Al-Rafidain J Med Sci. 2025;8(1):71-77.
DOI: https://doi.org/10.54133/ajms.v8i1.1642

AJMS

Online ISSN (2789-3219)

Research Article

Pluripotency Stem Cell Marker Expression and Apoptotic Changes in Placental Tissues of Normal and Intra-Uterine Growth Restriction (IUGR) Babies of Iraqi Mothers: A Comparative Study

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Received: 1 January 2025; Revised: 10 February 2025; Accepted: 12 February 2025

Abstract

Background: The placenta is a vital organ for embryo development, providing nutrition, waste elimination, and communication with the mother. It also facilitates nutrient transfer and stem cell creation. Intrauterine growth restriction (IUGR) can impact the unborn child's growth during pregnancy. ***Objective***: To examine the immunohistochemical expression of NANOG and CASP3 in both normal and IUGR placental tissues. ***Methods***: The study involved 90 healthy mothers and their babies at 38-40 weeks of pregnancy. Doppler ultrasonography confirmed idiopathic fetal IUGR. Placentas were categorized into control and IUGR groups, with each tissue block immunohistochemically stained for NANOG and CASP3 markers. ***Results***: Expression of the NANOG antibody in placental tissue of both normal and IUGR cases revealed a low intensity of expression in both normal and IUGR placentae and not significantly different. The expression of the CASP3 marker showed statistically significant results regarding staining intensity, percentage, and total score calculation between the normal and IUGR cases. It was of low intensity in most normal cases compared to the strong intensity of marker expression in most IUGR cases. ***Conclusions***: Embryonic stem cells showed a diminished expression by NANOG in placentae of both normal and IUGR cases attributed to the trimester of pregnancy that decreased its expression in the third trimester generally as the stem cells become less evident as we go through the trimesters of pregnancy. On the other hand, apoptosis is one of the important changes associated with placental pathology like IUGR.

Keywords: CASP3, IUGR, NANOG, Placenta, Pregnancy.

التعبير عن علامات الخلايا الجذعية متعددة القدرات والتغيرات المبرمجة في أنسجة المشيمة لأطفال تقييد النمو الطبيعي وداخل الرحم (IUGR) للأمهات العراقيات: دراسة مقارنة

الخلاصة

الخلفية: المشيمة عضو حيوي لنمو الأجنة، وتوفير التغذية والتخلص من النفايات والتواصل مع الأم. كما أنه يسهل نقل المغذيات وتكوين الخلايا الجذعية. يمكن أن يؤثر تقييد النمو داخل الرحم (IUGR) على نمو الجنين أثناء الحمل. **الهدف**: فحص التعبير الكيميائي المناعي ل NANOG و CASP3 في كل من أنسجة المشيمة الطبيعية و IUGR. **الطرائق**: شملت الدراسة 90 أما سليمة وأطفالهن في الأسبوع 38-40 من الحمل. أكد التصوير بالموجات فوق الصوتية دوبلر IUGR الجنين مجهول السبب. تم تصنيف المشيمة إلى مجموعات تحكم و IUGR ، مع تلطيخ كل كتلة نسيجية كيميائية مناعية لعلامات NANOG و CASP3. **النتائج**: كشف التعبير عن الجسم المضاد NANOG في أنسجة المشيمة لكل من الحالات الطبيعية وحالات IUGR عن كثافة منخفضة للتعبير في كل من المشيمة الطبيعية و IUGR ولا يختلف بشكل كبير. أظهر التعبير عن علامة CASP3 نتائج ذات دلالة إحصائية فيما يتعلق بشدة التلوين والنسبة المئوية وحساب الدرجات الإجمالية بين الحالات العادية والحالات IUGR. كانت منخفضة الكثافة في معظم الحالات العادية مقارنة بالشدة القوية للتعبير عن العلامات في معظم حالات IUGR. **الاستنتاجات**: أظهرت الخلايا الجذعية الجنينية تعبيرا متناقصا بواسطة NANOG في المشيمة لكل من الحالات الطبيعية و IUGR المنسوبة إلى الثلث الثالث من الحمل والتي قللت من تعبيرها في الثلث الثالث من الحمل بشكل عام حيث تصبح الخلايا الجذعية أقل وضوحا مع مرور الأشهر الثلاثة الأولى من الحمل. من ناحية أخرى ، يعد موت الخلايا المبرمج أحد التغييرات المهمة المرتبطة بأمراض المشيمة مثل IUGR.

