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Systematic Review

In Vivo Studies Exploring the Impact of IUGR in Rat Models on Offspring Malnutrition: A Systematic Review

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ABSTRACT

Introduction: Intrauterine growth restriction (IUGR), stemming from inadequate fetal nutrition due to maternal or placental factors, affects 10–15% of pregnancies worldwide. Animal models reflect human IUGR, showing malnutrition-associated outcomes like impaired growth, reduced body weight, delayed puberty, and disruptions in genetic and hormonal processes. Despite extensive research, gaps persist in understanding how IUGR impacts fetal outcomes, particularly regarding malnutrition-related outcomes.

Material and Methods: The literature search was conducted in four databases. Several outcomes were analyzed, including the timing of puberty, weight and body composition, and organ development, which represented the IUGR rat's malnutrition condition. Throughout the identification process, 11 articles were included in the systematic review.

Results: Research consistently demonstrates that IUGR in rat models results in delayed puberty, characterized by delayed vaginal opening and lower body weights compared to controls across early and later developmental stages. Additionally, IUGR adversely affects organ development, with studies showing reduced brain weights, diminished ovarian and mammary gland development, and altered sizes of reproductive organs. Furthermore, IUGR disrupts hormonal and genetic profiles, as evidenced by changes in ovarian follicle counts, altered gene expressions related to testicular development, and dysregulated metabolic pathways. All malnutrition-related outcomes significantly differed between IUGR and control rats ($p < 0.05$).

Conclusion: IUGR in rat models consistently induces developmental delays and physiological impairments in offspring, characterized by delayed puberty, reduced body weights, and compromised organ development, highlighting the adverse consequences of malnutrition associated with IUGR.

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INTRODUCTION

Intrauterine growth restriction (IUGR) manifests as abnormal fetal development in adverse prenatal conditions primarily caused by inadequate fetal nutrition, whether originating from maternal factors or placental issues [1]. This condition affects 10–15% of pregnancies globally [2]. It stems from the fetus's inability to achieve its genetic growth potential due to detrimental prenatal environments, resulting in a reduced fetal growth rate after the first trimester. The primary clinical indicator of IUGR is a low birth weight below the third or tenth percentile adjusted for gestational age and gender, often accompanied by abnormal cord blood flow observed in Doppler studies [3]. IUGR represents an initial stressor that unfavorably influences fetal developmental programming, heightening vulnerability to subsequent health challenges triggered by postnatal factors such as infection, stress, and, notably, malnutrition [4–6]. Given the diverse origins of IUGR in humans, numerous animal models have been established to investigate its effects on organ development and the onset of adult diseases.

Numerous clinical and experimental studies demonstrate that adverse uterine and early neonatal

environments profoundly alter development and increase the risk of lifelong health complications [7–9]. Experimental models replicating inadequate maternal conditions include corticosteroid exposure, bilateral uterine artery ligation to reduce blood flow, chronic hypertension, protein malnutrition, and uncontrolled type 1 diabetes mellitus [10–12]. For instance, rat models induced with streptozotocin-induced diabetes show that high maternal blood glucose levels (>300 mg/dL) lead to offspring born small for gestational age (SPA) due to intrauterine growth restriction (IUGR) [4,13]. Malnutrition in these models manifests as impaired growth, reduced body weight, delayed puberty, delayed organ development, and disruptions in genetic and hormonal processes [10,14,15]. Research by Baysal et al. (2022) using an IUGR rat model revealed significantly lower body and brain weights in IUGR infants than controls. Similarly, Engelbregt et al. (2004), using a Wistar rat model with uterine artery ligation-induced IUGR, found significantly lower body weights in IUGR rats just two days after birth, along with delayed vaginal opening and reduced testes weight at five days postpartum [16–18].

Importantly, intrauterine growth restriction (IUGR) has been shown to potentially adversely affect organ development—during fetal stages for IUGR and in the early neonatal period for preterm infants. Despite this knowledge, the mechanisms underlying the impact of IUGR on fetal outcomes, particularly regarding malnutrition, remain incompletely understood. Furthermore, there is a noticeable absence of updated systematic reviews that comprehensively evaluate how IUGR in rat models influences offspring malnutrition. Critical gaps persist in understanding the effects of IUGR on organ development, puberty timing, body weight regulation, and intricate genetic and hormonal profiles. To address these gaps, we recently conducted a systematic review to explore the induction of IUGR in rat models and its correlation with various malnutrition-related outcomes.

