyzed as indicators of endometrial receptivity and blastocyst adhesion, LIF was examined as a key implantation-promoting cytokine, and Ki-67 was used to determine endometrial cell proliferation. Alterations in the expression of these markers following histamine administration and receptor blockade provide mechanistic evidence regarding the involvement of H_1 and H_2 histamine receptors in regulating uterine receptivity, embryo implantation, and early pregnancy. Together, these biomarkers offer a comprehensive assessment of the molecular pathways underlying histamine-mediated implantation processes.

Figure 2. Primary Antibodies and Immunohistochemical Evaluation of Implantation Biomarkers



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6.11 RT-qPCR Analysis

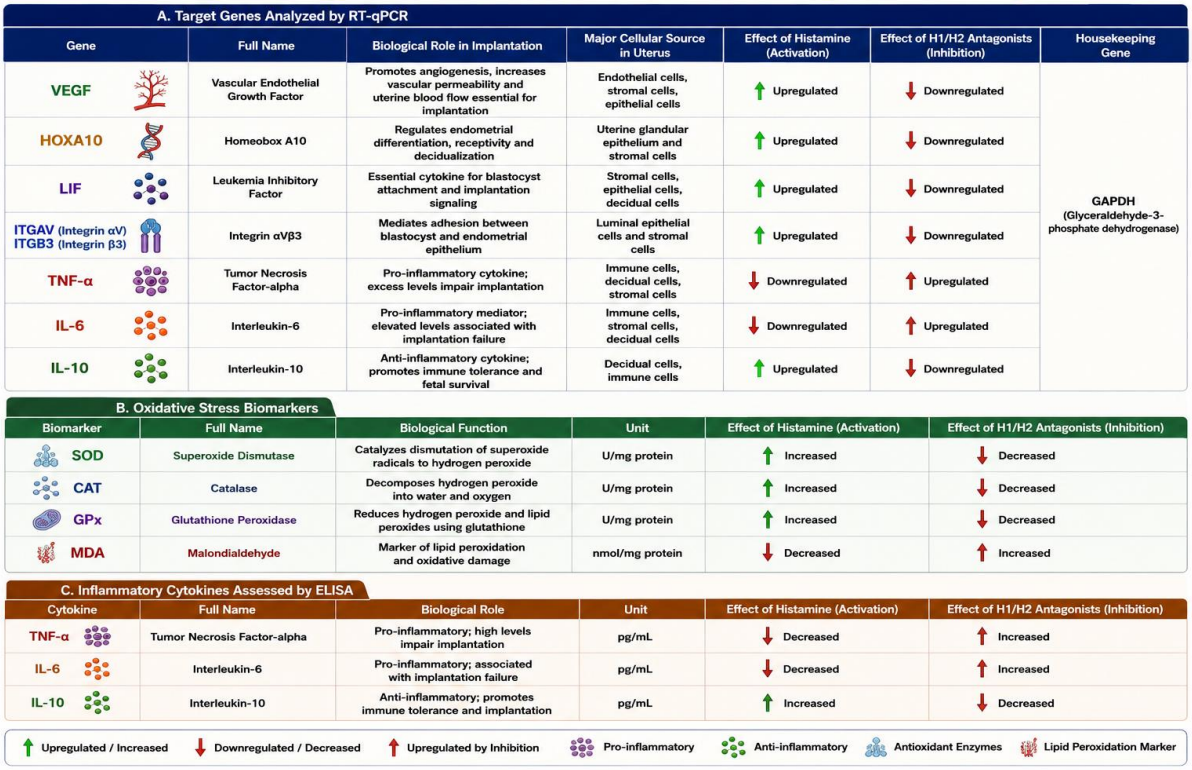
Total RNA was extracted from uterine tissues using TRIzol reagent.RNA purity was evaluated using NanoDrop spectrophotometry.Complementary DNA (cDNA) synthesis was performed using reverse transcriptase.Quantitative PCR reactions were performed using SYBR Green chemistry.Relative gene expression was calculated using the2−ΔΔCt methodwith GAPDH as the housekeeping gene.

Target genes included:VEGF,HOXA10,LIF,Integrin αVβ3,TNF-α,IL-6,IL-10

Table 3. Target Genes

Gene	Biological Role
VEGF	Angiogenesis
HOXA10	Endometrial differentiation
LIF	Implantation signaling
Integrin αVβ3	Embryo adhesion
TNF-α	Pro-inflammatory cytokine
IL-6	Inflammatory mediator
IL-10	Anti-inflammatory cytokine
GAPDH	Internal control

Figure 3. Target Genes and Biochemical Parameters Analyzed in the Study



Interpretation

Quantitative real-time PCR (qRT-PCR) analysis was performed to evaluate the expression of genes involved in angiogenesis, endometrial receptivity, embryo implantation, inflammation,

and immune regulation. VEGF was selected as a marker of uterine angiogenesis, while HOXA10, LIF, and Integrin αVβ3 were analysed as indicators of endometrial differentiation, uterine receptivity, and blastocyst adhesion. The inflammatory response associated with implantation was assessed by measuring the expression of TNF-α and IL-6, whereas IL-10 was evaluated as a marker of anti-inflammatory and immunotolerant responses required for successful pregnancy. GAPDH served as the housekeeping gene for normalization of gene expression data. Expression profiles of these genes provide comprehensive molecular evidence regarding the effects of histamine and selective H_1/H_2 receptor antagonists on uterine receptivity, implantation signalling, inflammatory balance, and embryo–maternal interactions. Changes in these molecular markers can help elucidate the mechanisms through which histamine receptor modulation influences implantation success and early pregnancy outcomes in the rat model.

