Estabraq A. Mahmoud et al /J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2952-2959

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Histopathological changes of placenta in pregnant women complicated with pregestational diabetes

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Abstract

The study presented the results of a study on 68 cases of pregnant women with normal and diabetes who delivered in Department of Obstetrics and Gynaecology, in three Hospital at Baghdad city during the period from 1 December 2016 to 1 may 2017. After delivery, placentae were collected for the histolohgy and histopathological studies. Numerous changes have been recorded significantly in placentae of pregestational diabetes (DM) women included: crowding of villi, increased villous number of immature intermediate villi, decreased terminal villi density, increased terminal villi size, numerous syncytial knots, basement membrane thickening in terminal villi, cytotrophoblast in terminal villi, decreased vascular-syncytial membrane thickness, extravillous fibrinoid between terminal villi, fibrosis in terminal and stem villi, fibrinoid necrosis of stem and terminal villi, stem villi with indented margin, continued trophoblastic layer and stroma with respectable cell population, basement membrane thickening in immature intermediate villous, immature intermediate villi with loose reticular stroma, increased of Hofbauer cells population in immature intermediate villi, calcification intracellular as well as extracellular, chorangiosis, thickening of villi vessels, fetal vessel thrombosis, nucleated fetal RBCs, mature intermediate villi with continuous trophoblastic layer and villous edema, edema in terminal and stem villi. Although the damages presented, when analyzed individually, may incidence in other diseases, when summarized they are highly suggestive for pregnancy related with diabetes we can diagnose.

Keywords: Placenta, Pregestational diabetes, Histopathology, Pregnancy.

INTRODUCTION

Diabetes mellitus is a chronic, lifelong condition that effects on body's ability to use the energy found in food. Diabetes in pregnancy is important because of increasing rates of the disease and its effect on maternal, fetal and neonatal health, such as preeclampsia, primary caesarean delivery, macrosomia and birth injury and clinical neonatal hypoglycaemia. Many studies of placentae in diabetes mellitus have created confusing and contradictory results due to the failure to account for confounding variables such as the type and severity of disease, degree of control of hyperglycaemia, premature delivery, and other associated abnormalities such as preeclampsia, fetal birth weight and diagnostic conditions [1].

The placenta is the organ accounting for the transfer of almost all nutrients and gases between mother and fetus. Thus, the placenta achieves fundamental role sustaining adequate fetal growth, and it has been implicated in abnormal fetal growth such as it is well recognized as a problem in pregnancy complicated by type 1 diabetes [2]. Type 2 diabetes insults at the onset of gestation have long-term effects on placental development. These adaptive responses of the placenta to the diabetic milieu, such as buffering excess maternal glucose or improved vascular resistance, may aid limit fetal growth within a normal range. If the period or extent of the diabetic insult, containing maternal hyperglycemia, hyperinsulinemia or dyslipidemia, exceeds the placental capacity to mount adequate responses, then excessive fetal growth may result [3]. Human pregnancy and in specific the first trimester, is a period highly susceptible towards confrontational insults such as oxidative stress, which may lead to insufficient embryonic and feto-placental development. Maternal type 1 and 2 diabetes increase cellular stress in the placenta between 7-9 weeks of pregnancy and because of underlying stressors may affect early and late placental development [4].

Thus, the present study aims to increase our knowledge about histopathological changes in placenta of pregestational diabetes due to very few studies have been done on histopathological changes in placenta of pregestational diabetic mother and prospect study in this field will go a long way in discovery a solution in assessing the distractive changes in diabetic placenta in different stages of pregnancy.

MATERIALS AND METHODS

The mothers' informed consents were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health. Fresh placentae were obtained from Department of Obstetrics and Gynaecology in three Hospital at Baghdad city during the period from 1 December 2016 to 1 may 2017. A total of 68 placentae were collected directly after delivery for the study and divided into two groups. First group, normal women and second group included women with pregnancies complicated by pregestational diabetes mellitus (DM) (cases n=34), (17 diabetes type 1, 17 diabetes type 2). Placentae were cut and sampled for histological examination. Two standard samples were taken from intact maternal surface and fetal surface and one from the central area and other from the peripheral area. Fresh placental tissue pieces were fixed with formalin (10%) [5]. Then tissue samples from placentae after delivery were prepared for histopathological studies according to routine paraffin methods [6]. Haematoxylin and Eosin staining was accomplished according to [7].