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Article citation: Noel KI, Abdulateef HH, Khamees NH. Pluripotency Stem Cell Marker Expression and Apoptotic Changes in Placental Tissues of Normal and Intra-Uterine Growth Restriction (IUGR) Babies of Iraqi Mothers: A Comparative Study. *Al-Rafidain J Med Sci*. 2025;8(1):71-77. doi: https://doi.org/10.54133/ajms.v8i1.1642

INTRODUCTION

The placenta is a crucial organ for the developing embryo because it provides nutrition, eliminates waste products, and communicates with the mother to facilitate the transfer of various elements, including nutrients [1]. The placenta is a vital location for the creation of stem cells, which might be vital for an embryo both during and after intrauterine life, in addition to all other material transfer. The growth of the unborn baby in the womb will be impacted by any insult to the placenta throughout the three trimesters of pregnancy; one such insult is the intrauterine growth restriction (IUGR) [2]. Intrauterine growth restriction

(IUGR) has been defined as the rate of fetal growth that is below normal considering the growth potential of a specific infant as per the race and gender of the fetus [1]. Since IUGR will decrease the resources provided to the embryo, the placenta and umbilical cord are abundant sources of embryonic stem cells, primarily found in the placenta's fetal section rather than its mother site. Since the option to save embryonic stem cells taken from a newborn baby's blood at birth to be used later in the baby's life has become more and more interesting, the study of the locations where stem cells are present is crucial to our ability to take these actions. Stem cells are totipotent cells that can give rise to all other body cells and tissues [3]. The placenta must compensate for this reduction in resources, which makes IUGR a serious insult to the newborn. The placenta must adapt and overcome the status resulting from the reduction in resources, and we must obtain a histological change in the placenta of these IUGR babies [4]. This complex placental vascular disease (IUGR) causes premature delivery, low birth weight, and higher-than-expected perinatal morbidity and mortality. There are various fetal, maternal, and placental factors that can cause IUGR. Failure of the terminal villi's capillary circulation to elongate, branch, or dilate causes fetal growth restriction [5]. Apoptosis is a type of cell death that differs from necrosis and is regarded as a physiological cell death [6]. Events that are associated with apoptosis include endocrine-dependent tissue atrophy, embryogenesis, cytotoxic immunological responses, turnover, and tissue homeostasis [7]. Furthermore, apoptosis occurs in cells exposed to specific stressors such as ionizing radiation, hypoxia, or heat. On the other hand, necrosis occurs when cells experience markedly different conditions from those of normalcy, which may result in damage to the plasma membrane [8]. The biological feature of apoptosis is the irreversible breakdown of genomic DNA, which certifies the death of the cell. It has been demonstrated that this DNA fragmentation occurs in a range of cell types due to the activation of an endogenous nuclear endonuclease that is dependent on Ca'+ and Mg2+. This enzyme specifically cleaves DNA at linker DNA sites, which are located between nucleosomal units, to yield mono- and oligonucleosomal DNA fragments [9]. Chromatin aggregation, nuclear and cytoplasmic condensation, and the division of the cytoplasm and nucleus into membrane-bound vesicles (apoptotic bodies) that include ribosomes, mitochondria, and nuclear material are morphological characteristics of cells going through apoptosis. These apoptotic particles are quickly consumed in vivo by macrophages or nearby epithelial cells without causing an inflammatory reaction or releasing harmful substances [10]. On the other hand, because of the disruption of the plasma membrane and the discharge of the cytoplasmic contents into the extracellular fluid, necrotic cell death is frequently linked to significant tissue damage. According to recent research, apoptosis takes place in the trophoblast and decidual tissue of mouse embryos after implantation [11]. It has been shown that the syncytiotrophoblast of unsuccessful first-trimester pregnancies has a higher incidence of apoptosis [12]. Smith and colleagues found that placental tissue from term gestations had a low incidence of apoptosis, whereas placental tissue from IUGR gestations had greater occurrences [13]. A member of the cysteine-aspartic acid protease (caspase) family, caspase 3 is found in nearly all cells as a zymogen (procaspase) and plays a role in the process leading up to apoptotic cell death [14]. Homeobox NANOG protein, which controls and preserves stem cells' ability to self-renew and remain pluripotent, is a crucial transcription factor that comes from the Irish mythology Tír na nÓg, Land of Eternal Youth [15]. The 305 amino acid NANOG protein has a homeodomain that binds to a specific region of DNA. On chromosome 12, the Nanog 1 gene (gi 13376297) encodes it. It consists of 4 exons and 3 introns 16. It is also believed that CDX2, a frequently used marker of trophoblast stem/progenitor cells, specifically points toward a trophoblast fate. It has been reported that these cells and villous mesenchymal stem/stroma cells display pluripotent markers, like NANOG [17]. ANOG has been demonstrated to be a crucial regulator for embryonic stem cells in the early stages of embryogenesis. Since inner mass cells without NANOG are unable to produce epiblast cells and can only produce partially endoderm-like cells, NANOG is essential for preserving self-renewal and pluripotency [18]. Apart from preserving pluripotency and self-renewal, NANOG has also been reported to control the stem cell cycle [19]. More descriptive research on NANOG expression in mesenchymal and trophoblast cells is mostly limited to the preimplantation stage and/or the mouse model. Moreover, none of the NANOG expression profiles have, as far as we are aware, been studied in placenta-associated prenatal disorders like IUGR. Considering that the trophoblast progenitor cells, which may have originated from the chorionic villi's mesenchymal compartment, would have to make up for the extravillous compartment or strained syncytium in some manner. This study aims to compare the expression profiles of NANOG progenitor cell markers during healthy pregnancy versus those observed during placenta-associated pregnancy IUGR. This immunohistochemistry analysis was performed to examine the frequency of apoptosis in placental tissue from IUGR gestations and to compare that frequency with that of simple gestations using the CASP3 marker.

