DOI: 10.24996/ijs.2026.67.5.18

ISSN: 0067-2904

Assessment of the Placental Growth Factors Role in the Development of Mice During the Mid and Late Gestation Period

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Received: 30/12/2024 Accepted: 27/5/2025 Published: 30/5/2026

Abstract

The placenta is the main controller of nutrient resources to the growing embryo during gestation. As such, adequate placental function is instrumental for developmental progression throughout intrauterine development. This study aimed to assess the role of placental growth factors: Placental growth hormone (PGH), placental lactogen (PL), Insulin-like growth factor1 (IGF1), and IGF2) in fetal development during mid and late gestation in albino mice. A total of 80 pregnant mice were divided into two groups based on gestational stages. The first group, second week, represents the mid-gestation period in embryonic day (ED), ED (8–14) day, and the second group, third week, represents late-gestation at (15–21) day. Some fetal metrics were measured: fetal weight (g), crown-rump length CRL (mm), and placental growth factors were measured using ELISA. Statistical analysis revealed significant differences in growth factors levels across gestational stages ($p \leq 0.05$). PGH levels increased significantly from 5.19 ± 0.09 ng/ml in the second week to 7.12 ± 0.14 ng/ml in the third week. Similarly, PL rose from 8.49 ± 0.18 ng/ml in the second week to 11.35 ± 0.25 ng/ml in the third week. In the second week, the IGF1 showed a marked increase from 5.27 ± 0.16 ng/ml compared to 11.13 ± 0.19 ng/ml in the third week, and IGF2 increased from 4.09 ± 0.08 ng/ml in the second week to 5.21 ± 0.09 ng/ml in the third week. The fetal weight and CRL also showed significant increases with the progress of development , the fetal weight increased more than doubling from 0.608 ± 0.03 g in the second week to 1.449 ± 0.03 g in the third week, and CRL increasing from 12.37 ± 0.46 mm in the second week to 20.34 ± 0.63 mm in the third week. The findings highlight the importance of placental growth factors in fetal growth and development, suggesting their potential application in improving pregnancy outcomes. Further research is recommended to explore the clinical implications of these findings in gestational health.

Keywords: Placental Growth Factors, Fetal Development, Mid and Late Gestation, Albino Mice.

تقييم عومل النمو المشيمية في اجنة الفئران خلال مرحلتي منتصف واواخر الحمل

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الخلاصة

تعد المشيمة المتحكم الرئيسي في توصيل الموارد الغذائية للجنين أثناء الحمل ، ونظرا لأهمية الوظيفة المشيمية في دعم التكوين الجنيني داخل الرحم ، هدفت هذه الدراسة إلى تقييم دور عوامل النمو المشيمية

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هرمون النمو المشيمي، اللاكتوجين المشيمي ، وعوامل النمو المشابهة للأنسولين 1 و2 في تكوين الجنين خلال المراحل المتقدمة من الحمل في الفئران البيض. تم تقسيم 80 فأرًا حاملًا إلى مجموعتين حسب مرحلة الحمل الأسبوع الثاني مثلت منتصف الحمل يوم الحضانة 14–8 في حين ان الأسبوع الثالث مثلت اواخر الحمل يوم الحضانة 21–15. تم قياس بعض القياسات للاجنة مثل وزن الجنين، الطول التاجي العصعصي، وبعض المؤشرات المشيمة باستخدام تقنية.ELISA

أظهرت التحليلات الإحصائية اختلافات معنوية (p ≤ 0.05) في عوامل النمو المشيمه بين مراحل الدراسة المختلفة. ارتفعت مستويات هرمون النمو المشيمي من 5.19 ± 0.09 نانوغرام/مل في الأسبوع الثاني إلى 7.12 ± 0.14 نانوغرام/مل في الأسبوع الثالث، بينما زادت مستويات اللاكتوجين المشيمي من 8.49 ± 0.18 نانوغرام/مل إلى 11.35 ± 0.25 نانوغرام/مل خلال الفترة ذاتها. كما أظهرت مستويات عوامل النمو المشابهة للأنسولين 1 زيادة ملحوظة من 5.27 ± 0.16 نانوغرام/مل إلى 11.13 ± 0.19 نانوغرام/مل، وعوامل النمو المشابهة للأنسولين 2 من 4.09 ± 0.08 نانوغرام/مل إلى 5.21 ± 0.09 نانوغرام/مل.