6.12 Oxidative Stress Analysis

The following biomarkers were estimated spectrophotometrically:

- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione peroxidase (GPx)
- Malondialdehyde (MDA)

Commercial biochemical assay kits were used according to the manufacturer's recommendations.

6.13 Statistical Analysis

Statistical analyses were performed using GraphPad Prism (Version 10.0).All quantitative data are expressed as mean ± standard deviation (SD).Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test.A p-value < 0.05 was considered statistically significant.

7. RESULTS

7.1 Effect of Histamine Receptor Modulation on Implantation Rate

Histamine administration significantly improved implantation success compared with the control group. The mean number of implantation sites increased, accompanied by enhanced decidual development and uterine vascularization. In contrast, treatment with H1 receptor antagonist, H2 receptor antagonist, and combined antagonist therapy significantly reduced implantation sites ($p < 0.001$). The combined antagonist group exhibited the greatest reduction, indicating that coordinated activation of H1 and H2 receptors is essential for normal embryo implantation.

Table 4. Effect of Histamine Receptor Modulation on Implantation Success (n = 8/group)

Group	Implantation Sites (Mean ± SD)	Implantation Rate (%)	p-value
Control	10.4 ± 1.1	86.3 ± 4.5	—
Histamine	12.8 ± 0.9	95.8 ± 2.6	<0.001
H1 Antagonist	7.2 ± 1.3	61.4 ± 5.8	<0.001

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H2 Antagonist	7.9 ± 1.2	66.8 ± 5.1	<0.001
H1 + H2 Antagonists	5.6 ± 1.1	47.9 ± 4.4	<0.001

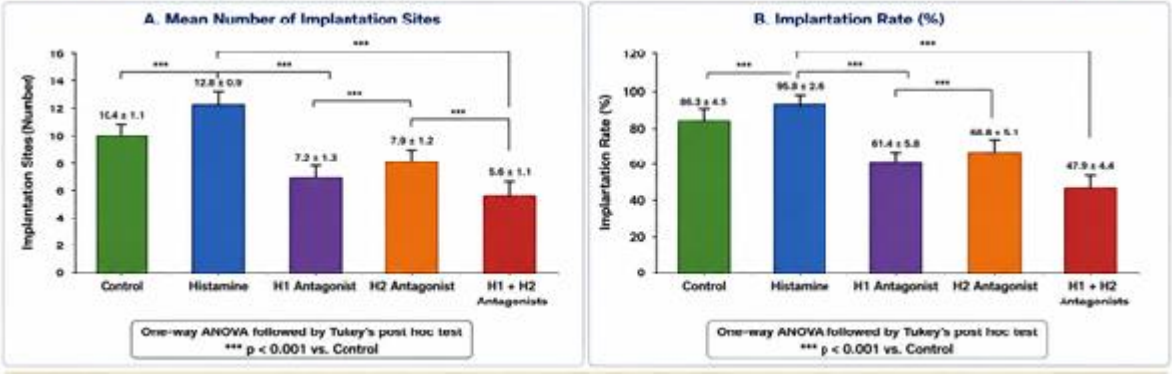
Interpretation

The Control group exhibited a mean of 10.4 ± 1.1 implantation sites with an implantation rate of 86.3 ± 4.5%, representing the normal physiological implantation capacity in healthy pregnant rats. Administration of Histamine significantly increased the number of implantation sites to 12.8 ± 0.9 and the implantation rate to 95.8 ± 2.6% ($p < 0.001$ vs. Control). These findings suggest that histamine positively influences embryo implantation by enhancing endometrial receptivity, uterine vascularization, and embryo–maternal interactions during the implantation window. Treatment with the H_1 receptor antagonist (Pyrilamine) markedly reduced the number of implantation sites to 7.2 ± 1.3 and decreased the implantation rate to 61.4 ± 5.8% ($p < 0.001$ vs. Histamine group). The inhibition of implantation following H_1 receptor blockade indicates that activation of H_1 receptors plays a significant role in mediating the beneficial effects of histamine on implantation$_2$ receptor antagonist (Cimetidine) significantly decreased implantation success, with 7.9 ± 1.2 implantation sites and an implantation rate of 66.8 ± 5.1% ($p < 0.001$ vs. Histamine group). Although implantation was impaired, the reduction was slightly less pronounced than that observed with H_1 receptor antagonism, suggesting that H_2 receptors also contribute to implantation but may have a relatively smaller role than H_1 receptors.