MATERIAL AND METHODS

This review was based on Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines.

Eligibility Criteria

Inclusion and exclusion criteria were decided upon before the literature search to improve the review's specificity. Literature search and screening were performed in duplicate by group, starting with the screening on the title and abstract. Subsequently, eligible articles were screened for final inclusion based on their full text. In case of disagreement, a third independent investigator was consulted. In vivo studies using IUGR rats as an animal model and their impact on malnutrition status were the predefined criteria analyzed in this comprehensive review.

Mainly, inclusion criteria were (1) studies performed in mammals, (2) proven restricted birth weight in IUGR offspring, (3) a control group of typical growth peers, (4) reporting on malnutrition status, and (5) timing puberty, weight and body composition, and organ development were set as the primary outcome. Meanwhile, the exclusion criteria were adopted: 1) irrelevant to the study's aim, 2) in vitro studies, and 3) non-English studies and grey literature.

Search Strategy

From Mei 22 to June 20, 2024, researchers searched the literature using numerous databases such as ScienceDirect, PubMed, SpringerLink, and Google Scholar Journal. The keywords used ("Rat" OR "Mice" OR "Mouse") AND ("IUGR" OR "Intrauterine Growth Restriction" OR "FGR" OR "Fetal Growth Restriction" OR "growth restricted") AND ("malnutrition" OR "malnourished" OR "undernutrition")).

Data Extraction and Analysis

In these reviews, the chosen studies were analyzed descriptively by authors in a Google Sheet, and then other authors evaluated the studies' correctness eligibility, oversaw the process, and documented them. Discussions were used to settle disagreements that arose during the writing process.

Risk of Bias Assessment

Assessment of study quality is conducted using the SYRCLE's tool to assess the risk of bias in the chosen studies. The other writers oversaw the procedure. The instrument has nine domains: randomization, baseline comparability, allocation concealment, random housing of the animals, blinding investigators, outcome assessment, outcome assessor blinding, incomplete outcome data, selective reporting, and other sources of bias. The domains were split into three categories based on the study's quality: low, moderate, and high risk of bias.

Outcome of Interest

This review focused on the timing of puberty, weight and body composition, and organ development, which represent the malnutrition condition of IUGR rats in each of the included studies. Meanwhile, gene expression and hormonal profiles were analyzed as the secondary outcomes. Outcomes were analyzed descriptively due to the high variation of data to enhance this review's comprehensiveness and thoroughly

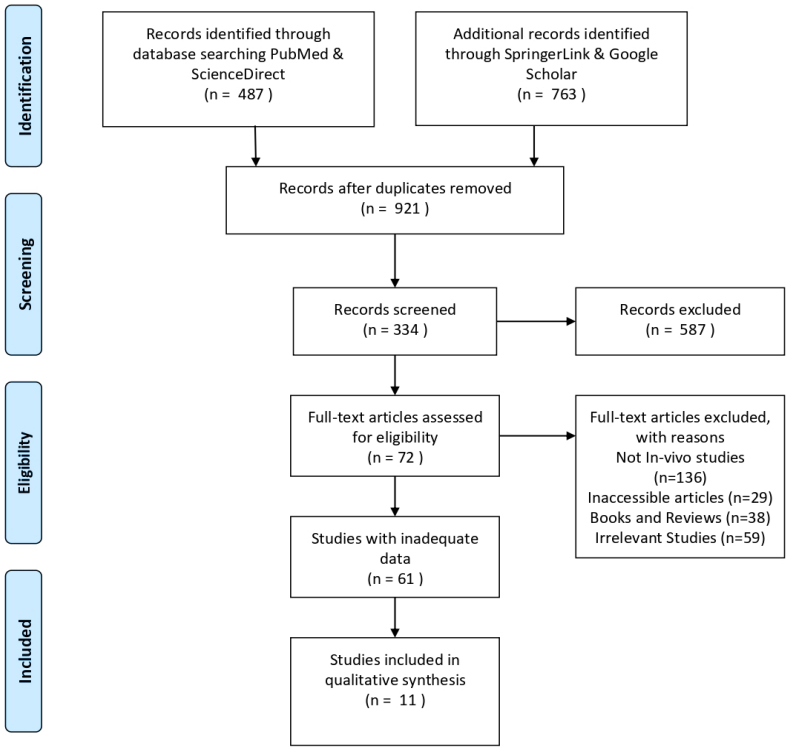


Fig. 1. PRISMA Flowchart

elucidate the correlation of IUGR as a primary contributor factor for malnutrition.