The sections were examined by compound light microscope (Meijitechno, Japan) with digital camera (Canon, Japan, 18 megapixels). Images were analysed independently with the help of expert pathologist by Multihead teaching microscope (Genex, USA). The images were captured with a Live View Pro digital camera directly into the computer in advanced embryology laboratory, Department of Biology, College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad then adding scale bars to all images by using ImageJ software.

The data analysis was performed using SPSS program version 15 and 18 (SPSS Inc., Chicago, IL, USA). Analysis of variance using independent t-test were used as appropriate test for morphological and histological parametric data. The level of significance was at the limits of agreement (95% confidence interval of the difference between the two groups, and values of $P \leq 0.05$ and $P \leq 0.001$ were considered statistically significant and highly significant, respectively between the studied groups.

RESULTS

Histopathological examination of the sections from the placentae of DM mothers showed numerous changes (Figure 1) including: crowding of villi (Fig.2-A), increased villous number of immature intermediate villi and decreased terminal villi density as well as increased terminal villi size (Fig.2-B), numerous syncytial knots (Fig. 2-C), basement membrane thickening, cytotrophoblast and decreased vascular-syncytial membrane thickness in terminal villi (Fig. 2-D), extravillous fibrinoid between terminal villi and fibrosis in terminal and stem villi (Fig. 2-E), fibrinoid necrosis of stem and terminal villi (Fig. 2-F), stem

villi with indented margin, continued trophoblastic layer and stroma with respectable cell population (Fig. 2-G), basement membrane thickening in immature intermediate villous (Fig. 2-H), immature intermediate villi with loose reticular stroma and increased of Hofbauer cells population in immature intermediate villi (Fig. 2-I), calcification intracellular as well as extracellular (Fig. 2-J), chorangiosis (Fig. 3-K), thickening of villi vessels and thrombosis(Fig. 3-L), nucleated fetal RBCs (Fig. 3-M), mature intermediate villi with continuous trophoblastic layer villous edema in mature intermediate (Fig. 3-N) edema in terminal (Fig. 3-O) and stem villi (Fig. 3-P) as compared to the placentae of normal mother (Figure 4, 5) which was found to be statistically significant differences with a value of P≤0.001 or P≤0.05.

