10.22070/jbcp.2025.20258.1199 http://jbcp.shahed.ac.ir

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, Online ISSN: 2345-4334
Vol. 14, No. 2, January 2026

A Semiannual Publication of
Shahed University Press

Effect of Prenatal Stress on Cytokine Profile in Rodent Fetus and Offspring: A Systematic Review and Meta-analysis

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Abstract

Background and Objective: Prenatal stress can lead to alterations in cytokine secretion in the offspring, which may enhance inflammatory processes. We conducted a meta-analysis of rodent studies to evaluate the effects of prenatal stress exposure on the pro- and anti-inflammatory cytokines in the fetus and offspring.

Materials and Methods: Data were extracted, and effect size and heterogeneity were assessed using random-effects models, Cochrane Q, and I2 statistics.

Results: Prenatal stress consistently increased most pro-inflammatory cytokines (IFN-ϒ, IL-1β, IL-6, IL-18, and TNF-α), while decreasing the level of anti-inflammatory cytokine (IL-10). Most of the cytokines (IL-1β, IL-6, IL-18, and TNF-α) increased in the short term after prenatal stress (≤ 24 hours), except for IL-10, which decreased in long-term stress ($P < 0.05$). Challenges in offspring following prenatal stress had no significant effect on IL-10, IL-5, IL-6, and TNF-α levels. Meta-analysis of the species subgroup indicated that significant changes in most cytokines (IFN-ϒ, IL-1β, IL-6, and TNF-α) only occurred in the rat fetus and offspring following prenatal stress. In contrast, only IL-10 significantly changed in both rats and mice.

Conclusion: This meta-analysis indicates important biological differences in the response to prenatal stress based on species, duration, and challenges faced by the offspring in immune response studies.

Keywords: Interleukin, Interferon, Stress Type, Stress Duration

1. Introduction

The perinatal period is a pivotal and susceptible phase in an individual's life, owing to the rapid development of the organism, which can be influenced by beneficial or harmful factors present in the uterine environment (1). During this stage, the fetus is directly affected by the mother's physiological changes, which are transmitted via the placenta through hormones, immunological mediators, or nutrients. Exposure to stress may disrupt the normal development and functioning of the immune system in progeny (2). Fetal development is characterized by the early presence of both innate and adaptive immune

Received: 15 March 2025 **Revised:** 20 April 2025 **Accepted:** 04 May 2025
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cells, which emerge progressively throughout gestation and grow substantially until delivery (2). Monocytes/macrophages, the first innate cells, appear in small quantities during the prenatal period. Granulocytes and natural killer (NK) cells follow, increasing significantly and peaking shortly after birth (3). B and T cell precursors also appear during gestation (4). Although innate and adaptive immune cells are present throughout fetal development, their effector functions remain underdeveloped during this period (5). A key factor is the type of antigens encountered during pregnancy, which originate from both the fetus and the mother (6). Both types of antigens must be tolerated to ensure fetal survival during pregnancy and prevent autoimmunity after birth. Thus, the fetal immune system is inherently tolerogenic to avoid harmful inflammatory responses (2). Notably, less is known about fetal immune development compared to newborns. Furthermore, comprehensive research on how prenatal exposures affect immune cell activation and maturation pathways remains limited, though emerging studies are beginning to address this gap.

As mentioned, prenatal maternal stress has long-lasting effects on the offspring's immunity. It has been associated with the emergence of long-term conditions such as asthma and allergies (7). Studies using animal models have shown that maternal stress causes a decrease in the immune response of the offspring. Maternal stress achieves this by altering lymphocyte proliferation and function, as well as limiting the ability of natural killer cells to kill target cells (8). Furthermore, there have been reports of impaired adaptive immune responses in newborns delivered to mothers who experienced anxiety during pregnancy (9). These neonates had diminished humoral immune responses and a decreased ability to induce TH1-type immunological responses. Variations in newborn immunity may arise from the transmission of stress hormones, such as glucocorticoids, across the placenta (9). These hormones possess immunomodulatory properties and, hence, have the potential to modify the process of embryonic immunological maturation (9).

The use of animal models is beneficial in comprehending the intricate processes that underlie the stress response. This knowledge may aid in clarifying the underlying causes of immunological illnesses, which are persistent outcomes of prenatal and early life adversity (10). The objective of this research was to conduct a systematic review and meta-analysis of rodent studies that examined the impact of prenatal stress on inflammatory cytokines in the fetus and offspring.