اما بالنسبة الى وزن الجنين وطوله التاجي العصعصي فقد كانت هناك زيادات ملحوظة، حيث تضاعف وزن الجنين من 0.608 ± 0.03 غرام إلى 1.449 ± 0.03 غرام، وزاد الطول التاجي العصعصي من 12.37 ± 0.46 ملم إلى 20.34 ± 0.63 ملم.

تؤكد هذه النتائج أهمية عوامل النمو المشيمية في تكوين الجنين ونموه، مما يدل إلى إمكانية تطبيق هذه المعرفة لتحسين الحمل. ويوصى بإجراء المزيد من البحوث لاستكشاف الآثار السريرية لهذه النتائج على صحة الحمل.

1.Introduction

Gestation in a normal condition occurs in mammalian and is characterized by many physiological and anatomical alterations or changes in order to prepare an appropriate environment for developing the embryo until birth [1]. Mammalian placental growth factors play a pivotal role in mediating developmental signaling through a complex network of cytokines and signaling pathways [2]. Placental growth factors are essential for maintaining pregnancy and are likely important gestationally when placentation is still converging on maturity [3]. This includes the latter part of the first trimester, a time of rapid placental development in humans [4]. Diseases associated with aberrant subcortical placental development, such as preeclampsia, can affect gestation at this time, and the gestational period may extend as placentation continues in multiple species[5]. In mouse gestation, the placenta is also short-lived, and, as in women, there are indices of placental development approximating mid-first to second trimester development in humans, which occurs during relatively late gestation in mid-gestation mice [6]. The placenta serves as a central regulator of maternal-fetal communication, playing an indispensable role in the transport of nutrients and gases while simultaneously secreting hormones crucial for maintaining pregnancy [7]. As pregnancy progresses, especially in the mid to late stages, placental growth factors placenta influence various developmental processes in the fetus. However, the specific roles of these growth factors during late gestation remain poorly understood [8]. Placental growth factors exert several functions that are essential for the development of the fetuses, especially for those tissues and organs that begin to grow extensively at mid and late gestation [9]. These functions can be subdivided into different categories [10]. Placental growth factors regulate cellular growth, mainly through the induction of proteins involved in the cellular uptake of amino acids, like cationic amino acid transporters, which are necessary for cellular protein synthesis [11]. Furthermore, these growth factors influence cellular differentiation, hormonal production, and the expression of other growth factors[12]. Moreover, these growth factors are also important in relation to cellular migration processes that are crucial for the correct development of several tissues during gestation, such as the lung, brain, and skeletal muscles [13].

2.Materials and Methods

This study involved 80 pregnant albino mice divided into two groups based on gestational stages: Mid-gestation (embryonic day [ED] 8–14) and late-gestation (ED 15–21). Parameters monitored included fetal weight (g) (Figure 1) and fetal crown-rump length (mm) (Figure 2). Placental growth hormone (PGH), Placental Lactogen (PL), insulin-like growth factor 1(IGF1), and insulin-like growth factor 2 (IGF2), all quantified using enzyme-linked immunosorbent assay (ELISA). It aimed at the mid-gestation stage, beginning when the organism develops at a rather fast rate and the placenta is still rather small in size, and ending at the time which is considered to be the beginning of the preparatory period to the birth, when the organs are mature. Likely to avoid variability, mice were housed and kept at an ambient temperature of 20–25°C, exposed to light for 12 hours daily, and fed on a standard diet and water [14].

Figure 1: The measurement of embryo weight at the third-week of gestation.