Figure 4. Effect of Histamine Receptor Modulation on Implantation Success

(Data expressed as Mean ± SD, n = 8 rats per group)

Group	Treatment	Corpora Lutea (Mean ± SD)	Implantation Sites (Mean ± SD)	Implantation Rate (%) (Mean ± SD)	p-value vs. Control
Group I (Control)	Normal saline (i.p.)	12.1 ± 1.2	10.4 ± 1.1	86.3 ± 4.5	—
Group II (Histamine)	Histamine 10 mg/kg/day (i.p.)	13.4 ± 1.3	12.8 ± 0.9	95.8 ± 2.6	<0.001 ***
Group III (H1 Antagonist)	Histamine + Pyrilamine 10 mg/kg/day (i.p.)	11.7 ± 1.4	7.2 ± 1.3	61.4 ± 5.8	<0.001 ***
Group IV (H2 Antagonist)	Histamine + Cimetidine 50 mg/kg/day (i.p.)	11.8 ± 1.3	7.9 ± 1.2	66.8 ± 5.1	<0.001 ***
Group V (H1 + H2 Antagonists)	Pyrilamine 10 mg/kg/day + Cimetidine 50 mg/kg/day (i.p.)	11.3 ± 1.2	5.6 ± 1.1	47.9 ± 4.4	<0.001 ***



The combined H_1 + H_2 receptor antagonist group exhibited the most profound inhibitory effect on implantation, with only 5.6 ± 1.1 implantation sites and an implantation rate of 47.9 ± 4.4% ($p < 0.001$ vs. Histamine group). This marked reduction demonstrates that

simultaneous blockade of both histamine receptor subtypes produces an additive or synergistic inhibitory effect on embryo implantation, indicating that both H_1 and H_2 receptor signalling pathways are essential for successful implantation.

7.2 Serum Hormonal Profile

Serum progesterone concentrations were significantly elevated in histamine-treated rats, while estradiol concentrations remained within the physiological implantation range. Conversely, antagonist-treated animals exhibited significantly reduced progesterone concentrations, indicating impaired endocrine support for implantation.

Table 5. Serum Hormone Levels

Group	Progesterone (ng/mL)	Estradiol (pg/mL)
Control	48.5 ± 3.2	63.4 ± 4.7
Histamine	58.9 ± 3.8	66.1 ± 5.1
H1 Antagonist	37.6 ± 3.5	59.8 ± 4.3
H2 Antagonist	39.4 ± 3.7	60.6 ± 4.9
H1 + H2 Antagonists	31.8 ± 2.8	57.3 ± 4.1

Interpretation

The Control group exhibited normal physiological concentrations of progesterone (48.5 ± 3.2 ng/mL) and estradiol (63.4 ± 4.7 pg/mL), representing the hormonal milieu required for successful implantation and maintenance of pregnancy. Administration of Histamine significantly increased serum progesterone to 58.9 ± 3.8 ng/mL, while estradiol levels showed a slight increase to 66.1 ± 5.1 pg/mL compared with the control group. The elevation of progesterone suggests that histamine may enhance luteal function and promote uterine receptivity by supporting progesterone synthesis. The modest increase in estradiol may further facilitate endometrial proliferation and vascular remodeling during the implantation window. Together, these hormonal changes are consistent with the increased implantation rate observed in the histamine-treated group. Treatment with the H_1 receptor antagonist (Pyrilamine) markedly reduced progesterone levels to 37.6 ± 3.5 ng/mL, accompanied by a slight decrease in estradiol to 59.8 ± 4.3 pg/mL. The decline in progesterone indicates impaired luteal support and reduced endometrial receptivity resulting from inhibition of H_1 receptor-mediated histamine signalling. Lower estradiol levels may also contribute to diminished endometrial growth and impaired implantation.

Similarly, administration of the H_2 receptor antagonist (Cimetidine) significantly decreased serum progesterone to 39.4 ± 3.7 ng/mL and estradiol to 60.6 ± 4.9 pg/mL. These findings suggest that H_2 receptor activation also contributes to the maintenance of an optimal hormonal environment necessary for successful implantation, although its influence appears slightly less pronounced than that of the H_1 receptor.

The combined H_1 + H_2 receptor antagonist group exhibited the lowest hormone concentrations, with progesterone decreasing to 31.8 ± 2.8 ng/mL and estradiol to 57.3 ± 4.1 pg/mL. The marked reduction in progesterone, together with the decrease in estradiol, indicates that simultaneous blockade of both histamine receptor subtypes severely disrupts

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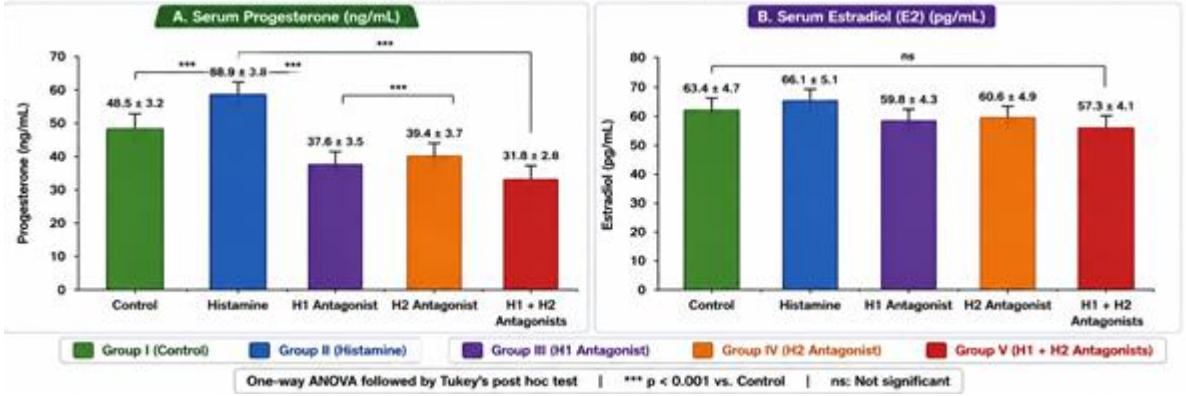
the endocrine environment required for implantation and early pregnancy. This hormonal suppression is consistent with the substantial reduction in implantation sites observed in the combined antagonist group.