RESULTS

Study Selection and Identification

A comprehensive literature search identified 1250 articles published across four databases. Of these, 329 duplicates were removed. An additional 849 articles were excluded due to ineligibility, including review articles, books, protocol studies, and inaccessible articles due to subscription constraints. Furthermore, 61 articles did not meet the predefined inclusion criteria due to inadequate data. As depicted in Fig. 1 of the PRISMA flowchart, these exclusions led to the inclusion of 11 articles in the systematic review for qualitative analysis.

Risk of Bias Assessment

Based on the risk of bias evaluation, only four studies were assessed to have a moderate risk of bias due to insufficiently detailed methodological descriptions in the first, fifth, eighth, and tenth domains of the SYRCLE's Tool. The remaining studies (n=7) were considered to have a low risk of bias (Table 2). Despite varying levels of bias across the included studies, comprehensive data were adequate for the study's analysis.

Summaries of the Included Studies

This review includes 11 in vivo studies conducted on Wistar and Sprague-Dawley rats to investigate intrauterine growth restriction (IUGR). Various methods were employed to induce IUGR, such as maternal low protein/restricted diets, lipopolysaccharide, dexamethasone, Pregnant mare's serum gonadotrophin (PMSG) serum injections, and uterine artery ligation [6,15,19]. The studies aimed to assess the effects of IUGR on development and physiological outcomes in offspring, including body weight regulation, organ development, puberty timing, and genetic and hormonal profiles. The total sample size across all studies was 210 animals, with interventions to induce IUGR typically involving dietary restrictions or pharmacological treatments administered during pregnancy. Each study contributed unique insights into the consequences of IUGR, providing valuable data for understanding developmental outcomes in rat models. Table 1 presents an overview of the included studies.

Impact of IUGR on Malnutrition Shown by Delayed Puberty Time and Lower Body Weight

The impact of intrauterine growth restriction (IUGR) on developmental outcomes, particularly delayed puberty and lower body weight, has been extensively studied using rat models. For instance, Baysal et al. (2022) reported that Wistar rats with IUGR exhibited delayed vaginal opening compared to controls (IUGR: 33.5 ± 0.9 days vs. Control: 30.2 ± 0.5 days, $p < 0.05$) [20]. Additionally, these IUGR rats consistently showed lower body weights throughout early development (IUGR vs. Control at day 21: 53% lower, $p < 0.001$) and into later stages (IUGR vs. Control at weaning: $p < 0.01$). Similarly, Engelbregt et al. (2002) observed delayed vaginal opening in IUGR rats compared to controls ($p < 0.05$), along with significantly lower body weights persisting from birth through weaning ($p < 0.01$) [12,21].

Further supporting evidence from Beinder et al. (2014) showed that rats subjected to maternal low-protein diets inducing IUGR displayed delayed development of mammary ducto-alveolar structures compared to controls ($p = 0.02$) [22]. Meanwhile, Faria et al. (2003) demonstrated delayed vaginal opening in rats exposed to protein-restricted diets (IUGR: 33.5 ± 0.9 days vs. Control: 30.2 ± 0.5 days, $p < 0.05$), accompanied by consistently lower body weights across developmental stages (PR vs. Control at day 21: 53% lower, $p < 0.001$) [23]. The impact of IUGR on puberty and body weight was detailed across multiple studies, revealing consistent delays in puberty (e.g., delayed vaginal opening) and lower body weights in affected rodents compared to controls provided in Table 1.