2. Materials and Methods

2.1. Search strategy

Two authors (HH and LN) independently searched PubMed, Scopus, ISI Web of Science, ProQuest, the Scientific Information Database (SID), MagIran, and Google Scholar (for grey literature) to identify relevant studies published up to January 15, 2025. The search terms included: ("maternal stress" OR "prenatal stress*" OR "maternal social stress" OR "maternal restraint" OR "maternal exposure to cold" OR "maternal food deprivation " OR "maternal prevention of sleep" OR "maternal overcrowding" OR "maternal forced swim" OR "maternal cat meowing" OR "maternal social isolation" OR "maternal cage tilting" OR "maternal foot shocks" OR "maternal bystander stress" OR "maternal noise stress" OR "maternal ultrasound stress" OR "gestational variable stress" OR "maternal/prenatal chronic unpredictable mild stress") AND ("cytokine" OR "interleukin" OR "interferon" OR "immunity" OR "immune" OR "TNF" OR "IL" OR "TGF" OR "INF") AND ("rat" OR "mice" OR "murine" OR "mouse" OR "rodent" OR "guinea pig" OR "hamster"). To ensure no studies were missed, we manually screened references from eligible studies and relevant reviews.

2.2. Study eligibility

In accordance with the subsequent criteria, studies were incorporated into this survey: (a) experimental studies employing control and treatment groups comprised of rodents (rat, mouse, hamster, and guinea pig); (b) studies utilizing acute/chronic prenatal stress in the offspring (i.e., physical, nutritional, psychological, or unpredictable) with or without an immune stimulation challenge; (c) studies that are written exclusively in English or Persian since the year 2000; (d) studies analyzing protein levels of cytokines in various tissues or blood solely using biochemical techniques (such as enzyme-linked immunosorbent assay, ELISA and cytometric bead array, CBA) or western blotting. To have sufficient data for meta-analysis, the choice of inflammatory cytokines was according to their presence in a minimum of four independent studies. The eligible outcomes included interferon

(IFN)-ϒ, interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-10, IL-18, and tumor necrosis factor (TNF)-α. Consensus discussions were employed to resolve any uncertainties about the inclusion or exclusion of studies.

2.3. Data extraction

The following data were extracted from all included articles by two independent authors (HH and LN): 'first author', 'publication year', 'species', 'number of subjects per group' 'prenatal stress protocol', 'prenatal stress period, 'prenatal stress duration', 'analyzed sample', 'pup challenge', 'outcome data', and 'method of measurement'. The data was extracted utilizing Engauge Digitizer software version 12.1 if the article exclusively presented its data through graphs and not in the form of text or a table.

2.4. Data analysis

Effect size (standardized mean difference, SMD) and confidence interval (CI) were determined. The estimation of summaries was performed utilizing random-effects models. The value of a standard error was transformed into a standard deviation whenever it was presented. The assessment of heterogeneity was conducted utilizing the Cochrane Q and I^2 statistics. 25%, 50%, and 75% heterogeneity measures were categorized as low, moderate, and high, respectively (11, 12). Subgroup analyses were performed utilizing parameters from the prenatal stress protocol (specifically, 'total duration of prenatal'), presence or absence of challenge, and type of species. To determine which study or studies may be contributing to the heterogeneity and to assess the impact of a specific study or number of studies on the overall effect, a sensitivity analysis was performed (13). Publication bias was assessed using Egger's regression asymmetry test and the Begg & Mazumdar adjusted rank correlation test (14). The statistical analyses were performed utilizing Stata 11.2 (Stata Corp., College Station, TX). $P < 0.05$ was deemed to be statistically significant.

2.5. Risk of bias assessment

The Risk of Bias (RoB) tool for animal studies, developed by the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE), was employed to evaluate the risk of bias in the studies that were included (15). The ten items comprising the RoB tool identify bias in the following areas: selection, performance, detection, attrition, and reporting. A "yes" score indicates a low risk of bias for the item in question, whereas a "no" score signifies a high risk. An item was deemed "unclear" and its potential for bias was unknown if it was not reported or explicitly stated.

3. Results

3.1. Search results

As shown in Figure 1, 1321 records were initially identified, and 503 duplicates were removed. After screening, 775 irrelevant records were excluded. Finally, 43 full-text articles were assessed, and 23 studies (16-41) *met the inclusion criteria.*

3.2. Study characteristics

Table 1 presents the attributes of 26 qualified studies conducted in Argentina, Brazil, Canada, China, France, Iran, Poland, the USA, South Africa, Thailand, and Germany. Rats were used in 65.4% of eligible studies (n = 17), while mice were used in 34.6% (n = 9). Regarding prenatal stress protocols, 'restraint' (n = 13) and 'restraint under bright light' (n = 6) stress were the most used, which corresponded to 50.0% and 23.1% of studies, respectively. Other stress protocols, including 'sleep deprivation' (n = 1), 'sound' (n = 2), 'ultrasound' (n = 1), 'nest material restriction/ diesel exhaust particle aspiration' (n = 1), 'social isolation stress' (n = 1), and 'unpredictable mild stress' (n = 1) corresponded to 26.9% of studies. Most studies utilized prenatal stress periods of 2-7 days (55.2%, n = 16), followed by 24.1% of studies using 8-14 days (n = 7) and 23.1% of studies using 15-21 days (n = 6). Noticeably, Chen et al. (2020) and Vargas et al. (2016) used two distinct time stages of prenatal stress protocol in their experiments. Also, most studies (69.2%, n = 18) induced prenatal stress for 3-18 hours, while the rest of studies (30.8%, n = 8) induced the stress for 18-504 hours. Brain tissues of the fetus or offspring (total brain, hippocampus, and frontal/prefrontal cortex) were the predominant samples analyzed (57.6%, n =19), followed by the serum samples of offspring (18.2%, n = 6) that were totally analyzed in 76.9% of the studies. The lymphocytes (9.1%, n = 4), bronchoalveolar lavage (6.1%, n = 2), spleen (3.0%, n = 1), and uterus (3.0%, n = 1) of offspring were the least samples analyzed in 30.8% of the studies. 57.7% of the studies (n = 15) used no challenge before cytokine assessment, while 42.3% of the studies