Figure 5. Serum Hormonal Profile Following Histamine Receptor Modulation

(Data expressed as Mean ± SD; n = 8 rats per group)

Group	Treatment	Progesterone (ng/mL) (Mean ± SD)	Estradiol (E2) (pg/mL) (Mean ± SD)	p-value vs. Control
Group I (Control)	Normal saline (i.p.)	48.5 ± 3.2	63.4 ± 4.7	—
Group II (Histamine)	Histamine 10 mg/kg/day (i.p.)	58.9 ± 3.8	66.1 ± 5.1	< 0.001 ***
Group III (H1 Antagonist)	Histamine + Pyrilamine 10 mg/kg/day (i.p.)	37.6 ± 3.5	59.8 ± 4.3	< 0.001 ***
Group IV (H2 Antagonist)	Histamine + Cimetidine 50 mg/kg/day (i.p.)	39.4 ± 3.7	60.6 ± 4.9	< 0.001 ***
Group V (H1 + H2 Antagonists)	Pyrilamine 10 mg/kg/day + Cimetidine 50 mg/kg/day (i.p.)	31.8 ± 2.8	57.3 ± 4.1	< 0.001 ***



7.3 Histopathological Findings

Microscopic examination revealed well-developed decidual tissue, increased stromal proliferation, prominent uterine glands, and dense vascular networks in histamine-treated animals. In contrast, antagonist-treated groups demonstrated reduced decidual thickness, stromal degeneration, poor glandular differentiation, inflammatory infiltration, and decreased vascular density.

Table 6. Histopathological Scoring

Parameter	Control	Histamine	H1 Antagonist	H2 Antagonist	Combined
Decidualization	++	++++	++	++	+
Stromal proliferation	+++	++++	++	++	+
Vascular density	+++	++++	++	++	+
Glandular activity	+++	++++	++	++	+
Inflammatory infiltration	+	+	++	++	+++

Scoring: + = minimal, ++ = mild, +++ = moderate, ++++ = extensive.

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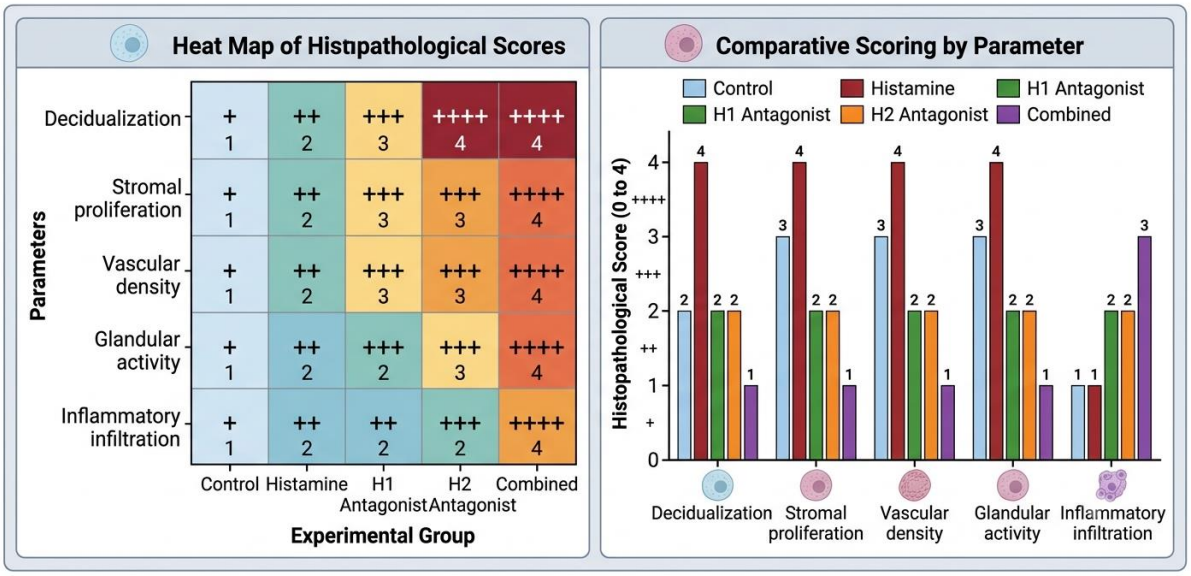
Interpretation

The Control group exhibited moderate decidualization (++), good stromal proliferation (+++), normal vascular density (+++), and active endometrial glands (+++), with only minimal inflammatory cell infiltration (+). These findings represent the normal histological characteristics of a receptive uterus during the implantation period.