Impact of IUGR on Malnutrition Shown by Delayed Organ Development

Several studies have evidenced that intrauterine growth restriction (IUGR) profoundly influences organ development. Baysal et al. (2022) revealed that rats with IUGR exhibited significantly lower brain weights at postnatal day 7 (PN7) compared to controls ($p < 0.05$) [20]. However, brain-to-body weight ratios did not differ substantially between protein diet groups and controls by PN35. Furthermore, Engelbregt et al. (2002) reported diminished ovarian weights in IUGR rats at euthanasia ($p < 0.001$), indicating impaired ovarian development [21]. Binder et al. (2014) observed reduced ducto-alveolar structures in the mammary fat pads of IUGR rats at day 28 ($p = 0.02$), suggesting compromised mammary gland development due to maternal low-protein diets. Additionally, Faria et al. (2003) documented decreased uterine weights in IUGR groups ($p < 0.001$), indicating delayed reproductive maturation [21]. Valverde et al. (2015) found significantly altered relative heart weights ($p < 0.05$) and trends toward reduced kidney sizes in male IUGR offspring, reflecting altered cardiovascular and renal development [5]. Importantly, Pampanini et al. (2017) reported significantly reduced testes weights in IUGR rats at five days postpartum compared to sham-operated rats (5.9 ± 1.2 vs 10.8 ± 1.9 mg, $p < 0.001$), along with alterations in testicular gene expression and hormone levels, highlighting disruptions in testicular development due to early growth restriction [10]. Together, these studies underscore the diverse and significant impacts of IUGR on organ development, emphasizing the critical role of maternal nutrition in ensuring optimal fetal growth and maturation of vital organs.

Impact of IUGR on Malnutrition Shown by Genetic and Hormonal Profiles

Studies on rats with intrauterine growth restriction (IUGR) reveal distinct hormonal and genetic differences compared to normal controls. Engelbregt et al. (2002) demonstrated that IUGR rats induced by PMSG injection exhibited significantly fewer ovarian follicles: primordial ($p < 0.05$), growing ($p < 0.01$), and antral follicles ($p < 0.01$), with no difference in corpora lutea numbers [21]. Pampanini et al. (2017) identified altered testicular gene expression in IUGR rats, including upregulation of citrate synthase (Cs) and downregulation of lactate dehydrogenase genes (Ldh-a, Ldh-b, Ldh-c) at five days postpartum ($p < 0.02$). By 40 days postpartum (dpp), these rats exhibited increased follicle-stimulating hormone receptor (Fshr) expression ($p < 0.05$) and decreased Kit ligand (Kitl) expression ($p < 0.02$), suggesting disrupted Sertoli and Leydig cell function. Shen et al. (2022) identified 815 differentially expressed genes in IUGR rats, notably upregulating Ppargc1a and downregulating Rxrg (fold-change >1.5, $p < 0.05$), implicating metabolic and signaling pathway dysregulation [24]. Additionally, Beinder et al. (2014) demonstrated that rats exposed to a maternal low protein (LP) diet-induced IUGR exhibited reduced duct-alveolar expansion in the mammary fat pad at day 28 ($p = 0.02$) and lower terminal end bud (TEB) proliferative activity at days 21 (34% lower) and 28 (49% lower), alongside higher serum progesterone levels ($p = 0.0023$) and elevated serum deoxycorticosterone levels ($p = 0.01$) at day 28, indicating hormonal dysregulation and impaired mammary gland development in IUGR conditions.

DISCUSSION

This review examines 11 in vivo studies on Wistar and Sprague-Dawley rats investigating intrauterine growth restriction (IUGR). Various methods, such as maternal low-protein diets, lipopolysaccharide administration, dexamethasone treatment, PMSG injections, and uterine artery ligation, were used to induce IUGR [12,20,25,26] These reviews evaluated how IUGR impacts offspring development based on

maturity, body weight, and organ development. These studies involved two hundred ten animals, with interventions typically involving dietary or pharmacological treatments during pregnancy. Each study provided unique insights into the consequences of IUGR in rat models, revealing consistent findings of delayed puberty marked by delayed vaginal opening and lower body weights compared to controls across various developmental stages. Additionally, IUGR was found to impair organ development, including reduced brain size, diminished growth of ovarian and mammary glands, and altered sizes of reproductive organs. Furthermore, IUGR disrupted hormonal and genetic profiles, affecting ovarian follicle counts, gene expressions related to testicular development, and metabolic pathways [9,12,18,24].