METHODS

Study design

This investigation was done with support from the obstetrics and gynecology department of Al-Yarmouk Teaching Hospital in Baghdad, Iraq, by the Department of Human Anatomy, Medical Faculty, University of Mustansiriyah, approved by each of these medical

institutes' scientific committees. In this research, 90 moms and their babies and placentas were included.

Inclusion criteria

The women were selected at 38–40 weeks of pregnancy, and based on their medical history, laboratory results, ultrasound scan, and clinical assessments, they appeared to be healthy and normal. They were also non-smokers, non-diabetic, and normotensive.

Exclusion criteria

Mothers with difficult or delayed labor were excluded. Mothers who have difficult or delayed labor are not eligible to participate in this work.

Setting

Doppler ultrasonography was also used to confirm the fetal condition as IUGR in the absence of any obvious fetal abnormalities. Each mother verbally gave her agreement to be included in this work. As there was no obvious fetal or maternal basis, all fetal IUGR was idiopathic. There were two sets of placentas. First, 45 placentas of newborns with a normal average body weight (control) were embraced, while 45 placentas of newborns with idiopathic IUGR were held in the second group. This group was selected based on factors such as fetal weight less than the gestational tenth percentile, decreased amnion, and evidence of diminished end diastolic flow velocity of the umbilical artery as confirmed by Doppler study and subsequent obstetric ultrasound examination [20].

Outcome measurements

Each placental tissue block was approximately 1 centimeter by 1 centimeter when it was fixed and immunohistochemically stained for NANOG and active CASP3 markers. Subsequently, five consecutive sections with a thickness of approximately 4 µm were taken from each tissue block and arranged on positively charged slides. The portions gradually rehydrated after dewaxing. Affinity-based biotin-peroxidase labeling is accomplished through immunohistochemical staining. To lower endogenous peroxidase, a blocking reagent containing 3% hydrogen peroxide was employed, maintained in a pH 6.0 sodium citrate buffer solution for 15 minutes to let the antigen be withdrawn. The sections were handled with rabbit polyclonal antibodies that were reactive with both active CASP3 (Elabscience®) and NANOG (Abnova®) and then left to stay at room temperature for an hour. After cleaning the sections with Tween-phosphate-buffered saline, they were allowed to sit at room temperature for 20 minutes while a secondary antibody was inserted and then for 30 minutes with a biotin-streptavidin complex. 3,3-diaminobenzidine tetrahydrochloride (DAB) was used to monitor the process. After that, the sections were fixed and given a hematoxylin counterstain [21]. The staining reaction's strengths were ranked by two pathologists who were blind to the study's goals [22]. The NANOG and active CASP3 antibody immunohistochemical reaction was estimated semi-quantitatively for stain intensity: 0/negative signifies no staining, 1+ indicates weak positive, 2+ designates moderately positive, and 3+ signifies significantly positive. The total staining score for immunohistochemistry is calculated by multiplying the staining percentage for each region in the high-power field by the staining intensity [23].

Ethical considerations

The study protocol was approved by the local research ethics committee of the College of Medicine, Mustansiriyah University (Certificate # 782, date: 17.12.2020). Written informed consent was obtained from the families of the patients who participated in the study.

Statistical analysis

Statistical analysis was performed with SPSS version [24]. The categorical data were displayed as percentages, and the continuous variables were represented as mean ± SD. To compare groups for immunohistochemistry results, the chi-square test was employed. A P-value of less than 0.05 was deemed statistically significant.

RESULTS

Expression of NANOG antibody in placental tissue of both normal and IUGR cases is obtained by double-blind examination regarding staining intensity, percentage, and calculation of total score of the marker expression, which revealed a low intensity of expression in both normal and IUGR placentae, and therefore it was a not significant result statistically (Tables 1, 2, and 3; Figures 1, 2, and 3).

Table 1: Samples and marker type in relation to staining intensity (chi square), Intrauterine growth restriction (IUGR), Homeobox protein NANOG, CASP3 (caspase protein)

Staining Intensity	Control (%)		IUGR (%)		*P* value
	CASP 3	NANO G	CASP 3	NANO G	
0	13%	7%	0%	13%	<0.000
1+	80%	80%	0%	74%	1
2+	7%	13%	26%	13%	
3+	0%	0%	74%	0%	

Table 2: Sample and marker type in relation to staining percentage (t-test). Intrauterine growth restriction (IUGR), Homeobox protein NANOG, CASP3 (caspase protein)

Sample Type	IHC Marker	Staining (%)	*p*-value
Control	CASP3	42.7±20.8	0.2600
	NANOG	59.3±18.8	
IUGR	CASP3	82±6.5	<0.0001
	NANOG	48±26.1	

Values are expressed as mean±SD.