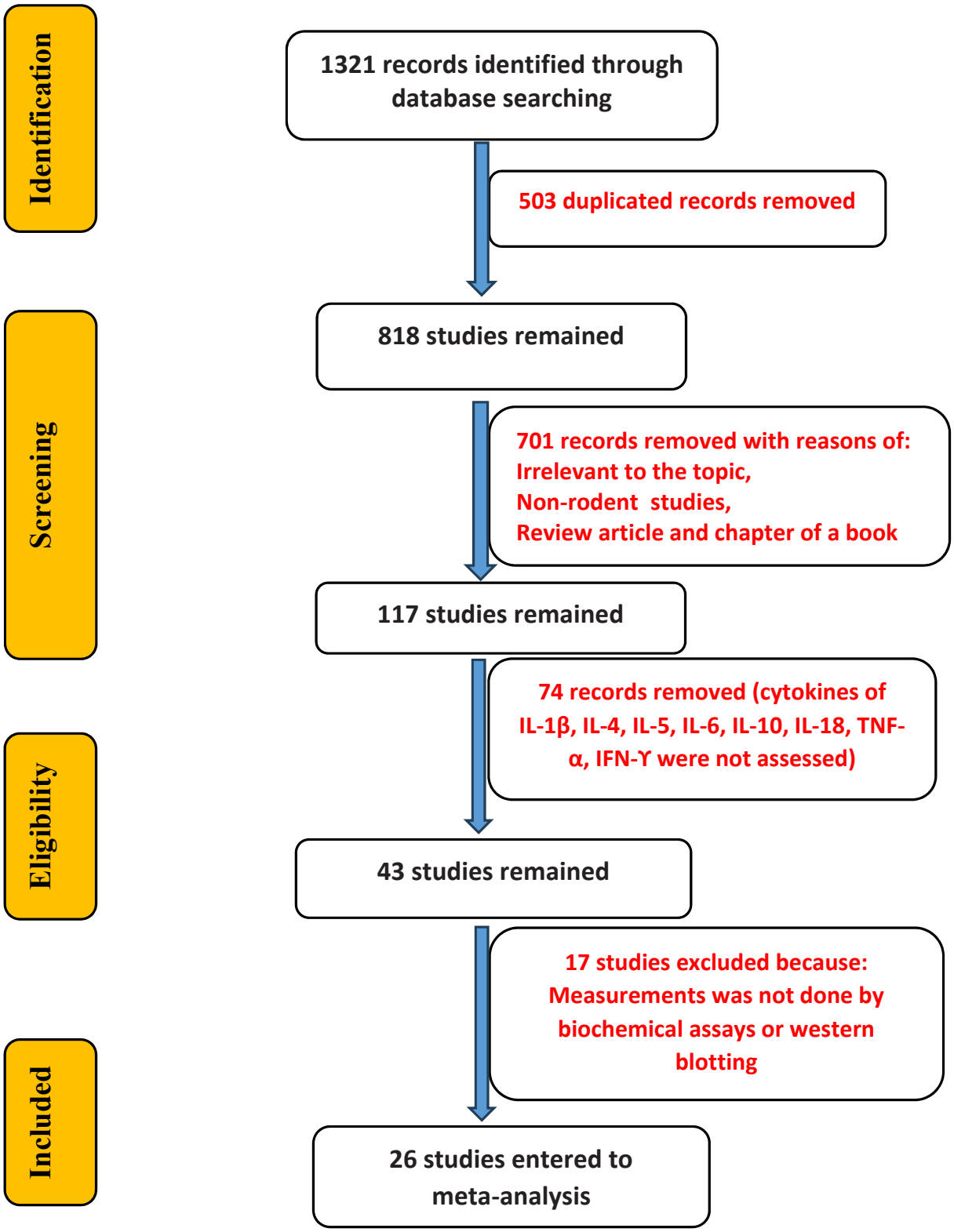

Fig. 1. Flow diagram of study identification, inclusion, and exclusion.