In epidemiological terms, intrauterine growth restriction (IUGR) refers to infants born with a birth weight below the third percentile adjusted for gestational age and gender, often accompanied by abnormal cord blood flow detected through Doppler studies [27,28]. This condition arises from maternal undernutrition, high-altitude living, or placental insufficiency, leading to inadequate delivery of nutrients and oxygen to the fetus. Complex biological mechanisms underlying IUGR include reduced placental growth and vascularity, impaired function in transporting amino acids and glucose, oxidative stress in the placenta and conceptus, decreased hemoglobin levels due to iron deficiency impacting oxygen transport, and impaired cell signaling for protein synthesis regulation in both placenta and fetus [29]. Recent studies also emphasize the roles of nitric oxide (NO) and polyamines, suggesting potential interventions like L-arginine supplementation to enhance fetal outcomes in cases of IUGR by increasing NO bioavailability in the vasculature and improving placentation [13,30].

Placental transport capacity, crucial for nutrient transfer from mother to fetus, relies on factors such as the expansive fetal villous trophoblast placental surface area and the enhanced concentration and affinity of transport proteins for glucose, amino acids, and fatty acids [13,31–33]. By the second trimester, a significant portion of maternal oxygen and glucose entering the uterus is consumed by these transport activities, leaving only a fraction available for fetal nutrition. As pregnancy progresses towards term, the proportion of nutrients available for fetal use steadily increases. However, vascular changes in the placenta could alter substrate supply to the fetus [14,28,34]. In pregnancies affected by intrauterine growth restriction (IUGR), disruptions in both fetal-maternal substrate transfer and fetal-placental metabolism are observed, often shifting towards increased catabolism. These changes have profound implications, impacting cardiovascular, metabolic, and neurological development in the fetus, with potential long-term effects extending into adulthood [3,20].

Alisjahbana et al. (2019) conducted a study in Indonesia that underscored significantly higher infant mortality rates among those born with IUGR, particularly within the first 90 days of life. Their findings emphasize the importance of using birth weight and birth length to identify at-risk infants and advocating for nutritional support during the critical first 1000 days of life to mitigate high morbidity rates associated with adverse environmental conditions [35]. Long-term studies by Barker et al. in southern UK cohorts have established links between lower birth weights due to IUGR and increased risks of mortality from coronary disease, along with a higher prevalence of metabolic syndrome in adulthood [1,5,36–38]. Similarly, a study by Valverde et al. in New Zealand highlighted the association between IUGR and elevated risks of hypertension and cardiovascular diseases in later life, underscoring the need for early detection and intervention strategies to improve long-term health outcomes in affected individuals [5,39–41].

Certain limitations should be acknowledged. Due to a lack of studies discussing the impact of IUGR on malnutrition in animal models, we included journals published more than ten years ago. Additionally, the number of animal studies may not be adequate, and there is heterogeneity in the interventions used to induce IUGR in rat models, as well as variability in the outcomes measured among these studies, precluding a meta-analysis and potentially introducing bias. Despite these limitations, the study convincingly demonstrated the impact of IUGR on developmental delays and physiological impairments in offspring. Further studies with larger samples and analysis in human trials are needed to understand the impact of IUGR on malnutrition comprehensively.

CONCLUSION

In summary, the reviewed studies on intrauterine growth restriction (IUGR) in rat models consistently reveal significant developmental delays and physiological impairments in offspring. These include delayed puberty, lower body weights, and compromised organ development such as reduced brain, ovarian, testes, and mammary gland growth, indicative of malnutrition conditions. Moreover, hormonal dysregulation and changes in gene expression patterns further emphasize the impact of IUGR. These findings underscore the profound influence of prenatal conditions, especially maternal nutrition, on the health outcomes of offspring. They highlight the critical need for early-life interventions to mitigate the long-term consequences of IUGR-induced malnutrition.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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Table 1. Summaries Included Studies