Table 3: Sample and marker type in relation to total score (t-test). Intrauterine growth restriction (IUGR), Homeobox protein NANOG, CASP3 (caspase protein)

Sample Type	IHC Marker	Total score	*p*-value
Control	CASP3	0.5±0.2	0.5687
	NANOG	0.7±0.3	
IUGR	CASP3	2.2±0.4	<0.0001
	NANOG	0.6±0.5	

Values are expressed as mean±SD.

The expression of the CASP3 marker showed statistically significant results regarding marker staining intensity, percentage, and total score calculation between the normal and IUGR cases. It was of low intensity in most normal cases compared to the strong intensity of marker expression in most of the IUGR cases, as shown in Tables 1, 2, and 3 and Figures 1, 2, and 3.

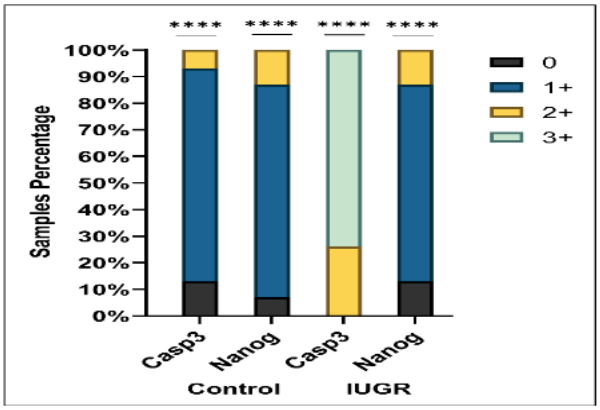

Figure 1: Staining intensity distribution of IHC markers according to sample type. **** *p*-value < 0.0001.

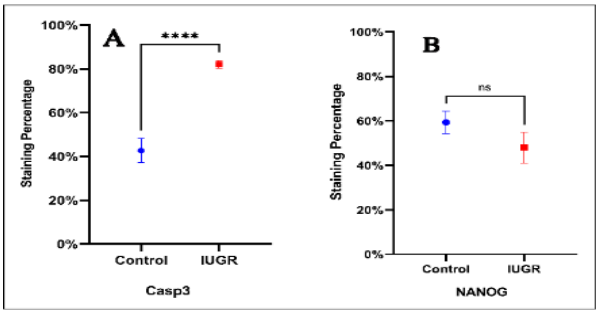

Figure 2: Staining percentage of IHC markers according to sample type. (**A**) Casp3; (**B**) NANOG.**** $p < 0.0001$, ns: $p > 0.05$.

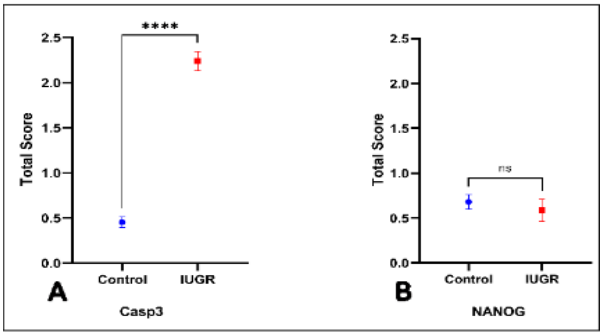

Figure 3: Total score of IHC marker staining according to sample type. (**A**) Casp3; (**B**) NANOG. ****= $p < 0.0001$, ns= $p > 0.05$.

Histologically, the placenta of IUGR pregnancies, characterized by many histological findings, includes chronic villitis, avascular villi, villous infarction, cytotrophoblast hyperplasia, and syncytial knots as seen in Figure 4 compared to normal placentae.

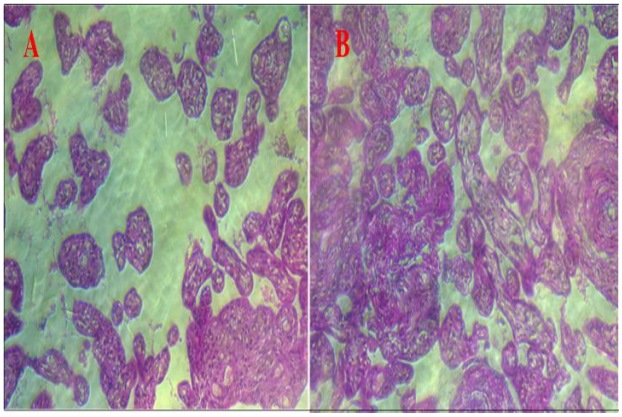

Figure 4: Normal placenta (**A**); IUGR placenta (**B**). H & E (400X).