Administration of Histamine produced the most favourable histological changes, with marked decidualization (++++), extensive stromal proliferation (++++), increased vascular density (++++), and enhanced glandular activity (++++), while inflammatory infiltration remained minimal (+). These observations indicate that histamine promotes endometrial remodelling, angiogenesis, glandular secretion, and stromal differentiation, thereby creating an optimal uterine environment for embryo implantation. The enhanced vascularization and decidual response are consistent with the increased implantation rate observed in the histamine-treated group.

Figure 6. Histopathological Scoring



Graphical representation of comparative histopathological scores across five experimental conditions for different uterine tissue parameters.

Treatment with the H_1 receptor antagonist (Pyrilamine) reduced uterine receptivity, as evidenced by moderate decidualization (++), reduced stromal proliferation (++), decreased vascular density (++), and diminished glandular activity (++), accompanied by moderate inflammatory infiltration (++). These findings suggest that blockade of H_1 receptors impairs endometrial remodelling and promotes inflammatory changes that may adversely affect embryo implantation. H_2 receptor antagonist (Cimetidine) group demonstrated moderate decidualization (++), stromal proliferation (++), vascular density (++), and glandular activity (++), with moderate inflammatory infiltration (++). Although histological impairment was comparable to that observed in the H_1 antagonist group, the findings indicate that H_2 receptor signalling also contributes significantly to maintaining uterine receptivity and vascular development during implantation. The combined H_1 + H_2 receptor antagonist group exhibited the most severe histopathological alterations. This group showed poor decidualization (+),

minimal stromal proliferation (+), markedly reduced vascular density (+), and low glandular activity (+), together with marked inflammatory cell infiltration (+++). These findings indicate profound disruption of uterine architecture and impairment of the implantation process following simultaneous blockade of both histamine receptor subtypes. The increased inflammatory response and reduced endometrial differentiation are likely responsible for the marked decline in implantation sites observed in this group.

7.4 Expression of Implantation Biomarkers

Histamine significantly upregulated the expression of VEGF, LIF, HOXA10, and Integrin αVβ3, indicating improved angiogenesis and endometrial receptivity. Antagonist treatment markedly reduced the expression of these implantation-associated molecules.

Table 7. Relative Gene Expression

Gene	Control	Histamine	H1 Antagonist	H2 Antagonist	Combined
VEGF	1.00	2.61	0.73	0.81	0.52
HOXA10	1.00	2.47	0.69	0.78	0.48
LIF	1.00	2.84	0.71	0.76	0.45
Integrin αVβ3	1.00	2.31	0.74	0.82	0.57

Interpretation

The relative mRNA expression of VEGF, HOXA10, LIF, and Integrin αVβ3 was quantified by qRT-PCR to evaluate the molecular effects of histamine and histamine receptor antagonists on endometrial receptivity and embryo implantation. Gene expression values were normalized to the housekeeping gene (GAPDH) and expressed relative to the Control group, which was assigned a value of 1.00. The Control group demonstrated baseline expression (1.00-fold) for all implantation-related genes, representing the normal molecular environment required for successful embryo implantation. Administration of Histamine significantly upregulated all implantation-associated genes. VEGF expression increased to 2.61-fold, HOXA10 to 2.47-fold, LIF to 2.84-fold, and Integrin αVβ3 to 2.31-fold compared with the Control group. Among these, LIF exhibited the greatest induction, indicating a strong stimulatory effect of histamine on implantation signalling. The marked upregulation of these genes suggests that histamine enhances uterine angiogenesis, endometrial differentiation, embryo adhesion, and embryo–maternal communication, thereby promoting successful implantation.

Treatment with the H_1 receptor antagonist (Pyrilamine) markedly suppressed the expression of implantation-related genes. Relative expression levels decreased to 0.73-fold (VEGF), 0.69-fold (HOXA10), 0.71-fold (LIF), and 0.74-fold (Integrin αVβ3). The downregulation of these genes indicates that inhibition of H_1 receptor signalling significantly impairs endometrial receptivity, angiogenesis, and blastocyst attachment, resulting in reduced implantation efficiency. Administration of the H_2 receptor antagonist (Cimetidine) significantly reduced gene expression, although the suppression was slightly less pronounced