No	Study	Study Design	Sample	IUGR	Control	Manipulation	Primary Outcome	Secondary Outcome
1	Baysal et al. (2022) [20]	In Vivo	Wistar rats	24	12	animals were fed a 10% low protein diet and injected with 300 µg/kg intraperitoneal lipopolysaccharide.	At postnatal day 7 (PN7), infants affected by intrauterine growth restriction (IUGR) had notably reduced body and brain weights compared to the control group ($p < 0.05$). However, the brain-to-body weight ratios at PN7 were similar between the standard protein (SP) and high protein (HP) groups ($p = 0.9$), but both groups exhibited significantly lower ratios compared to the control group (SP vs. control: $p = 0.012$; HP vs. control: $p = 0.009$). By PN35, the SP group's brain-to-body weight ratios were comparable to those of the control group ($p = 0.271$).	Secondary outcomes revealed that the number of TUNEL-positive cells, indicating apoptosis, in the periventricular white matter was significantly elevated in both IUGR groups at PN7 and PN35 compared to controls ($p < 0.05$). Additionally, immunoreactivity for MBP and O4, markers of myelination, was significantly diminished in the IUGR groups at both time points ($p < 0.01$). These results suggest delayed neurodevelopment in infants with IUGR, marked by disrupted brain growth, heightened apoptosis, and impaired myelination compared to healthy controls.
2	Beinder et al. (2014) [22]	In Vivo	Wistar rats	8	8	IUGR induced by maternal low protein (LP) diet	On day 28, animals on a low protein diet (LP) exhibited a significantly smaller area of ducto-alveolar structures within the mammary fat pad compared to animals on a standard protein diet (NP) ($p = 0.02$). This indicates reduced ducto-alveolar expansion in LP animals relative to NP animals at this stage of development.	Animals on a low protein diet (LP) showed significantly reduced TEB proliferative activity compared to those on a standard protein diet (NP). Specifically, TEB proliferation was 34% lower at day 21 and approximately 49% lower at day 28 in LP animals versus NP animals. By day 28, LP animals also exhibited higher serum progesterone levels ($p = 0.0023$) and elevated serum deoxycorticosterone levels ($p = 0.01$), with a significant increase from day 21 to day 28 within the LP group ($p = 0.0389$). In contrast, NP animals experienced a 16% decrease in serum deoxycorticosterone levels from day 21 to day 28.
3	Faria et al. (2003) [23]	In Vivo	Wistar rats	9	9	IUGR induced by a protein-restricted diet containing 8% protein	Both the protein-restricted (PR) and energy-restricted (ER) groups showed consistently lower body weights compared to controls throughout the study (day 4 to day 40), with significant differences observed on day 21 (PR: 53% lower, ER: 42% lower) and day 40 (PR: 21% lower, ER: 19% lower) ($p < 0.001$). At day 21, PR animals also exhibited lower body weight and linear growth compared to the ER group ($p < 0.05$). At euthanasia, both PR and ER groups had significantly reduced uterine (PR: 42% lower, ER: 40% lower, $p < 0.001$) and ovarian (PR: 26% lower, ER: 19% lower, $p < 0.05$) weights compared to controls.	Vaginal opening was significantly delayed in both PR and ER groups compared to controls (Control: 30.2 ± 0.5 days; PR: 33.5 ± 0.9 days; ER: 32.6 ± 0.8 days, $p < 0.05$), indicating delayed sexual maturation in malnourished groups.