Immunohistochemically, the expression of NANOG and CASP3 in the placental tissue of normal and IUGR cases was shown in Figures 5 and 6, where different staining intensities were shown.

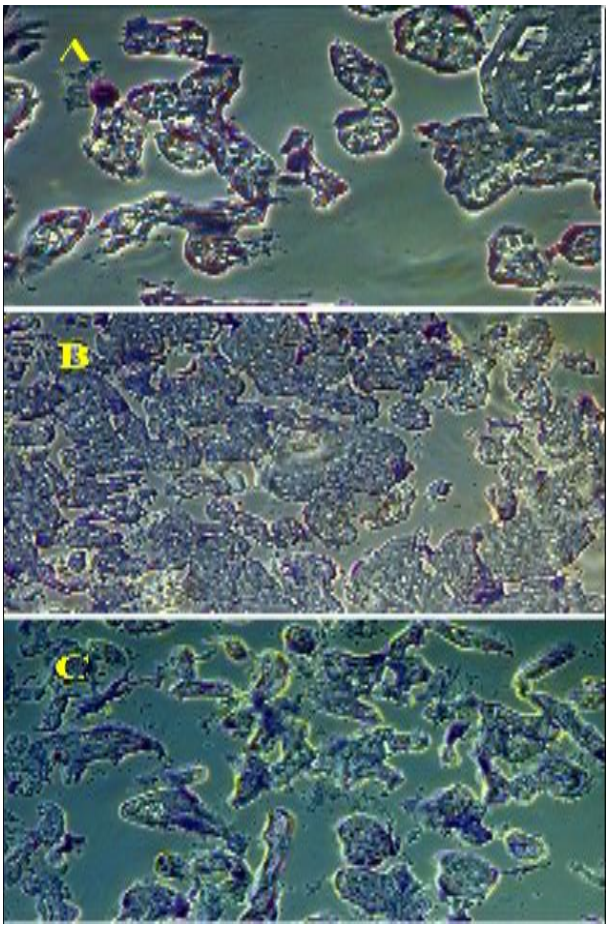

Figure 5: NANOG expression in normal and IUGR placenta. (**A**) NANOG expression in normal placenta (1+ nuclear reaction); (**B**) NANOG expression in IUGR placenta (-ve); (**C**) NANOG expression in IUGR placenta (1+ nuclear reaction). (400X).

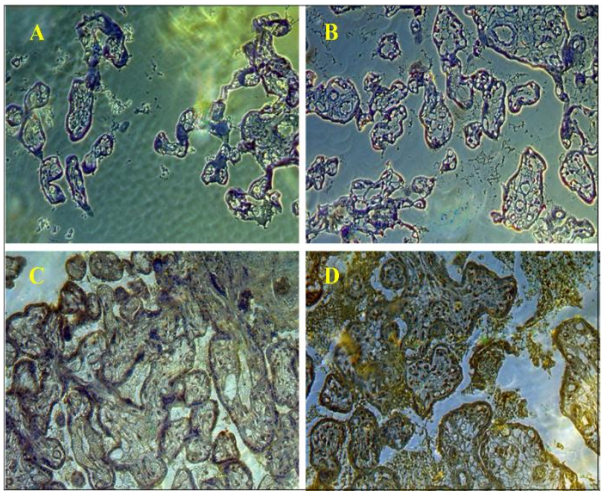

Figure 6: CASP3 expression in normal and IUGR placenta. (A) CASP3 expression in normal placenta (-ve reaction); (**B**) CASP3 expression in normal placenta (1+ membrane and cytoplasmic reaction); (**C**) CASP3 expression in IUGR placenta (2+ membrane and cytoplasmic reaction); (**D**) CASP3 expression in IUGR placenta (3+ membrane and cytoplasmic reaction) (400X).