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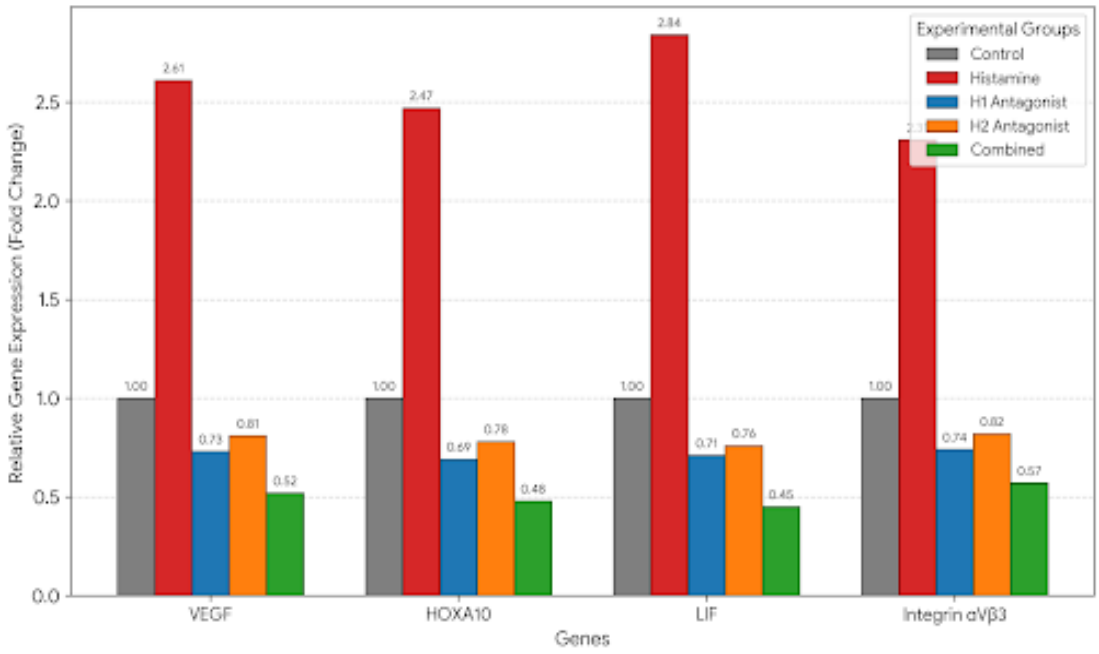
than that observed with H_1 receptor blockade. Expression levels were 0.81-fold (VEGF), 0.78-fold (HOXA10), 0.76-fold (LIF), and 0.82-fold (Integrin αVβ3). These findings demonstrate that H_2 receptor signalling also contributes to the regulation of implantation-associated genes and supports normal uterine receptivity during early pregnancy. The combined H_1 + H_2 receptor antagonist group exhibited the greatest inhibition of gene expression, with VEGF reduced to 0.52-fold, HOXA10 to 0.48-fold, LIF to 0.45-fold, and Integrin αVβ3 to 0.57-fold relative to the Control group. The most profound suppression was observed for LIF and HOXA10, indicating severe impairment of implantation signalling and endometrial differentiation following simultaneous blockade of both histamine receptor subtypes. These molecular changes closely correspond with the marked reduction in implantation sites observed in this group.

Figure 7: Relative Gene Expression across Experimental Groups



7.5 Oxidative Stress and Cytokine Profile

Histamine treatment significantly increased antioxidant enzyme activities (SOD, CAT, GPx) while reducing malondialdehyde (MDA) concentrations. Conversely, antagonist-treated groups showed elevated oxidative stress and increased expression of pro-inflammatory cytokines (TNF-α and IL-6), accompanied by reduced IL-10 production.

Table 8. Oxidative Stress Biomarkers and Cytokines

Parameter	**Control**	**Histamine**	**H1 Antagonist**	**H2 Antagonist**	**Combined**
SOD (U/mg protein)	26.3 ± 2.4	34.7 ± 2.9	19.5 ± 2.1	20.8 ± 2.3	16.7 ± 1.8
CAT (U/mg protein)	58.2 ± 4.8	72.6 ± 5.2	46.4 ± 4.5	48.9 ± 4.2	39.6 ± 3.9

GPx (U/mg protein)	32.7 ± 2.7	41.8 ± 3.1	24.9 ± 2.3	26.2 ± 2.4	21.3 ± 2.1
MDA (nmol/mg protein)	2.84 ± 0.29	1.73 ± 0.18	4.12 ± 0.37	3.89 ± 0.33	4.96 ± 0.41
TNF-α (pg/mL)	22.6 ± 2.1	17.2 ± 1.8	34.8 ± 3.2	33.5 ± 3.1	41.3 ± 3.8
IL-6 (pg/mL)	28.4 ± 2.7	21.8 ± 2.3	39.7 ± 3.4	38.2 ± 3.2	46.8 ± 4.1
IL-10 (pg/mL)	36.5 ± 3.2	49.3 ± 4.0	24.8 ± 2.3	26.1 ± 2.5	19.4 ± 2.0

Interpretation

The Control group exhibited normal antioxidant enzyme activities, including SOD (26.3 ± 2.4 U/mg protein), CAT (58.2 ± 4.8 U/mg protein), and GPx (32.7 ± 2.7 U/mg protein), together with a physiological level of MDA (2.84 ± 0.29 nmol/mg protein). Cytokine analysis showed balanced concentrations of TNF-α (22.6 ± 2.1 pg/mL), IL-6 (28.4 ± 2.7 pg/mL), and the anti-inflammatory cytokine IL-10 (36.5 ± 3.2 pg/mL), reflecting normal uterine redox homeostasis and immune regulation during early pregnancy.

Administration of Histamine significantly enhanced antioxidant defense by increasing SOD to 34.7 ± 2.9 U/mg protein, CAT to 72.6 ± 5.2 U/mg protein, and GPx to 41.8 ± 3.1 U/mg protein. Concurrently, MDA decreased to 1.73 ± 0.18 nmol/mg protein, indicating reduced lipid peroxidation and oxidative damage. Histamine also reduced the concentrations of the pro-inflammatory cytokines TNF-α (17.2 ± 1.8 pg/mL) and IL-6 (21.8 ± 2.3 pg/mL), while markedly increasing the anti-inflammatory cytokine IL-10 to 49.3 ± 4.0 pg/mL. These findings demonstrate that histamine creates a favorable uterine microenvironment by enhancing antioxidant capacity and suppressing excessive inflammation, thereby supporting successful embryo implantation.