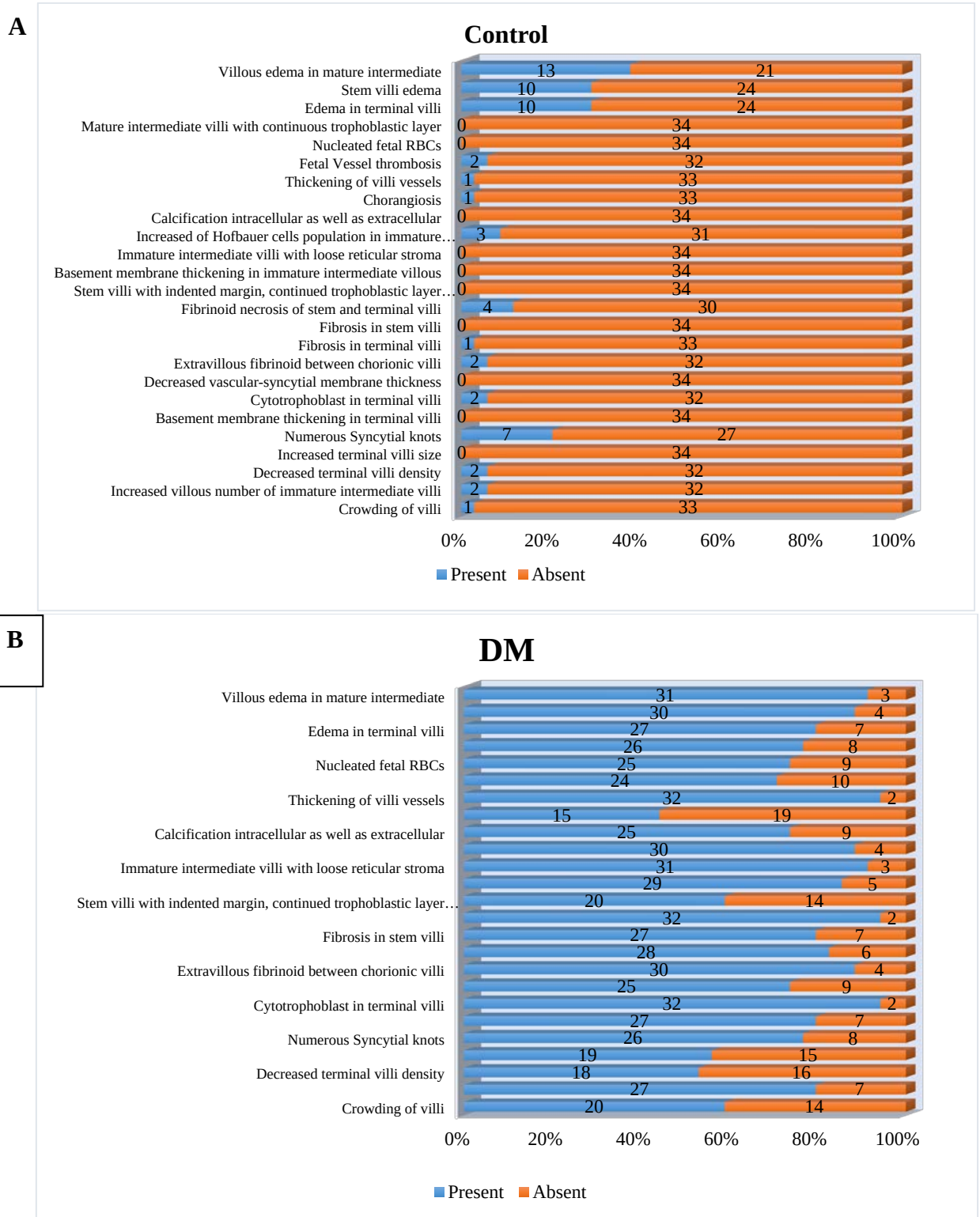

Figure 1: Comparison of histopathological features of placenta in control (A) and DM (B) groups