DISCUSSION

IUGR is one of the pregnancy-associated disorders that have many fetal outcomes that affect the intrauterine growth of the baby. NANOG is a marker for the pluripotency expression of embryonic stem cells. During the first trimester of pregnancy, embryonic stem cells are particularly noticeable; as the pregnancy goes on, these cells begin to diminish until the third trimester, when they are still visible in the embryonic tissues. Although the fetal site of the placenta produces more embryonic cells than the mother region, the mother portion of the placenta also expresses stem cells. In a typical soft pregnancy, these facts are present. Although we assume that even stem cells become less expressed in placental pathology in IUGR pregnancies where the placenta's function is restricted, most of the cases in our study showed little to no difference in NANOG expression between IUGR and control cases, which is not statistically significant. The fact that we looked at NANOG expressions on the mother side rather than the fetal site may have contributed to these results. As we mentioned at the beginning of this session, the number of stem cells is already declining by the third trimester of pregnancy. Therefore, the expression of stem cells in IUGR-affected placentas was unaffected. Other researchers have also discussed these results [25]. The primary factor contributing to improper pregnancy in IUGR is altered uteroplacental blood flow [26]. The term "apoptosis" describes the distinct morphology of cell death, which can occur naturally or because of pathological situations [27]. In both IUGR placentas [28-30] and normal human placentas [31,32], trophoblast apoptosis has been seen. There is debate on the significance of placental apoptosis in IUGR pregnancy since diverse procedures and measurement methods have led to differing assessments of placental apoptosis in the literature [33,34]. While it is widely acknowledged that aberrant pregnancies result in increased placental apoptosis [31,32,35,36], other research indicates that IUGR patients experience less placental apoptosis [37]. In the current investigation, patients with IUGR had considerably higher levels of the apoptotic marker caspase-3 in their villous trophoblasts as compared to the control group. Similar findings to these studies point to elevated placental apoptosis in pregnancies complicated by IUGR. Aban et al. found that in pregnancies complicated by IUGR and preeclampsia, there was increased placental apoptosis as indicated by M30 and caspase-3 staining, along with increased NF-κB and decreased bcl-2 expression [35]. Increased apoptosis throughout FAS antigen and bcl-2 has been shown by Ishihara et al. in human term placentas afflicted by either IUGR or preeclampsia [36]. Nonetheless, certain research has also documented a reduction in placental apoptosis in pregnancies complicated by IUGR and preeclampsia. Apoptotic mediators BNip3 and Nix are reduced in the villous trophoblast cells of individuals with IUGR, HELLP syndrome, and preeclampsia, according to a study by Stepan et al. [27]. They proposed that the reduced apoptosis was due to the placenta's resilience to long-term hypoxia, which may have prevented the expected reduction in trophoblast apoptosis. These placental apoptotic characteristics are contentious due to methodological constraints brought about by the limitations of research on human tissue and animal studies. Placental bed biopsies cannot be taken during the early stages of human pregnancy due to the pathophysiology of IUGR and preeclampsia [37]. Because experimental animals differ in their trophoblastic invasion, it is also challenging to establish a model of pregnancy in humans using animal experimentation [24,25]. Most research on placentas has been on nuclear alterations that take place during the apoptotic process, which are comparatively arbitrary parameters. But in human trophoblasts, apoptosis is a highly intricate process that involves numerous signal transduction routes, caspases, bcl-2 regulators, and substrates 20. Further, more quantitative techniques like image analysis ought to be favored to reduce bias in the quantification of immunohistochemical staining in the placenta [38,39]. These disagreements concerning the apoptosis of the human placenta in abnormal pregnancies are therefore based on a complex cascade of apoptosis in the placenta, challenges in obtaining placental bed biopsies, a scarcity of study materials, and variations in trophoblastic invasion in experimental animals. From our point of view and regarding our result we suggest that the increase in the expression of CASP3 in the IUGR than the control group which indicate the increase in the apoptotic index of the trophoblastic cells of placental villi is considered as a part of a pathophysiology of the IUGR condition may be attributed to the chronic hypoxia that the cells subjected to it and as you know any tissue in the body must

confirm the balance between cell proliferation, cell maturation and cell death even when it is under normal conditions and you can imagine the effect of chronic hypoxia on this balance, which we consider an acceleration of the cell turn over to confirm the balance as an end result, or it may attributed to oxidative damage due to release of reactive oxygen species (ROS) which may exaggerate apoptosis in trophoblast in cases of IUGR [40], and so we obtain an increased apoptosis which is a physiological death rather than a necrosis (pathological cell death).

Study limitations

One of the important limitations of this study is that the placentas from mothers in the first and second trimesters are not included due to difficulties in obtaining these placentas.

Conclusion

Embryonic stem cells showed a diminished expression by NANOG in placentae of both normal and IUGR cases, attributed to the trimester of pregnancy that decreased its expression in the third trimester generally as the stem cells become less evident as we go through the trimesters of pregnancy. On the other hand, apoptosis is one of the important changes associated with placental pathology like IUGR.

Conflict of interests

No conflict of interest was declared by the authors.