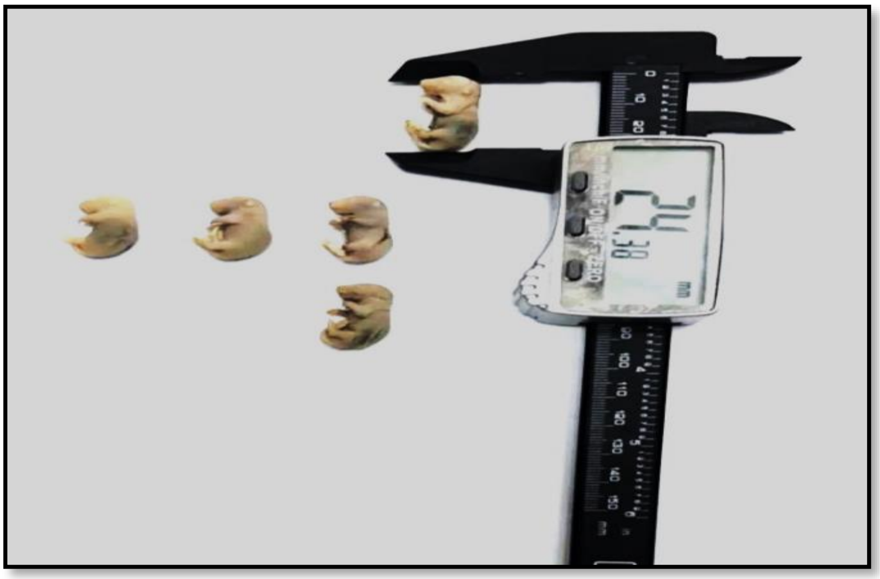

Figure 2: The measurement of embryo crown-rump length at the third week of gestation.

3. Statistical Analysis

The statistical analysis system- SAS (2018) program was used to detect the effect of different groups on study parameters. Least significant difference (LSD) and T-test were used to significantly compare the means. Estimate of the correlation coefficient between variables in this study. Results were considered statistically significant if the p-value was≤ 0.05.

Ethical Approval

Ethical approval for this study was obtained from the College of Science Research Ethics Committee, University of Baghdad (Reference No. CSEC/0824/0058, approved on August 14, 2024). All procedures adhered to ethical standards, ensuring the welfare and humane treatment of albino mice. The committee was informed of the study's design and will receive updates, including the final report, as per institutional guidelines.

4. Results

4.1 Comparison of mothers' weight before and after mating

The analysis of the mothers' weights before and after mating (Table 1). Before mating, the second week, and third week had highly significant weights of (29.87 ± 0.62 g) and (28.99 ± 0.44 g), respectively. After mating, both groups demonstrated a highly significant increase in weight ($P \leq 0.01$), as shown in Figures 3 and 4.

Table 1: Comparison between groups of study in mean of weight before and after mating

Age of fetus (days)	**Weight (g)** *Mean ±SE*	
	before mating	**after mating**
Second week (8-14 day)	29.87 ±0.62 a	39.63 ±0.72 a
Third week (15-21 day)	28.99 ±0.44 b	43.38 ±0.64 a
L.S.D.	1.688	2.027 **
P-value	0.0410	0.0001

Means having with the different letters in same column differed significantly. ** (P≤0.05), ** (P≤0.01).*