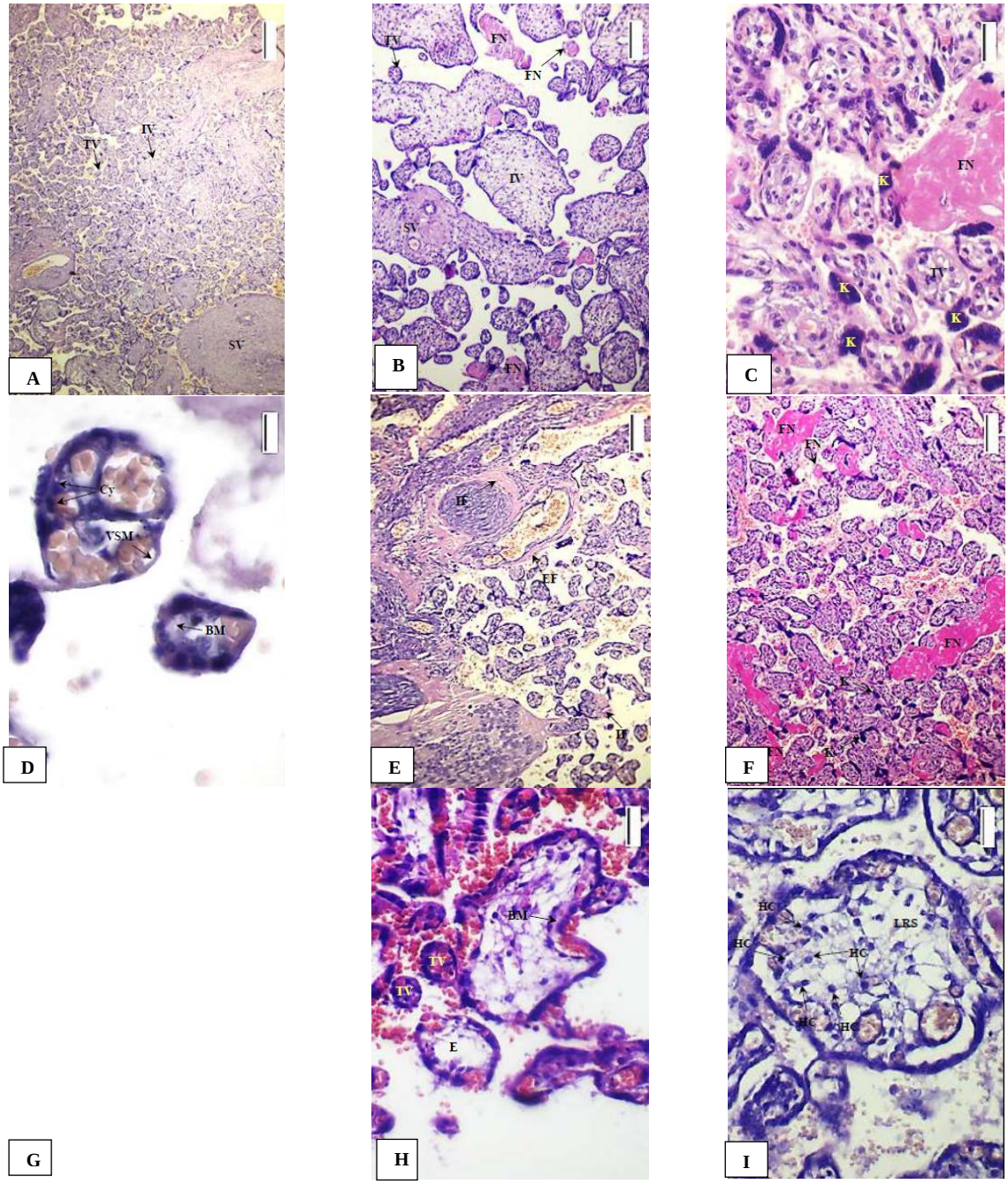

Figure 2: A-I Section of placenta in women complicated with DM showing: A- Crowded villi, (4X); B- Decreased terminal villi density and increased size as well as increased immature intermediate villi (10X); C- Numerous syncytial knots (40X); D- Basement membrane thickness in terminal villi, more cytotrophoblast and decreased vascular-syncytial membrane thickness in terminal villi (100X); E- Present extravillous fibrinoid between chorionic villi and fibrosis inside terminal and stem villi (10X), F- Present fibrinoid necrosis in terminal and stem villi (40X), G- Stem villi with indented margin, continued trophoblastic layer and stroma with respectable cell population (arrow head) (10X), H- Thickening of syncytiotrophoblast basement membrane in immature intermediate villi (40X), I- Immature intermediate villi with more loose reticular stroma and Hofbauer cells population (40X); stem villous (SV) and mature intermediate villous (MV), immature intermediate villous (IV), terminal villous (TV), fibrinoid necrosis (FN), fibrosis inside (IF) chorionic villi, syncytial knots (K), basement membrane (BM), cytotrophoblast (Cy), vascular-syncytial membrane (VSM), syncytiotrophoblast (Sy), Hofbauer cell (HC), fetal blood vessel (FBV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (IMC), edema (E), loose reticular stroma (LRS); (H&E) (4X, Scale bar 500 µm); (10X, Scale bar 200 µm); (40X, Scale bar 50 µm); (100X, Scale bar 20 µm).