4	Engelbregt et al. (2002) [12]	In Vivo	Wistar rats	9	11	50 IU Pregnant mare's serum gonadotrophin (PMSG) was injected intraperitoneally in IUGR rats	Body weight in intrauterine growth-restricted (IUGR) rats was significantly lower than controls on day two after birth ($p < 0.01$) and remained significantly lower at weaning on day 24 ($p < 0.01$). At the vaginal opening, IUGR rats exhibited a delayed considerably age of maturation ($p < 0.05$) and lower body weight ($p < 0.05$) compared to controls.	Following stimulation with PMSG (pregnant mare serum gonadotropin) in the first cycle, IUGR rats had significantly fewer total follicles in the ovaries ($p < 0.01$) compared to controls. Specifically, the numbers of primordial ($p < 0.05$), growing ($p < 0.01$), and antral follicles ($p < 0.01$) were significantly lower in IUGR rats. However, the number of corpora lutea did not differ from that in controls.
5	Engelbregt et al. (2004) [21]	In Vivo	Wistar rats	10	12	IUGR was induced by bilateral ligation of the uterine arteries on day 17	IUGR rats showed lower body weight than controls by day 2 ($p < 0.01$) and maintained this deficit at weaning (day 24, $p < 0.01$). At the weaning and vaginal opening, both IUGR and FR rats had significantly lower body weights compared to controls ($p < 0.01$). IUGR rats also had delayed vaginal opening compared to controls ($p < 0.05$).	In IUGR rats, total bone mineral content adjusted for total body weight (TBBMC) was significantly lower than controls. However, no differences in total fat mass, percentage fat, or percentage lean were observed between the IUGR or FR rats and the controls.
6	Gereltsetseg et al (2012) [12]	In Vivo	Pregnant Sprague-Dawley Rats	9	8	dams received 50% of the daily food intake of the mNN group from embryonic day 15 until delivery. Maternal	Offspring in the mUN group, where dams received 50% of the daily food intake compared to the mNN group, exhibited significantly lighter body weights from day 16 through day 21 (% of day 16) compared to the mNN group (two-way ANOVA: $p < 0.01$ on day 16, $p < 0.001$ on day 21). Additionally, pups in the mUN group had significantly lighter body weights than those in the mNN group on postnatal day 1 ($p < 0.0001$).	Ovarian weights did not show significant differences among the four groups studied. However, uterine weights in the IUGR-NN group (offspring from undernourished mothers and fed a regular diet) were significantly lighter compared to the other groups ($p < 0.05$). Moreover, the cumulative rate of vaginal opening (VO) in the control groups (mNN) was significantly higher than that in the IUGR groups (mUN and mNN) from day 33 through day 37 ($p < 0.05$).
7	Iwasa et al. (2011) [15]	In Vivo	Pregnant Sprague-Dawley Rats	9	9	dexamethasone (1microgram/mL) induced IUGR rats	Dexamethasone-treated dams exhibited significantly less body weight gain during pregnancy than untreated dams, and this difference persisted with a significant reduction in body weight from birth to postnatal day 28 (two-way ANOVA; $p < 0.01$).	Offspring in the Dexamethasone (DEX) group showed delayed onset of puberty, with vaginal opening occurring at 34.4 ± 0.4 days compared to 31.1 ± 0.3 days in the control group ($P < 0.01$). Additionally, on postnatal day 28, the DEX group had significantly lower ovarian weight than the control group. However, there were no significant differences between the control and DEX groups in serum concentrations of LH (luteinizing hormone), FSH (follicle-stimulating hormone), or leptin.
8	Kinouchi et al. (2012) [25]	In Vivo	Pregnant Sprague-Dawley Rats	8	8	dexamethasone (1microgram/mL) induced IUGR rats	The CD group, which received central administration of dexamethasone, exhibited significantly less weight gain from day 30 to day 34 than the CS group (two-way ANOVA; $P < 0.05$). Additionally, the CD group demonstrated a delayed onset of puberty, with a VO (vaginal opening) ratio on day 34 of 12.5% compared to 88.9% in the CS group ($p < 0.01$).	No significant differences were found between the CS and CD groups regarding serum LH (luteinizing hormone) concentrations or hypothalamic mRNA levels of Kiss1, GnRH, or NPY on day 34. However, the CD group showed significantly lower ovarian and uterine weights, reduced hypothalamic Kiss1r mRNA levels, and higher serum leptin concentrations compared to the CS group.