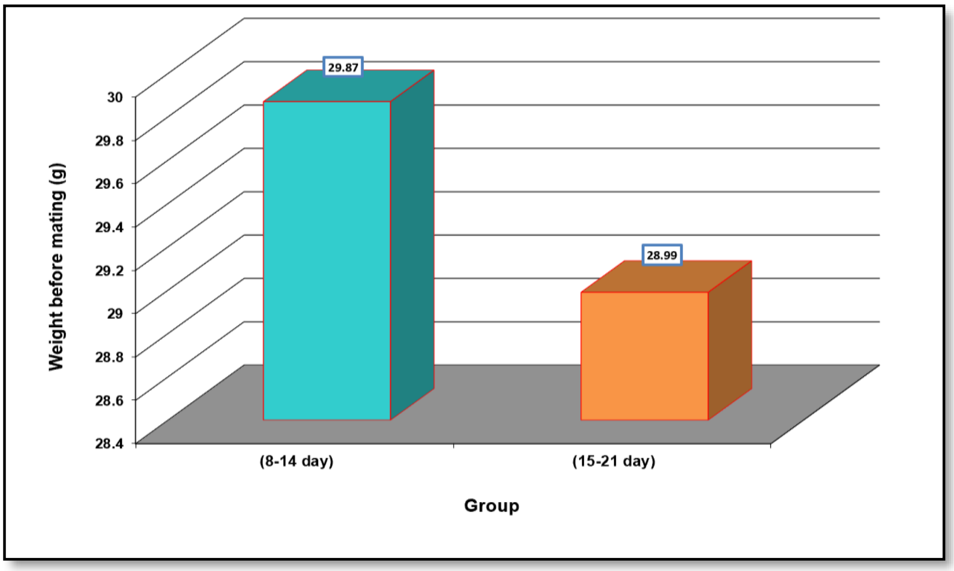

Figure 3: Comparison between studied groups in weight before mating

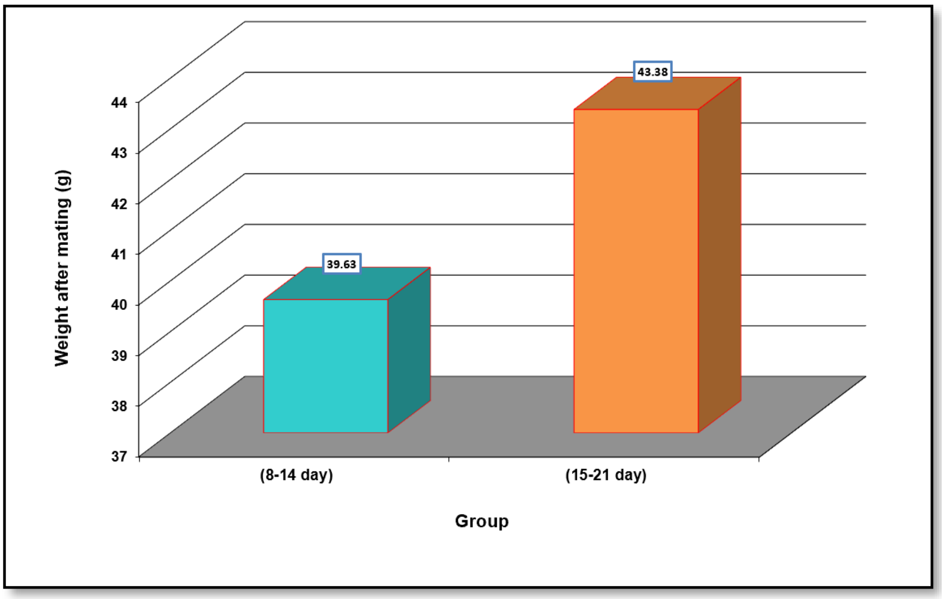

Figure 4: Comparison between studied groups in weight after mating

4.2 Placental development markers across gestational weeks

The results in Table 2 showed the levels of placental development markers (PGH, PL, IGF1, and IGF2) during the second and third gestational stages in albino mice. A significant increase was observed in the concentrations of all markers as gestation progressed ($P \leq 0.01$). The PGH concentration increased significantly from 5.19 ± 0.09 ng/ml in the second week to its peak at 7.12 ± 0.14 ng/ml in the third week, suggesting a consistent rise in PGH levels as pregnancy advanced. PL levels rose from 8.49 ± 0.18 ng/ml in the second week, peaking at 11.35 ± 0.25 ng/ml in the third week, indicating its key role in supporting fetal growth during mid to late pregnancy. IGF1 showed a marked increase from 5.27 ± 0.16 ng/ml in the second week to 11.13 ± 0.19 ng/ml in the third week, highlighting its importance in promoting fetal and placental growth in the later stages. The IGF2 concentration significantly increased from 4.09 ± 0.08 ng/ml in the second week to 5.21 ± 0.09 ng/ml in the third week, emphasizing its crucial role in fetal development, especially during late gestation. The trends for these markers are shown in Figures 5 to 8.

Table 2: Comparison between placental development markers with gestational weeks

Group	*Mean ±SE*			
	PGH (ng/ml)	**PL (ng/ml)**	**IGF1 (ng/ml)**	**IGF2 (ng/ml)**
Second week (8-14 day)	5.19 ±0.09 b	8.49 ±0.18 b	5.27 ±0.16 b	4.09 ±0.08 b
Third week (15-21 day)	7.12 ±0.14 a	11.35 ±0.25 a	11.13 ±0.19 a	5.21 ±0.09 a
L.S.D.	0.291 **	0.606 **	0.478 **	0.273 **
P-value	0.0001	0.0001	0.0001	0.0001

Means having the different letters in the same column differed significantly. *** (P≤0.01).*