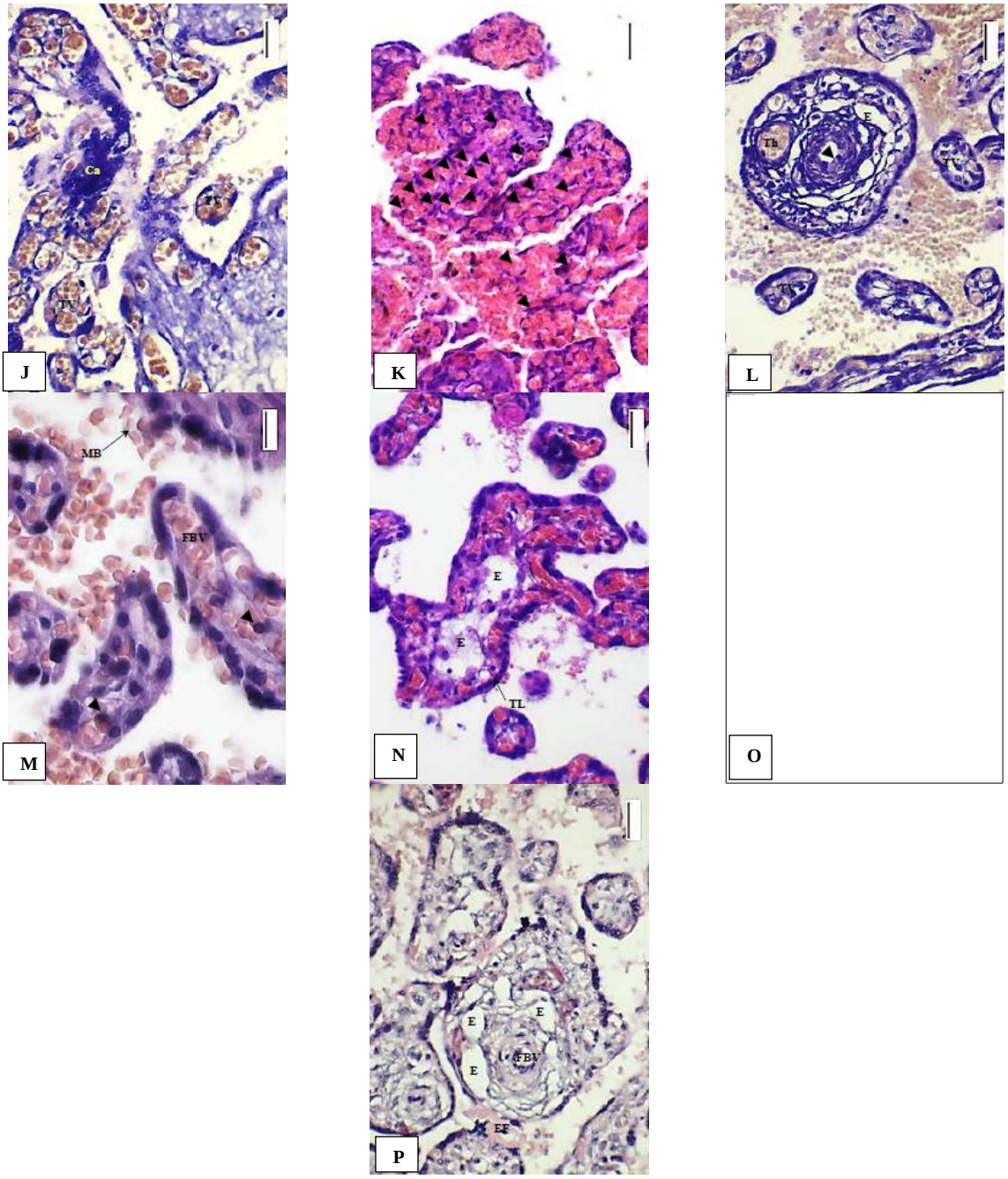

Figure 3: J-P Section of placenta in women complicated with DM showing: J- Placental calcification (40X); K- Chorangiosis (arrow head) (40X), L- Thickening of villi vessels (arrow head) and vessel thrombosis (40X), M- Present nucleated fetal RBCs (arrow head) (100X), N- Contained mature intermediate villi with continuous trophoblastic layer and edema in mature intermediate (40X), O- Edema in terminal villi (40X), P- Edema in stem villi (40X); calcification (CA), vessel thrombosis (Th), terminal villous (TV), edema (E), extravillous fibrosis, maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (TL), syncytial knot (K), fibrinoid necrosis (FN), extravillous fibrosis (EF).