9	Valverde et al. (2015) [5]	In Vivo	Pregnant Sprague-Dawley Rats	12	12	dams received 50% of the daily food intake of the mNN group from embryonic day 15 until delivery. Maternal	Offspring from maternal undernutrition (MUN) rats had significantly smaller body weights at birth than the control (C) group, with males and females showing a 36% reduction. By postnatal day 7, MUN offspring still exhibited lower body weights than their sex-matched controls, although they showed accelerated growth compared to earlier stages.	Relative heart weight (ventricular weight) was significantly increased in male and female MUN offspring compared to controls. Male MUN offspring showed a trend towards smaller kidneys compared to controls, approaching statistical significance ($p = 0.06$). Relative liver weight was significantly elevated in MUN offspring compared to controls. Additionally, subcutaneous fat levels were significantly higher in male and female MUN rats than in controls.
10	Pampanini et al. (2017) [10]	In Vivo	Pregnant Sprague-Dawley Rats	7	4	intrauterine growth restriction induced by uterine artery ligation on the postnatal rat testis.	Testes from intrauterine growth restriction (IUGR) rats exhibited significantly reduced weight at five days postpartum (dpp) compared to sham-operated rats (5.9 ± 1.2 vs. 10.8 ± 1.9 mg, $p < 0.001$). Additionally, IUGR rats had a significantly lower mean body weight at five dpp than shams (9.6 ± 1.9 vs 13.2 ± 0.6 g, $p < 0.05$).	In IUGR rats at 5 dpp, Citrate synthase (Cs) and Hydroxy acyl-CoA dehydrogenase (Hadh) were upregulated compared to controls ($p < 0.02$). By 40 dpp, Lactate dehydrogenase genes (Ldh-a, Ldh-b, Ldh-c) were downregulated in IUGR rats ($p < 0.002$, $p < 0.02$, $p < 0.02$, respectively). Sox9 expression, indicative of Sertoli cell (SC) function, was decreased at 5 dpp ($p < 0.02$), while Follicle-stimulating hormone receptor (Fshr) was elevated at 20 and 40 dpp ($p < 0.05$ and $p < 0.02$, respectively). Kit ligand (Kitl) was downregulated at 40 dpp ($p < 0.02$). Intra-testicular testosterone (ITT) levels were significantly higher at 20 dpp in IUGR rats ($p < 0.05$), suggesting significant disruptions in testicular gene expression and hormone levels due to early growth restriction.
11	Shen et al. (2022) [24]	In Vivo	Pregnant Sprague-Dawley Rats	6	6	dams received 50% of the daily food intake of the mNN group from embryonic day 15 until delivery.	Infants born with intrauterine growth restriction (IUGR) exhibited a significant 30% decrease in average birth weight compared to infants born at appropriate gestational age (AGA) ($p < 0.001$).	Analysis of gene expression in IUGR compared to AGA groups revealed 815 significantly differentially expressed genes (fold-change >1.5, $p < 0.05$). Of these, 347 genes were upregulated, and 468 genes were downregulated in IUGR rats. Key genes identified included Ppargc1a, Prkaa2, Slc2a1, Rxrg, and Gcgr, which were central in enrichment and protein-protein interaction network analyses. Pathways such as the PPAR signaling pathway and FoxO signaling pathway were prominently affected, suggesting their potential involvement in the development of metabolic syndrome associated with IUGR.

Table 2. SYRCLE's Tool for In Vivo Studies

No	Study	Study Design	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Overall Appraisal
1	Baysal et al. (2022) [20]	In Vivo	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Unclear	Yes	Yes	8 / 10 Low Risk of Bias
2	Beinder et al. (2014) [22]	In Vivo	No	Yes	Yes	Yes	Unclear	Yes	Yes	No	Yes	No	6/10 Moderate Risk of Bias
3	Faria et al. (2003) [23]	In Vivo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	9 / 10 Low Risk of Bias
4	Engelbregt et al. (2002) [12]	In Vivo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10 / 10 Low Risk of Bias
5	Engelbregt et al. (2004) [21]	In Vivo	No	No	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Unclear	6/10 Moderate Risk of Bias
6	Gereltsetseg et al. (2012) [12]	In Vivo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	9 / 10 Low Risk of Bias
7	Iwasa et al. (2011) [15]	In Vivo	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	9 / 10 Low Risk of Bias
8	Kinouchi et al. (2012) [25]	In Vivo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10 / 10 Low Risk of Bias
9	Valverde et al. (2015) [5]	In Vivo	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10 / 10 Low Risk of Bias
10	Pampanini et al. (2017) [10]	In Vivo	No	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Unclear	6/10 Moderate Risk of Bias
11	Shen et al. (2022) [24]	In Vivo	No	Yes	Yes	Yes	No	Yes	Yes	Unclear	Yes	No	6/10 Moderate Risk of Bias