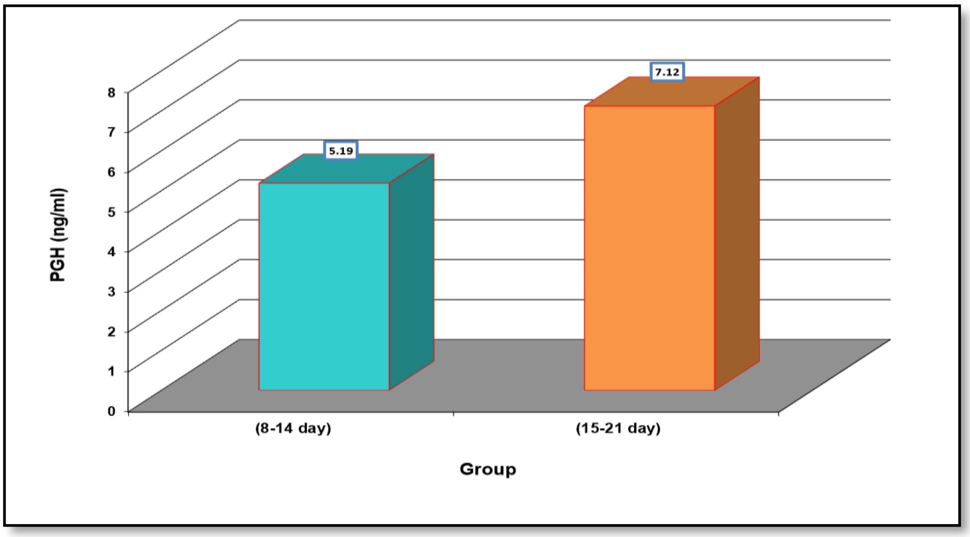

Figure 5: Comparison between the studied groups in PGH

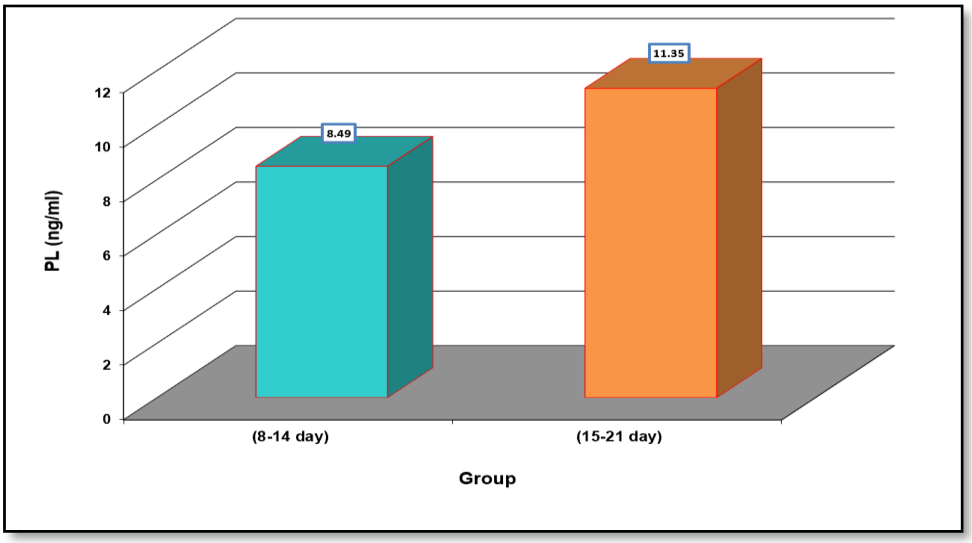

Figure 6: Comparison between the studied groups in PL

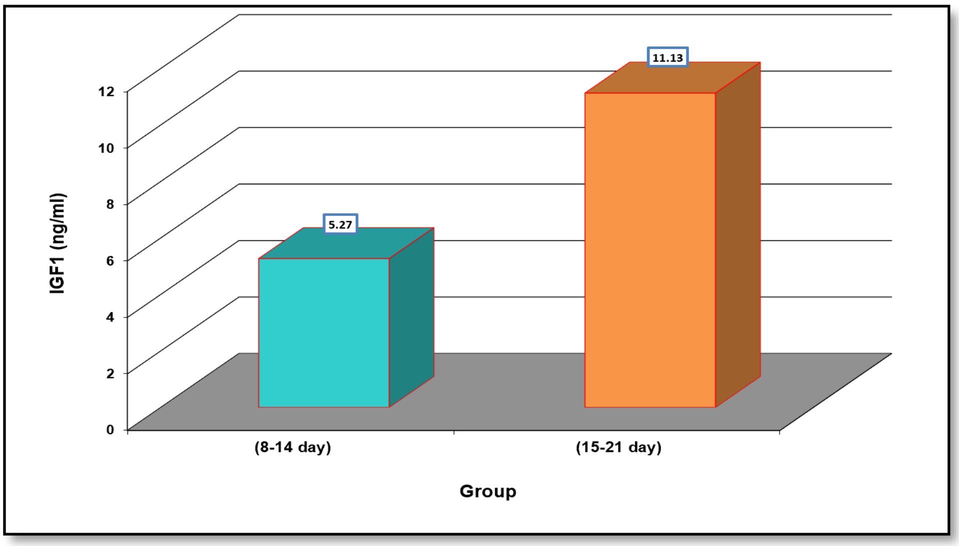

Figure 7: Comparison between the studied groups in IGF1

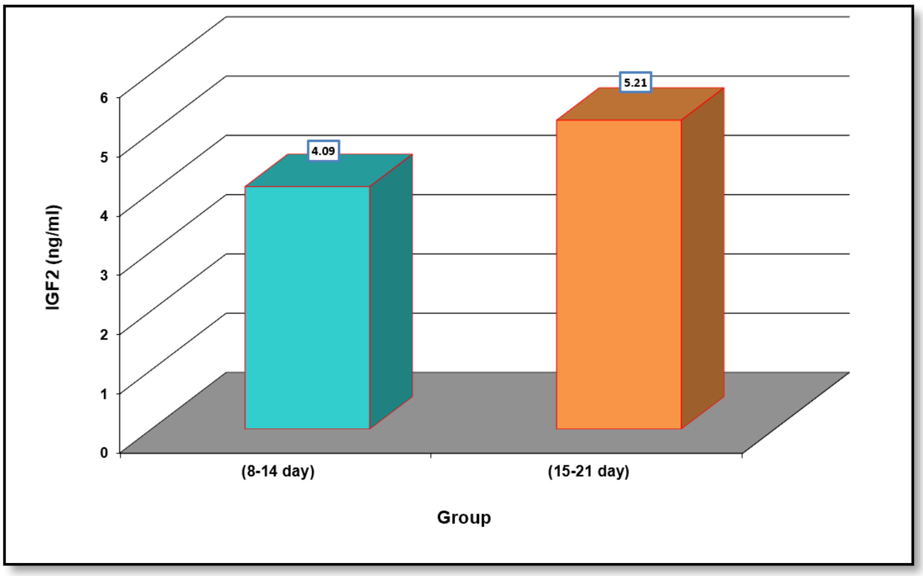

Figure 8: Comparison between the studied groups in IGF2

4.3 Fetal and placental growth parameters in the second and third week of gestation

This study analyzed some development parameters of the embryos and fetus weight, crown-rump length (CRL), and placental weight in mice during the second and third gestational weeks to assess the progression of fetal development (Table 3). As shown in Figures 9 and 10 the morphology of retrieved embryos proved morphological differences between different embryonic days. The mean embryo weight showed a substantial increase from 0.608 ± 0.03 g in the second week to 1.449 ± 0.03 g in the third week, indicating a more than two-fold increase (Figure 11). Similarly, the CRL significantly increased from 12.37 ± 0.46 mm in the second week to 20.34 ± 0.63 mm in the third week, as shown in Figure 12.

Figure 9: Comparison between groups of study in a number of implanted fetus.

Figure 10: Comparison between groups of study in a number of implanted fetus.