Table 1. Specifications of experimental studies included in the meta-analysis

Study	Year	Species	No. Of subjects/ Group	Prenatal stress protocol	Prenatal stress period	Prenatal stress duration	Offspring challenge	Analyzed sample	Outcome data	Method of measurement
de los Angeles´ Aldirico et al.	2023	Mouse	5	Restraint	GD:15-20	60 min; 1x/day; TD: 6 h	Asthma induction with ovalbumin	Bronchoalveolar lavage of offspring	IL-4, IL-5	ELISA
Amani et al.	2021	Rat	10	Restraint under bright light (60W)	GD: 5-19	30 min; 3x/day; TD: 7.5 h	None	Brain of offspring	IL-6	ELISA
Baratta et al.	2020	Rat	3-6	Sleep deprivation	GD:16-18	5h/ day; TD: 3 h	None	Brain of fetus	IL-1β, IL-6	ELISA
Bolton et al.	2013	Mouse	8	Nest material restriction	GD: 14-19	24hours/day; TD:144 h	None	Brain of fetus	IL-1β, Il-10	ELISA
				Diesel exhaust particles aspiration	GD: 2-17	1 min; Every 3 days; TD: 5min				
Chen et al.	2020	Rat	18	Restraint under bright light (6500 lx)	GD: 8-14 GD: 15-21	45 min; 3x/day; TD: 15.75 h	None	Hippocampus of offspring	IL-18	ELISA, Western blot
Ho et al.	2013	Mouse	8-13	Sound (70 db, 300 Hz)	GD: 12, GD: 14	24h/ day; TD: 48 h	Atherosclerosis development	Lymphocyte of offspring	TNF-α, IFN-ϒ	ELISA
Kohman et al.	2008	Mouse	5	Restraint	GD: 15-17	240 min; 1x/day; TD: 12 h	None, LPS	Serum, Spleen of offspring	IL-1β	ELISA
Li et al.	2024	Rat	8	Unpredictable mild stress	GD: 1-21	Crowding (24 h), Shaking (30 min), Humid sawdust (24 h), Cage tilting (24 h), Restraint (2 h), Fasting and water deprivation (24 h), Swimming in ice water (5 min) TD: 295.75 h	None	Serum of offspring	IL-6, TNF-α, TGF-β	ELISA
Lopes et al.	2022	Rat	5	Social isolation stress	GD: 1-21	All times; TD: 504 h	None	Uterus of offspring	IL-1β, IL-6	Magnetic bead-based assay
Mkhize et al.	2017	Rat	3	Restraint	GD: 14-20	1 h/day; TD: 7 h	None, Febrile seizures	Hippocampus of offspring	IL-1β, TNF-α, IL-6, IL-10	Magnetic bead-based assay, ELISA

Study	Year	Species	No. Of subjects/ Group	Prenatal stress protocol	Prenatal stress period	Prenatal stress duration	Offspring challenge	Analyzed sample	Outcome data	Method of measurement
Nemati et al.	2020	Rat	10	Restraint	GD: 13-21	1 h /day; TD: 9 h	None	Hippocampus of offspring	IL-10, IL-1β, IL-6, TNF-α	ELISA
Pascuan et al.	2017	Mouse	38	Restraint	GD: 15-20	2 h /day: TD: 10 h	None	Lymphocyte of offspring	IL-4, IL-10, IFN-ϒ	ELISA
Pavlov et al.	2023	Mouse	5	Ultrasound (20-45 Hz, 50 ± 5 db)	GD: 1-21	24 h/day TD: 504 h	LPS	Serum of offspring	Ifnγ, IL-1β, IL4, IL-5, IL-6, IL-10, TNF-α	ELISA
Pincus-Knackstedt et al.	2006	Mouse	5-15	Sound (70 db, 300 Hz)	GD: 12-14	24 h/day TD: 72 h	Asthma induction with ovalbumin	Serum, Lymphocytes of offspring	IL-4, IL-5, TNF-α, IFN-ϒ	ELISA
Qulu et al.	2012	Rat	6	Restraint	GD: 14-20	45 min; 3x/day; TD: 15.75 h	None, febrile seizures induction	Serum of offspring	IL-1β	ELISA
Qulu et al.	2016	Rat	6	Restraint	GD: 14-20	45 min; 3x/day; TD: 15.75 h	None, febrile seizures induction	Serum of offspring	IL-1β	ELISA
Slusarczyk et al.	2015	Rat	6	Restraint under bright light (150W)	GD: 14-21	45 min; 1x/day; TD: 6 h	None	Hippocampus, Frontal cortex of offspring	IL-1β, IL-18, TNF-α, IL-6	ELISA
Slusarczyk et al.	2016	Rat	6	Restraint under bright light (150W)	GD: 14-21	45 min; 3x/day; TD: 18 h	None	Hippocampus, Frontal cortex of offspring	IL-1β, IL-18, TNF-α, IL-6	ELISA
Sun et al.	2021	Rat	6	Restraint	GD: 14-20	45 min; 3x/day; TD: 15.75 h	None	Hippocampus	IL-1β	Western blot
Suwaluk et al.	2022	Rat	6	Restraint	GD: 14-20	4 h /day; TD: 28 h	None	Medial prefrontal cortex	IL-6	Western blot
Szczesny et al.	2014	Rat	6-8	Restraint under bright light (150 W)	GD: 14-21	45 min; 3x/day; TD: 18 h	None, LPS	Hippocampus, Frontal cortex of offspring	IL-1β, IL-6, IL-10, TNF-α, IFN-γ	ELISA
Trojan et al.	2019	Rat	5-6	Restraint under bright light (150 W)	GD: 14-21	45 min; 3x/day; TD: 18 h	None	Hippocampus, Frontal cortex of offspring	IL-1β, IL-18	ELISA
Trojan et al.	2023	Mouse	3-4	Restraint	GD: 12.5-18.5	45 min; 1x/day; TD: 5.25 h	None	Hippocampus, Frontal cortex of offspring	TNF-α, IL-6	ELISA