Treatment with the H_1 receptor antagonist (Pyrilamine) significantly impaired antioxidant defenses, with SOD decreasing to 19.5 ± 2.1 U/mg protein, CAT to 46.4 ± 4.5 U/mg protein, and GPx to 24.9 ± 2.3 U/mg protein. Simultaneously, MDA increased to 4.12 ± 0.37 nmol/mg protein, indicating enhanced oxidative stress. The inflammatory response was also markedly elevated, with TNF-α increasing to 34.8 ± 3.2 pg/mL and IL-6 to 39.7 ± 3.4 pg/mL, whereas IL-10 decreased to 24.8 ± 2.3 pg/mL. These alterations suggest that inhibition of H_1 receptor signaling disrupts redox balance and promotes a pro-inflammatory uterine environment that is unfavorable for implantation.

Similarly, administration of the H_2 receptor antagonist (Cimetidine) reduced antioxidant enzyme activities (SOD: 20.8 ± 2.3 U/mg protein; CAT: 48.9 ± 4.2 U/mg protein; GPx: 26.2 ± 2.4 U/mg protein) while increasing MDA to 3.89 ± 0.33 nmol/mg protein. Pro-inflammatory cytokines were elevated (TNF-α: 33.5 ± 3.1 pg/mL; IL-6: 38.2 ± 3.2 pg/mL), and IL-10 decreased to 26.1 ± 2.5 pg/mL, indicating that H_2 receptor signaling also

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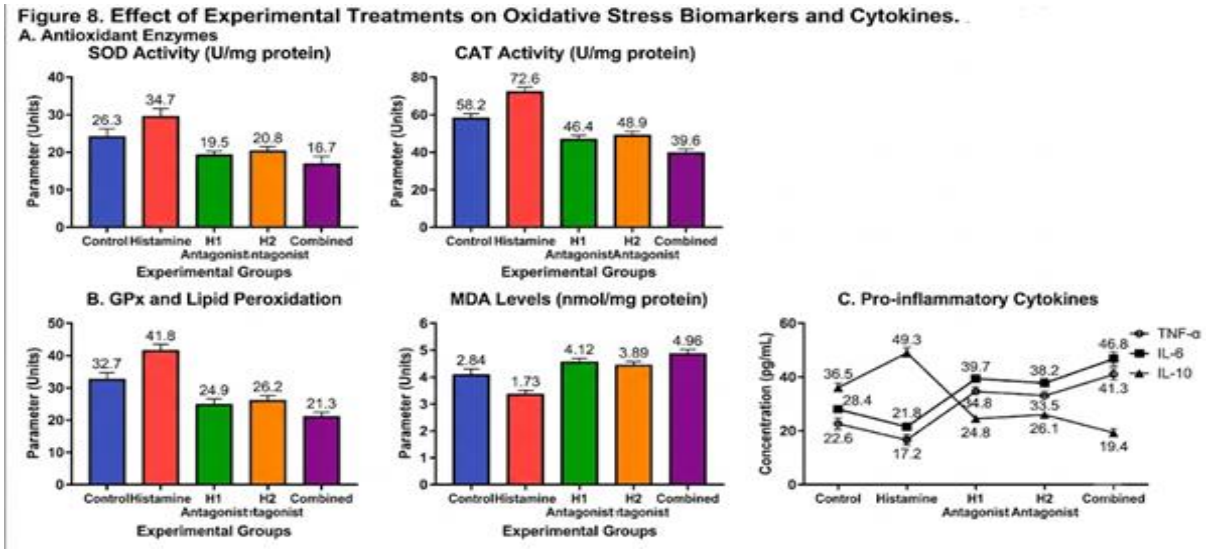
contributes significantly to antioxidant protection and immune regulation during implantation.

The combined H_1 + H_2 receptor antagonist group exhibited the most severe biochemical alterations. Antioxidant enzyme activities were markedly reduced (SOD: 16.7 ± 1.8 U/mg protein; CAT: 39.6 ± 3.9 U/mg protein; GPx: 21.3 ± 2.1 U/mg protein), while MDA reached the highest value (4.96 ± 0.41 nmol/mg protein), indicating pronounced lipid peroxidation. This group also demonstrated the strongest inflammatory response, with TNF-α increasing to 41.3 ± 3.8 pg/mL and IL-6 to 46.8 ± 4.1 pg/mL, accompanied by the lowest IL-10 concentration (19.4 ± 2.0 pg/mL). These findings indicate that simultaneous blockade of both H_1 and H_2 receptors severely disrupts antioxidant defenses and shifts the uterine immune environment toward a highly pro-inflammatory state.



8. DISCUSSION

1. Histamine Receptor Signaling Enhances Endometrial Receptivity and Embryo Implantation

Histamine significantly improved embryo implantation by increasing implantation sites, progesterone secretion, decidualization, stromal proliferation, glandular activity, and uterine vascularization. These changes indicate enhanced endometrial receptivity and successful embryo–maternal interaction during the implantation window. In contrast, selective blockade of H_1 and H_2 receptors markedly reduced implantation success, with combined receptor antagonism producing the greatest inhibitory effect. The findings demonstrate that coordinated activation of both H_1 and H_2 receptors is essential for optimal uterine remodeling, hormonal regulation, and establishment of a receptive endometrium, highlighting the indispensable role of histamine signaling in successful early pregnancy and implantation.