Table 3: Comparison between weight embryos and CRL in the study groups

Age of fetus (day)	*Mean ±SE*	
	weight (g)	**CRL (mm)**
Second week (8-14)	0.608 ±0.03 b	12.37 ±0.46 b
Third week (15-21)	1.449 ±0.03 a	20.34 ±0.63 a
T-test	0.0819 **	1.565 **
P-value	0.0001	0.0001

*Means having the different letters in the same column differed significantly. ** (P≤0.01).*

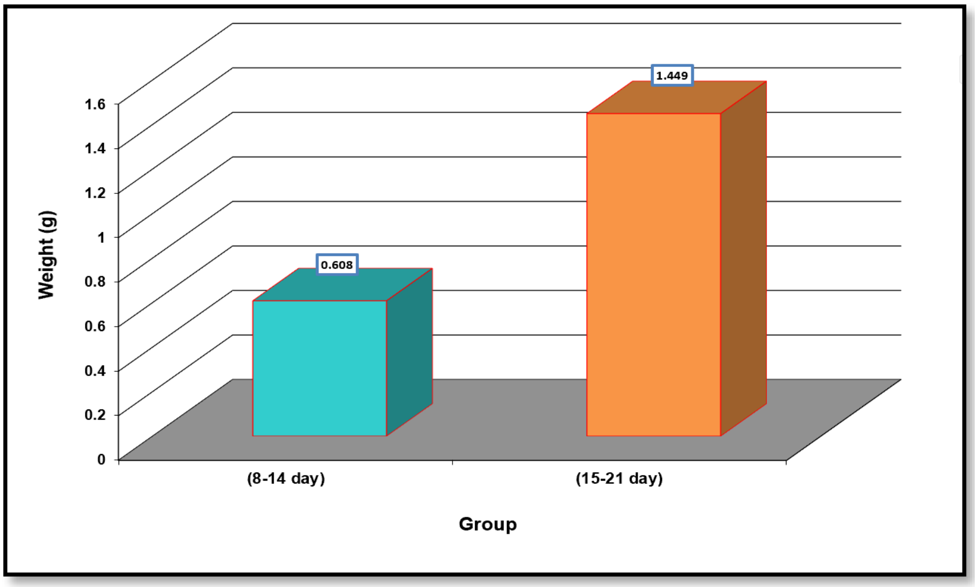

Figure 11: Comparison between the studied groups (second week and third week) in weight

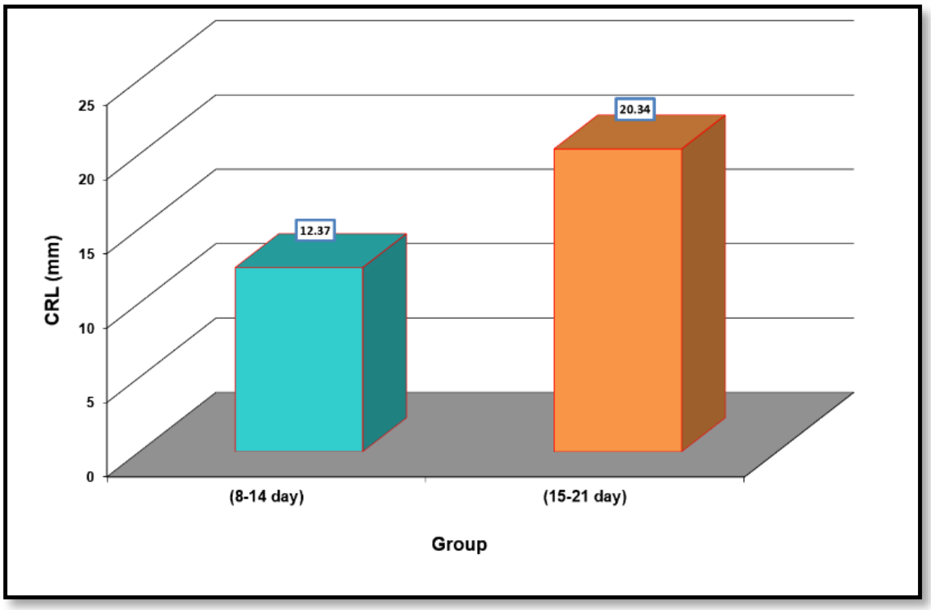

Figure 12: Comparison between studied groups (second week and third week) in CRL

Discussion

The results of the present study showed that maternal weight gain significantly increases after mating, particularly during the second and third weeks of gestation, aligning with the findings of Sanches *et al.,* [15], who reported that the weight gain in the early to mid-gestational period reflects enhanced placental development and nutrient availability to the fetus. The significant increase in weight after mating for the second and third weeks, suggests a strong role of gestational hormones and placental growth factors in supporting maternal and fetal needs as pregnancy progresses, as observed by Lufkin *et al.,*[16].