(n = 11) applied a challenge in at least one part of their experiments. These challenges included the injection of lipopolysaccharide (LPS) as a bacterial toxin (n = 3), the injection of LPS + kainic acid inducing febrile seizures (n = 3), the administration of ovalbumin inducing asthma (n = 3), in vitro using a T-cell-specific mitogen (PHA, n = 1), and the use of apolipoprotein E-deficient dams to develop atherosclerosis (n = 1). 84.6% of the studies (n = 22) using the ELISA method to measure cytokine proteins, followed by 15.4% of the studies (n = 4) using western blotting and 11.5% (n = 3) using magnetic bead-based assay. There is a mismatch between the overall number of studies and the stated percentages due to the inclusion of research that has assessed several data points.

3.3. Risk of bias assessment

The SYRCLE Risk of Bias tool (Fig. 2) was used to evaluate the risk of bias. Regarding selection bias, which encompasses items of sequence generation, baseline characteristics, and allocation concealment, the baseline characteristics were scored as "yes" for most eligible studies (n = 21). In relation to performance bias including items of random housing and performance blinding, no study scored "no" in two items, while the remaining highest score was "unclear" (18 and 22 studies for two items, respectively). Also, when it comes to detection bias, including items of random outcome assessment and blinding, most studies took a score of "unclear" (n = 20 and 21 for two items, respectively). Most included studies scored "yes" on attrition bias (i.e., incomplete outcome data) (n = 22) and reporting bias (i.e., selective outcome reporting) (n = 17). Finally, most studies scored (n = 16) "unclear" on items regarding other biases.

3.4. Publication bias

According to the Begg & Mazumdar adjusted rank correlation test, no effect of publication bias was observed for IFN-ϒ (P = 0.088), IL-4 (P = 0.493), and IL-5 (P = 0.805). Moreover, the Egger regression asymmetry test demonstrated no sign of publication bias for IFN-ϒ (P = 0.101), IL-4 (P = 0.935), and IL-5 (P = 0.557). The mentioned tests evidenced publication bias in IL-1β, IL-6, IL-10, IL-18, and TNF-α. The presence of publication bias suggests a potential overestimation of the effect size. Therefore, the trim-and-fill method was applied, which corrected asymmetry by adding many studies (or data points) for each cytokine (2 studies for IL-1β, IL-18, and TNF-α; 1 study for IL-6 and IL-10).

Study	Year	Species	No. Of subjects/ Group	Prenatal stress protocol	Prenatal stress period	Prenatal stress duration	Offspring challenge	Analyzed sample	Outcome data	Method of measurement
Vanbesien-Mailliot et al.	2007	Rat	8	Restraint	GD: 11-21	45 min; 3x/day; TD: 24.75 h	PHA	Peripheral blood mononuclear cells of offspring	IFN-ϒ	ELISA
Vargas et al.	2016	Mouse	6	Restraint	GD: 7-21 GD: 14-21	30 min; 1x/day; TD1: 7.5 h TD2: 4 h	Asthma induction with ovalbumin	Bronchoalveolar lavage	IL-4, IL-5, IL13, IL-10, INF-ϒ	Magnetic bead-based assay
Zheng et al.	2019	Rat	3-8	Restraint	GD: 14-20	45 min; 3x/day; TD: 15.75 h	None	Hippocampus of offspring	IL-1β, IL-6, IL-10, TNF-α,	ELISA, western blot

ELISA, enzyme-linked immunosorbent assay; GD, gestation days; IFN, Interferon; IL, interleukin; PHA, phytohaemagglutinin; TNF, tumor necrosis factor; TD, total duration.