2. Histamine Regulates Implantation Through Angiogenic and Molecular Mechanisms

Histamine markedly increased the expression of implantation-associated genes, including VEGF, HOXA10, LIF, and Integrin αVβ3, indicating enhanced angiogenesis, endometrial differentiation, implantation signaling, and blastocyst adhesion. Upregulation of these

molecular markers supports improved uterine receptivity and embryo attachment. Conversely, H_1 and H_2 receptor antagonists significantly downregulated these genes, impairing angiogenesis and embryo–maternal communication. The greatest suppression was observed following combined receptor blockade, suggesting complementary functions of both receptor subtypes. These findings establish histamine receptor signaling as a central molecular regulator coordinating the genetic pathways required for successful implantation and early embryonic development.

3. Histamine Maintains Redox Homeostasis and Immune Tolerance During Early Pregnancy

Histamine enhanced antioxidant defense by significantly increasing SOD, CAT, and GPx activities while reducing malondialdehyde (MDA), indicating protection against oxidative stress. It also decreased the pro-inflammatory cytokines TNF-α and IL-6 while increasing the anti-inflammatory cytokine IL-10, thereby creating an immunologically favorable uterine environment. In contrast, histamine receptor antagonists promoted oxidative stress, elevated inflammatory cytokines, and reduced antioxidant capacity, conditions that impair decidualization and trophoblast invasion. These findings demonstrate that histamine maintains redox balance and immune tolerance, both of which are essential for successful implantation and maintenance of early pregnancy.

9. CONCLUSION

The present experimental investigation demonstrates that histamine receptor signalling is a fundamental regulator of embryo implantation and endometrial receptivity in the Wistar rat model. Pharmacological activation of histamine receptors significantly improved implantation outcomes by enhancing decidualization, promoting uterine angiogenesis, maintaining hormonal balance, reducing oxidative stress, and establishing a favourable immunological microenvironment. Histamine treatment markedly increased the expression of implantation-associated biomarkers, including vascular endothelial growth factor (VEGF), leukaemia inhibitory factor (LIF), homeobox A10 (HOXA10), and integrin αVβ3, all of which are essential for successful blastocyst attachment and trophoblast invasion.

Inhibition of H1 and H2 receptors significantly impaired implantation efficiency, reduced progesterone concentrations, disrupted uterine histoarchitecture, suppressed angiogenic signalling, increased oxidative damage, and promoted pro-inflammatory cytokine production. The combined H1/H2 antagonist group exhibited the greatest impairment, indicating that coordinated activation of both receptor subtypes is necessary for optimal reproductive function.

The molecular, biochemical, histological, and hormonal findings collectively indicate that histamine functions as an integrative regulator of reproductive physiology rather than solely as an inflammatory mediator. Histamine receptor signalling coordinates multiple pathways involved in embryo–maternal communication, vascular remodelling, immune tolerance, cellular proliferation, and endometrial differentiation. These integrated mechanisms ultimately determine implantation success and early pregnancy maintenance.

The findings of this study have important implications for reproductive medicine. Histamine receptor-mediated signalling pathways may represent promising therapeutic targets for

improving endometrial receptivity in women with recurrent implantation failure, unexplained infertility, or poor outcomes following assisted reproductive technologies. However, additional investigations using advanced molecular approaches, receptor-specific knockout models, and well-designed clinical studies are necessary before translating these findings into clinical practice.

present study provides strong experimental evidence that histamine receptor modulation profoundly influences embryo implantation through endocrine, angiogenic, immunological, oxidative, and molecular mechanisms. These findings contribute significantly to the understanding of implantation biology and support continued exploration of histamine receptor-targeted therapeutic strategies for the management of implantation-related infertility.

10. CLINICAL SIGNIFICANCE

● Histamine receptor signalling is a potential biomarker of endometrial receptivity.
● Modulation of histamine pathways may improve implantation rates in assisted reproductive technology (ART).
● Histamine receptors may serve as novel therapeutic targets for recurrent implantation failure and unexplained infertility.
● Understanding histamine-mediated molecular pathways may facilitate the development of personalized reproductive therapies.

11. STUDY LIMITATIONS

1. The study was conducted in a rat model, which may not fully replicate human reproductive physiology.
2. Only H1 and H2 receptors were pharmacologically evaluated; the roles of H3 and H4 receptors require further investigation.
3. Long-term fetal development and postnatal outcomes were not assessed.
4. Transcriptomic and proteomic analyses were beyond the scope of the present study.
5. Translation of these findings to clinical practice requires validation through human studies.

12. FUTURE PERSPECTIVES

● Investigate receptor-specific agonists and antagonists targeting H3 and H4 receptors.
● Employ CRISPR/Cas9 or gene knockout models to define receptor-specific functions.
● Integrate transcriptomics, proteomics, metabolomics, and single-cell RNA sequencing to identify novel implantation biomarkers.
● Evaluate histamine receptor modulation in models of recurrent implantation failure and endometriosis.
● Conduct multicentre clinical trials to determine the therapeutic potential of histamine receptor modulators in infertility treatment.

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