The levels of placental growth markers (PGH, PL, IGF1, and IGF2) gradually increase with increasing gestational weeks, as reported by Salmeri *et al.,* [17], who asserted that these factors are critical in the development of placental function and fetal growth. The marked rise in PGH and PL in the third week in our study is consistent with Stern *et al.,* [18] , who therefore showed that the last phases of fetal development correspond to a rise in the endocrine activity of the placenta in response to the enhanced requirements of the developing fetus. Furthermore, it is worthwhile to point out that an increase in IGF1 and IGF2 by the end of the third week showed a positive association with the study of Szydlowska-Gladysz *et al.,* [19]. These growth factors are important in fetus organogenesis and weight gain during the later period of fetal development, as was demonstrated by the experiments performed. Particularly, IGF1 has been identified to enhance nutrient transport in placenta, hence increasing fetal growth as indicated by Davenport *et al.,* [20] .Therefore, the current results are different from Baxter [21] , who argued that IGF2 levels do not rise in the second week, and even in the third week, there was a tendency to appear flat. This difference might be due to variation in the experimental conditions, including the effects of the environment or the differences in mice strains, in which IGF2 is regulated. Additionally, it was reported that placental lactogen levels are highest in the second week of gestation, contrasting with the current findings of a continuous increase through the third week [22].

The gradual rise in PGH, PL, IGF1, and IGF2 during the gestational weeks in the present investigation also suggests temporal progression in placental and fetal growth regulation. These findings agree with Ortega *et al.,*[11], who showed the function of placental growth factors that are central to fetal development and contribute to good pregnancy outcomes. Moreover, in late gestation stems from the increasing metabolic and growth requirements of the developing fetus. These markers have differential and complementary functions in supporting fetal maturation during pregnancy, particularly in the third trimester [17]. The rise in PGH during late gestation enhances placental nutrient transport and vascular development, aligning with findings from Stern *et al.,* [18]. Similarly, PL plays a vital role in modulating maternal insulin resistance and ensuring adequate glucose supply to the fetus, as reported by Moazzam *et al.,* [22]. The significant increase in IGF1 and IGF2 during late gestation supports fetal weight gain and organ development, consistent with Szydlowska-Gladysz *et al.,* [19], who noted the critical role of IGF1 in nutrient transport. Davenport *et al.,* highlighted IGF2's involvement in placental vascularization [20].These findings align with Ortega *et al.,* [11] , who demonstrated that the IGF2 is critical for placental development and growth. It promotes trophoblast invasion and placental vascularization, ensuring efficient nutrient and oxygen delivery. In the last trimester, IGF2's role becomes prominent in supporting placental structure, which is vital for sustaining the growing fetus [16]. In humans, similar mechanisms are detected. Both PGH and PL levels increase gradually with pregnancy and reach their highest levels in the third trimester of pregnancy to meet the high demands of fetal metabolism. IGF1 and IGF2 are important for fetal growth and the placenta [23].

The increase in fetal weight, CRL, and placental weight between the second and third gestational weeks is consistent with the findings of Seo *et al.,* [24], who observe that fetal grows rapidly in the late gestation weeks because of the improved placental function and nutrient transport. The significant increase in the embryo weight and CRL in the third week proves that late gestation is the critical developmental stage of fetal development due to better placental functioning and nutrient availability, as explained by Mirza *et al.,* [25] . Moreover, the rapid increase in CRL during the third gestational week corroborates the study by Dos Santos *et al.,* [26] which identified this period as a peak phase for skeletal and muscular development in rodents.

Conclusion

This present work aims to explain the effects of placental growth factors (PGH, PL, IGF 1, and IGF 2) in fetal development at mid and late gestation among albino mice. Therefore, the observed rises in these aspects concurrently with a significant increment in fetal weight and crown-rump length are deemed to play central roles in the enhancement of pregnancy. These findings highlight the importance of identifying placental growth factors in fetal and placental growth and development and provide suggestions for further research on fetal growth regulators and pregnancy outcomes.

Conflict of interest

There is no Conflict of interest.

Referances

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