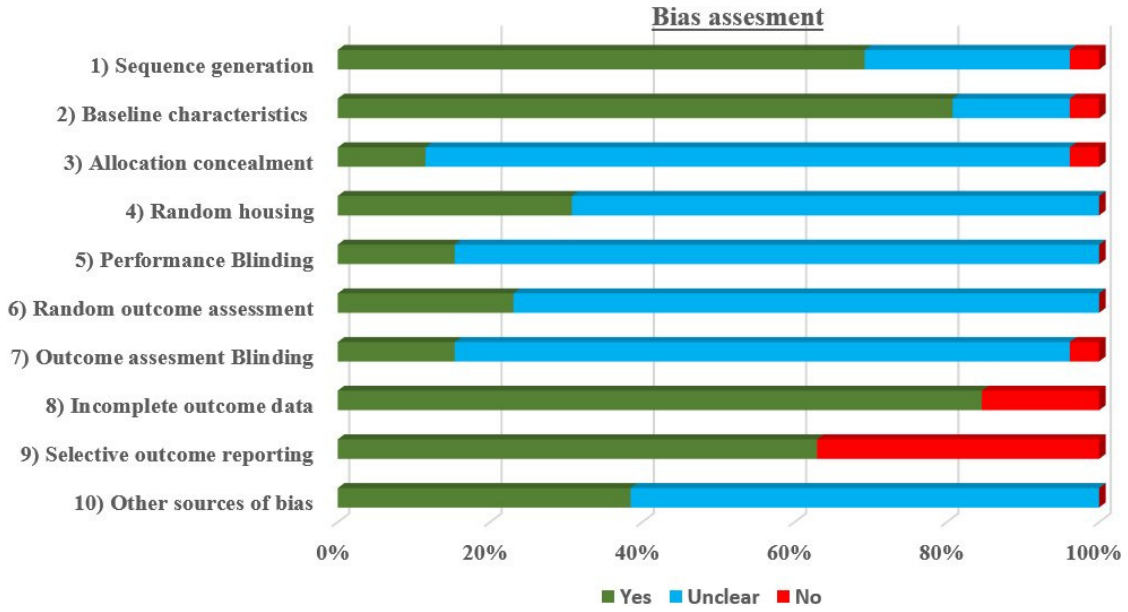

Fig. 2. Flow diagram of study identification, inclusion, and exclusion.

3.5. Effect of prenatal stress on cytokine profile

The overall analysis of cytokines is presented in Table 2. The random effect model of analysis revealed significant elevations in levels of IFN-ϒ, IL-6, IL-18, and TNF-α and a reduction in the level of IL-10 following prenatal stress in the fetus and offspring. The changes in IL-4 and IL-5 levels were not significant. Sensitivity analysis revealed that many data points in studies of Pascuan et al. (2017) and Vargas et al. (2016) were the sources of heterogeneity for IFN-ϒ. After excluding the aforementioned data points, the SMD of this cytokine was significant, and its heterogeneity was decreased to a low level. However, high levels of heterogeneity were detected for IL-4, IL-5, IL-6, IL-18, and TNF-α; moderate levels for IL-1β and IL-10; and low levels for IL-4 according to the results of I^2 index and Cochrane Q test. The sensitivity analyses for these cytokines failed to identify any study that contributed to the observed heterogeneity.

Table 2. Effect of prenatal stress on cytokine profile

Outcome	No. of studies	No. of data points	Effect size (95% CI)	P value	I^2 (%)	Q-statistics (P)
IFN-ϒ	8	13	0.27 (0.02 to 0.57)	0.038	0.0	0.705
IL-1β	15	42	0.95 (0.53 to 1.36)	< 0.001	73.4	< 0.001
IL-6	12	29	0.76 (0.07 to 1.45)	0.031	82.2	< 0.001
IL-18	5	13	1.89 (1.09 to 2.69)	< 0.001	86.3	< 0.001
TNF-α	11	27	0.92 (0.29 to 1.55)	0.004	84.1	< 0.001
IL-10	8	24	-0.41 (-0.81 to -0.01)	0.042	73.1	< 0.001
IL-4	5	12	0.42 (-0.34 to 1.19)	0.279	0.0	0.998
IL-5	4	8	0.29 (-0.20 to 0.76)	0.251	80.8	< 0.001

3.6. Analyses of subgroups

Table 3 presents the results of subgroup analyses, which include the total duration of prenatal stress, type of species, and offspring challenge. The study

revealed that most of the cytokines (IL-1β, IL-6, IL-18, and TNF-α) significantly increased in the short-term of prenatal stress (≤ 24 hours) except for IL-10, which decreased in long-term stress ($P < 0.05$).