Funding source

The authors did not receive any source of funds.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Guttmacher AE, Maddox YT, Spong CY. The human placenta project: placental structure, development, and function in real time. *Placenta*. 2014;35(5):303-304. doi: 10.1016/j.placenta.2014.02.012.
2. Ortega MA, Fraile-Martínez O, García-Montero C, Sáez MA, Álvarez-Mon MA, Torres-Carranza D, et al. The pivotal role of the placenta in normal and pathological pregnancies: A focus on preeclampsia, fetal growth restriction, and maternal chronic venous disease. *Cells*. 2022;11(3):568. doi: 10.3390/cells11030568.
3. Chia WK, Cheah FC, Abdul Aziz NH, Kampan NC, Shuib S, Khong TY, et al. A review of placenta and umbilical cord-derived stem cells and the immunomodulatory basis of their therapeutic potential in bronchopulmonary dysplasia. *Front Pediatr*. 2021;9:615508. doi: 10.3389/fped.2021.615508.
4. Zhang S, Regnault TR, Barker PL, Botting KJ, McMillen IC, McMillan CM, et al. Placental adaptations in growth restriction. *Nutrients*. 2015;7(1):360-389. doi: 10.3390/nu7010360.
5. Fasoulakis Z, Koutras A, Antsaklis P, Theodora M, Valsamaki A, Daskalakis G, et al. Intrauterine growth restriction due to gestational diabetes: From pathophysiology to diagnosis and management. *Medicina (Kaunas)*. 2023;59(6):1139. doi: 10.3390/medicina59061139.
6. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect Immun*. 2005;73(4):1907-1916. doi: 10.1128/IAI.73.4.1907-1916.2005.
7. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol*. 2007;35(4):495-516. doi: 10.1080/01926230701320337.
8. Jiao Y, Cao F, Liu H. Radiation-induced cell death and its mechanisms. *Health Phys*. 2022;123(5):376-386. doi: 10.1097/HP.0000000000001601.
9. Oberhammer F, Wilson JW, Dive C, Morris ID, Hickman JA, Wakeling AE, et al. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. *EMBO J*. 1993;12(9):3679-3684. doi: 10.1002/j.1460-2075.1993.tb06042.x.
10. Oberhammer FA, Hochegger K, Fröschl G, Tiefenbacher R, Pavelka M. Chromatin condensation during apoptosis is accompanied by degradation of lamin A+B, without enhanced activation of cdc2 kinase. *J Cell Biol*. 1994;126(4):827-837. doi: 10.1083/jcb.126.4.827.
11. Noel KI, Khamees NH, Abdulateef HH. Assessment of the effect of glucocorticoid injections on the expression of apoptotic active CASP3 and ALDH1A1 renal cell markers in New Zealand rabbits. *Eastern J Med*. 2024;29(4):397-405. doi: 10.5505/ejm.2024.39049.
12. Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in health and disease. *Am J Reprod Immunol*. 2010;64(3):159-169. doi: 10.1111/j.1600-0897.2010.00837.x.
13. Smith SC, Baker PN, Symonds EM. Increased placental apoptosis in intrauterine growth restriction. *Am J Obstet Gynecol*. 1997;177(6):1395-1401. doi: 10.1016/s0002-9378(97)70081-4.
14. Ponder KG, Boise LH. The prodomain of caspase-3 regulates its own removal and caspase activation. *Cell Death Discov*. 2019;5:56. doi: 10.1038/s41420-019-0142-1.
15. Abdulateef HH, Shukri EG, Ahmed BS. Immunohistopathological co-expression of NANOG and OCT4 in normal tissue around papillary thyroid cancer and multinodular goiter. *Biochem Cell Arch*. 2020;20(2):4477-4485.
16. Zhang W, Ni P, Mou C, Zhang Y, Guo H, Zhao T, et al. Cops2 promotes pluripotency maintenance by stabilizing nanog protein and repressing transcription. *Sci Rep*. 2016;6:26804. doi: 10.1038/srep26804.
17. Hambiliki F, Ström S, Zhang P, Stavreus-Evers A. Co-localization of NANOG and OCT4 in human pre-implantation embryos and in human embryonic stem cells. *J Assist Reprod Genet*. 2012;29(10):1021-1028. doi: 10.1007/s10815-012-9824-9.
18. Al-Kaabi M, Noel K, Al-Rubai A. Evaluation of immunohistochemical expression of stem cell markers (NANOG and CD133) in normal, hyperplastic, and malignant endometrium. *J Med Life*. 2022;15(1):117-123. doi: 10.25122/jml-2021-0206.
19. Novac MV, Iliescu DG, Tudorache S, Manolea M, Meetescu RE, Vrabie S, et al. Ultrasound evaluation of fetal biometry and doppler parameters in the third trimester of pregnancy suspected of intrauterine growth restriction. *Curr Health Sci J*. 2018;44(1):23-28. doi: 10.12865/CHSJ.44.01.04.
20. Al-Rubai A, Mohammed IM, Fakhir HA, Noel K, Eleiwi SA. Comparison of placental expression of basic fibroblast growth factor and insulin-like growth factor-1 in placentae of normal, pregnancy-induced hypertension, and preeclamptic pregnancies in Iraqi mothers. *Med J Babylon*. 2023;20(4):681-688. doi: 10.4103/MJBL.MJBL_40_23.
21. Hameed AF, Noel KI, Akkila SS. Placental angiogenesis, IUGR & CMV awareness in Iraqi women. Curr Issues Pharm Med Sci. 2022;35(3):147-151. doi: 10.2478/cipms-2022-0027.
22. Noel KI, Ibraheem MM, Ahmed BS, Hameed AF, Khamees NH, Akkila SS. Expression of OCT4 stem cell marker in benign prostatic hyperplasia and normal tissue around the prostatic carcinoma in a sample of Iraqi patients. *Egypt J Histol*. 2020;43(1):245-254. doi: 10.21608/ejh.2019.13791.1130.