DISCUSSION

In current study, we evaluate the histopathological changes of the 34 placentae from women with diabetes in comparison with normal woman was to quantify as much as possible the broadest range of changes in placental structures registered over time in the literatures. Crowding of villi was seen to be increased significantly higher in DM group in comparison with control group. [8] reported that histological anomalies such as presence crowding of villi was more frequently observed in diabetic placenta this finding agreed with our study. Previous studies have detected an increased occurrence of immature intermediate villi in placentae affected by either DM [9] compared to normal pregnancies. Due to defect in placental maturation have been related with chronic fetal hypoxia [10], a larger rate of immature intermediate villi may be revealing of a better preuterine hypoxic environment. Increasing the size of villi especially terminal and immature intermediate villi, gives the false impress of increased terminal villous density [11]. Number of studies reported an association between the frequency of immature villi and insufficient or absent terminal villi in pregestational diabetes [9]. Terminal villous size significantly increased in both diabetes groups compared to controls this result agreed with [12; 13] studies. Increased numerous of syncytial knots, bridges and sprouts are called as syncytial knotting or Tenny-Parker changes [14]. In past studies, done by [8; 11; 15], as compared with the normal placenta, placentae from pregnant

women with diabetes showed an increased incidence syncytial knots. [16; 17; 18] noticed increasing thickening trophoblastic basement membrane were present in most of the diabetic placenta in comparison of normal women. This histological change of placenta are mainly due to metabolic disturbances that leads to accumulation of carbohydrate and fat in the placenta. Whereby, this thickening is the consequence of mucopolysaccharide storage and it could be attributed to distributed villous trophoblastic activity such as increased production or decreased transformation of basement membrane molecules, as it is recognized that components of basement membrane constituents are produced by the secretion of trophoblast [14].