Table 3. Effect of prenatal stress on cytokines based on subgroup analyses

Outcome	Variables	No. of data points	Effect size (95% CI)	P value	I^2 (%)	Q-statistics (P)
IFN-ϒ	**Total time of prenatal stress**					
	≤ 24 hours	8	0.19 (-0.11, 0.50)	0.211	0.0	0.469
	> 24 hours	5	0.45 (-0.02, 0.92)	0.059	0.0	0.818
	Offspring challenge					
	No	3	0.36 (-1.99, 0.919)	0.207	29.9	0.240
	Yes	10	0.29 (-0.043, 0.63)	0.088	0.0	0.732
	Type of rodent					
	Rat	5	0.67 (0.19, 1.16)	0.006	0.0	0.89
	Mouse	8	0.11 (-0.19, 0.41)	0.456	0.0	0.76
IL-1β	**Total time of prenatal stress**					
	< 24 hours	31	1.39 (0.84, 1.94)	< 0.001	74.6	< 0.001
	> 24 hours	11	0.04 (-0.40, 0.48)	0.866	42.8	0.064
	Offspring challenge					
	No	32	0.96 (0.44, 1.49)	< 0.001	78.0	< 0.001
	Yes	10	0.97 (0.53, 1.40)	< 0.001	3.5	0.408
			Type of rodent			
	Rat	25	1.72 (0.98, 2.45)	< 0.001	80.4	< 0.001
	Mouse	17	0.20 (-0.07, 0.48)	0.151	0.0	0.575
IL-4	**Total time of prenatal stress**					
	≤ 24 hours	8	0.40 (-0.79, 1.58)	0.512	90.9	< 0.001
	> 24 hours	4	0.63 (-0.13, 1.40)	0.104	63.1	0.043
	Offspring challenge					
	No	3	1.07 (-0.54, 2.67)	0.193	94.1	< 0.001
	Yes	9	0.18 (-0.75, 1.11)	0.704	82.6	< 0.001
	Type of rodent					
	Rat	0	-	-	-	-
	Mouse	12	0.42 (-0.34, 1.18)	0.279	87.2	< 0.001
IL-5	**Total time of prenatal stress**					
	≤ 24 hours	6	0.10 (-463, 0.654)	0.738	83.4	< 0.001
	> 24 hours	2	0.99 (-0.07, 2.05)	0.068	76.8	0.038
	Offspring challenge					
	No	1	2.77 (0.78, 4.77)	0.006	0.0	-
	Yes	7	-0.13 (-1.29, 1.03)	0.825	80.1	< 0.001

Outcome	Variables	No. of data points	Effect size (95% CI)	P value	I^2 (%)	Q-statistics (P)
	Type of rodent					
	Rat	0	-	-	-	-
	Mouse	8	0.17 (-0.98, 1.32)	0.770	80.8	< 0.001
IL-6	**Total time of prenatal stress**					
	≤ 24 hours	24	0.84 (0.10, 1.59)	0.027	82.1	< 0.001
	> 24 hours	5	0.76 (0.07, 1.45)	0.953	85.5	< 0.001
	Offspring challenge					
	No	25	0.98 (0.25, 1.71)	0.008	81.7	< 0.001
	Yes	4	-1.11 (-3.45, 1.22)	0.350	84.0	< 0.001
	Type of rodent					
	Rat	22	0.80 (0.00, 1.60)	0.050	83.6	< 0.001
	Mouse	7	0.64 (-0.81, 2.09)	0.386	78.2	< 0.001
IL-10	**Total time of prenatal stress**					
	≤ 24 hours	15	-0.60 (-1.27, 0.08)	0.081	81.7	< 0.001
	> 24 hours	9	-0.41 (-0.76, -0.07)	0.019	0.0	0.743
	Offspring challenge					
	No	20	-0.60 (-1.07, -0.13)	0.013	75.5	<0.001
	Yes	4	0.06 (-0.91, 1.03)	0.903	55.5	0.083
	Type of rodent					
	Rat	9	-1.40 (-2.96, 0.16)	0.079	87.3	< 0.001
	Mouse	15	-0.19 (-0.47, 0.09)	0.179	30.0	0.130
IL-18	**Total time of prenatal stress**					
	≤ 24 hours	13	1.89 (1.09, 2.69)	< 0.001	86.3	< 0.001
	> 24 hours	0	-	-	-	-
	Offspring challenge					
	No	13	1.89 (1.09, 2.69)	< 0.001	86.3	< 0.001
	Yes	0	-	-	-	-
	Type of rodent					
	Rat	13	1.89 (1.09, 2.69)	< 0.001	86.3	< 0.001
	Mouse	0	-	-	-	-
TNF-α	**Total time of prenatal stress**					
	≤ 24 hours	18	1.69 (0.82, 1.56)	< 0.001	78.9	< 0.001
	> 24 hours	9	-0.16 (-0.98, 0.65)	0.696	85.4	< 0.001
	Offspring challenge					
	No	16	1.82 (0.82, 2.82)	< 0.001	80.5	< 0.001
	Yes	11	- 0.12 (-0.73, 0.50)	0.711	76.6	< 0.001
	Type of rodent					
	Rat	13	1.98 (0.87, 3.09)	< 0.001	82.8	< 0.001
	Mouse	14	0.15 (-0.53, 0.83)	0.664	80.5	< 0.001

IFN, Interferon; IL, interleukin; TNF, tumor necrosis factor.