23. Noel KI, Ibraheem MM, Ahmed BS, Hameed AF, Khamees NH, Akkila SS. CD133 and CD166 expression predicting the possibility of prostatic cancer development in cases of BPH. *Biomed Pharmacol J.* 2019;12(3):1403-1416. doi: 10.13005/bpj/1769.
24. Weber M, Göhner C, San Martin S, Vattai A, Hutter S, Parraga M, et al. Unique trophoblast stem cell- and pluripotency marker staining patterns depending on gestational age and placenta-associated pregnancy complications. *Cell Adh Migr*. 2016;10(1-2):56-65. doi: 10.1080/19336918.2016.1142035.
25. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod*. 2003;69(1):1-7. doi: 10.1095/biolreprod.102.014977.
26. Levy R, Nelson DM. To be, or not to be, that is the question. Apoptosis in human trophoblast. *Placenta*. 2000;21(1):1-13. doi: 10.1053/plac.1999.0450.
27. Stepan H, Leo C, Purz S, Höckel M, Horn LC. Placental localization and expression of the cell death factors BNip3 and Nix in preeclampsia, intrauterine growth retardation and HELLP syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2005;122(2):172-176. doi: 10.1016/j.ejogrb.2005.01.017.
28. Levy R, Smith SD, Yusuf K, Huettner PC, Kraus FT, Sadovsky Y, et al. Trophoblast apoptosis from pregnancies complicated by fetal growth restriction is associated with enhanced p53 expression. *Am J Obstet Gynecol*. 2002;186(5):1056-1061. doi: 10.1067/mob.2002.122250.
29. DiFederico E, Genbacev O, Fisher SJ. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *Am J Pathol*. 1999;155(1):293-301. doi: 10.1016/S0002-9440(10)65123-1.
30. Allaire AD, Ballenger KA, Wells SR, McMahon MJ, Lessey BA. Placental apoptosis in preeclampsia. *Obstet Gynecol*. 2000;96(2):271-276. doi: 10.1016/s0029-7844(00)00895-4.
31. Smith SC, Baker PN, Symonds EM. Increased placental apoptosis in intrauterine growth restriction. *Am J Obstet Gynecol*. 1997;177(6):1395-1401. doi: 10.1016/s0002-9378(97)70081-4.
32. Mayhew TM, Huppertz B, Kaufmann P, Kingdom JC. The 'reference trap' revisited: examples of the dangers in using ratios to describe fetoplacental angiogenesis and trophoblast turnover. *Placenta*. 2003;24(1):1-7. doi: 10.1053/plac.2002.0878.
33. Huppertz B, Kingdom J, Caniggia I, Desoye G, Black S, Korr H, et al. Hypoxia favours necrotic versus apoptotic shedding of placental syncytiotrophoblast into the maternal circulation. *Placenta*. 2003;24(2-3):181-190. doi: 10.1053/plac.2002.0903.
34. Aban M, Cinel L, Arslan M, Dilek U, Kaplanoglu M, Arpaci R, et al. Expression of nuclear factor-kappa B and placental apoptosis in pregnancies complicated with intrauterine growth restriction and preeclampsia: an immunohistochemical study. *Tohoku J Exp Med*. 2004;204(3):195-202. doi: 10.1620/tjem.204.195.
35. Ishihara N, Matsuo H, Murakoshi H, Laoag-Fernandez JB, Samoto T, Maruo T. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. *Am J Obstet Gynecol*. 2002;186(1):158-166. doi: 10.1067/mob.2002.119176.
36. Kadyrov M, Schmitz C, Black S, Kaufmann P, Huppertz B. Pre-eclampsia and maternal anaemia display reduced apoptosis and opposite invasive phenotypes of extravillous trophoblast. *Placenta*. 2003;24(5):540-548. doi: 10.1053/plac.2002.0946.
37. Ghanem ME, Eleiwe SA, Tayeh NK. Association of idiopathic IUGR with placental histological morphometry. *Indian J Public Health Res Dev*. 2019;10(7):943-948. doi: 10.5958/0976-5506.2019.01699.1.
38. Ghanem ME, Eleiwe SA. Expression of e-NOS in human placentas of idiopathic intra uterine growth restriction at term. *Medico-Legal Update*. 2020;20(1):440-445. doi: 10.37506/v20/i1/2020/mlu/194660.
39. Sharp AN, Heazell AE, Crocker IP, Mor G. Placental apoptosis in health and disease. *Am J Reprod Immunol*. 2010;64(3):159-169. doi: 10.1111/j.1600-0897.2010.00837.x.
40. Noel KI. A reciprocal relationship between oxidative stress, antioxidants, and cancer: A review. *Siriraj Med J*. 2024;76(8):550-556. doi: 10.33192/smj.v76i8.268647.