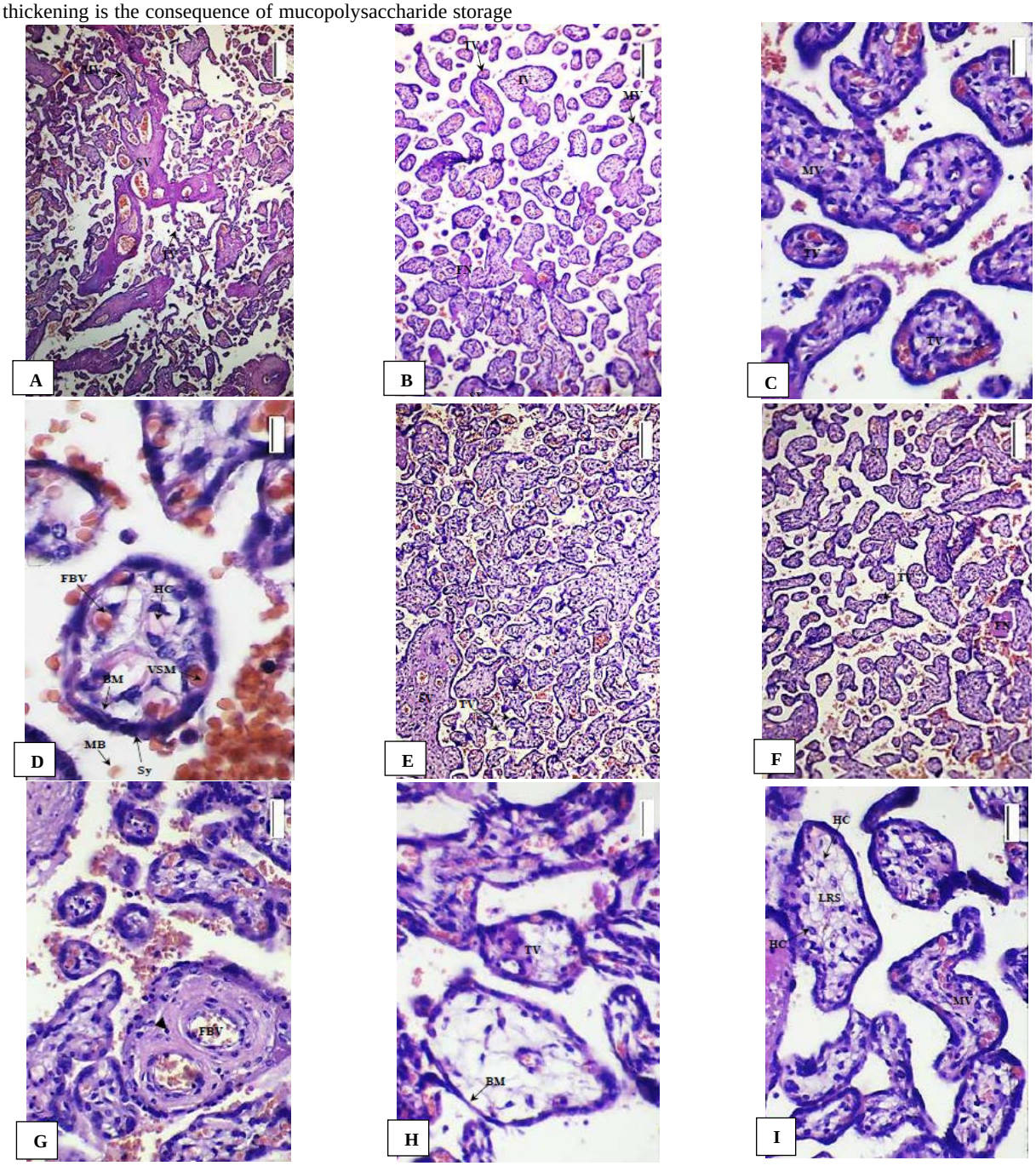

Figure 4: A-I Section of placenta in normal women showing: A- Normal crowded villi, (4X); B- Normal terminal villi density and size as well as normal immature intermediate villi (10X); C- Few numerous syncytial knots (40X); D- Normal basement membrane in terminal villi, few cytotrophoblast and normal vascular-syncytial membrane thickness in terminal villi (100X); E- No extravillous fibrinoid between chorionic villi and fibrosis inside terminal and stem villi (10X), F- Few fibrinoid necrosis in terminal and stem villi (40X), G- Stem villi with no indented margin, no continued trophoblastic layer and stroma with normal respectable cell population (arrow head) (10X), H- Normal syncytiotrophoblast basement membrane in immature intermediate villi (40X), I- Immature intermediate villi with normal loose reticular stroma and Hofbauer cells population (40X); stem villous (SV) and mature intermediate villous (MV), immature intermediate villous (IV), terminal villous (TV), fibrinoid necrosis (FN), syncytial knots (K), basement membrane (BM), cytotrophoblast (Cy), vascular-syncytial membrane (VSM), syncytiotrophoblast (Sy), Hofbauer cell (HC), fetal blood vessel (FBV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (IMC), loose reticular stroma (LRS); (H&E) (4X, Scale bar 500 µm); (10X, Scale bar 200 µm); (40X, Scale bar 50 µm); (100X, Scale bar 20 µm).