The results of meta-analysis showed that challenges in offspring following prenatal stress had no significant effect on levels of IL-10, IL-5, IL-6, and TNF-α, while these cytokines changed in the prenatal stress-induced offspring without any challenge ($P < 0.05$). On the other hand, the results of changes in IL-1β, IFN-ϒ, and IL-4 following prenatal stress were not influenced by the absence or presence of challenge.

The subgroup meta-analysis of type of rodent indicated that significant changes in most cytokines (IFN-ϒ, IL-1β, Il-6, and TNF-α) only occurred in the rat fetus and offspring following prenatal stress. In contrast, only IL-10 significantly changed in both rats and mice.

4. Discussion

In the present study, we investigated the impact of prenatal stress on the cytokine profile of rodent fetuses or offspring. Specifically, we focused on these consequences. Our study found that prenatal stress significantly increased the levels of pro-inflammatory cytokines IL-1β, IL-6, IL-18, IFN-ϒ, and TNF-α, while also decreasing the levels of anti-inflammatory cytokine IL-10 in various tissues of the offspring, including the brain, lymphocytes, bronchi, and uterus. Cytokines play a crucial role in modulating inflammatory processes, which are closely linked to the preservation of cell integrity that they are responsible for. If unchecked, elevated pro-inflammatory and reduced anti-inflammatory cytokines can be harmful. This may lead to chronic inflammation and exacerbate cellular damage (42, 43). Considering the substantial role that the immune system plays in the genesis of inflammation under this sort of stress, the fact that our research found that prenatal stress caused a rise in five pro-inflammatory cytokines and a drop in one anti-inflammatory cytokine is intriguing data.

The findings of our study showed that there is a significant disparity in the immunological responses and the majority of cytokine secretions that occur in the offspring of rats and mice in response to prenatal stress. It has previously been observed that there are variations of this sort (44, 45). On the other hand, the appropriate selection of species in immunological investigations may be the primary factor determining the outcomes. It appears that the immune system of rat offspring reacts to prenatal stress by secreting cytokines more prominently.

Short-term induction of prenatal stress was shown to be more effective in enhancing cytokine release than long-term induction of prenatal stress, according to the current meta-analysis. Acute or short-term stress experienced at the time of immune activation can enhance innate and adaptive immune responses. On the other hand, chronic or long-term stress has been shown to suppress immunity by reducing the number of immune cells and their function, as well as by increasing the number of immunosuppressive mechanisms that are active (46). However, our findings determined that the duration of prenatal stress may have a significant influence on immunological tests; moreover, this parameter may be an essential ethical consideration in reducing the rate of animal suffering.

Many eligible studies in the present meta-analysis applied different challenges in the offspring following prenatal stress. Our findings demonstrated that the challenges do not affect the immune responses by increasing the release of cytokines. The findings of Kemme et al. (47), which are in keeping with these results, indicate that prenatal stress has an effect on postnatal resilience and that prenatal stress does not hinder the ability to deal with problems later in life. Based on their findings, endocrine responses that occur later in life reflect usual responses to difficult circumstances. According to Serpeloni et al. (48), prenatal stress influences postnatal resilience through epigenetic changes. These changes include an increase in methylation in genes that code for the glucocorticoid receptor and its repressor, which indicates an enhanced ability to terminate hormonal stress responses in children who were prenatally stressed. These epigenetic alterations, on the other hand, may take place for cytokine genes and cause changes in the expression of those genes (49).

Publication bias was found in the majority of the meta-analyses that we conducted. As previously stated (50), the reason for this is the failure to disclose the results of research depending on the direction or strength of the study's findings. This suggests that only research with statistically significant positive outcomes is published, whereas studies with statistically insignificant or negative findings are not published. Possible reasons contributing to the risk of publishing bias include rejection by editors and reviewers, lack of interest in revising the article, conflicting interests, and a lack of enthusiasm to write despite doing the research.

While this study offers a thorough analysis of

cytokine levels related to prenatal stress, it is important to acknowledge certain limitations. These include potential publication bias, insufficient heterogeneity across different stress paradigms, and a lack of adequate data for analyzing variables such as sex and age.

Conclusion

In conclusion, prenatal stress may alter levels of pro-inflammatory (IFN-ϒ, IL-1β, IL-6, IL-18, and TNF-α) and anti-inflammatory (IL-10) cytokines. This study also suggests, based on subgroup analyses, that these alterations were more apparent in the rat species and short-term stress. Challenges following prenatal stress did not have predominant effects on most cytokine levels.

Acknowledgment

The authors are grateful for the support of the Research Vice-chancellorship of Shahrekord University (Shahrekord, Iran).

Compliance with ethical standards

Not applecible

Conflict of interest

The authors declare that they have no competing interest.

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