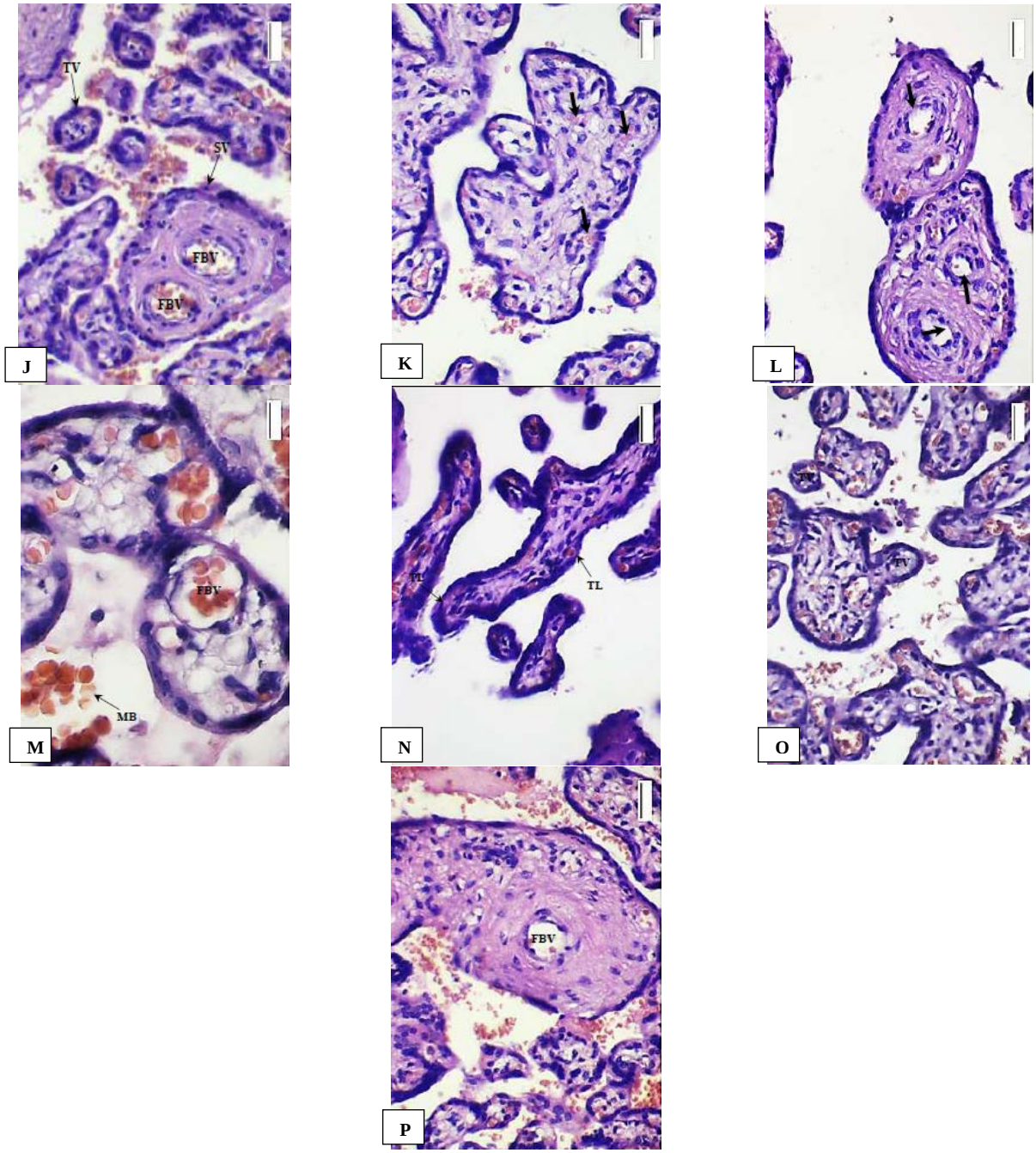

Figure 5: J-P Section of placenta in normal women showing: J- No calcification (40X); K- No chorangiosis (arrow) (40X), L- Normal thickening of villi vessels (arrow) and no vessel thrombosis (40X), M- No nucleated fetal RBCs (100X), N- Mature intermediate villi without continuous trophoblastic layer and few edema (40X), O- Few edema in terminal villi (40X), P- Few edema in stem villi (40X); terminal villous (TV), maternal blood (MB), fetal blood vessel (FBV), trophoblastic layer (TL).

However, in diabetic placentae, most of the times, one can observe the presence cytotrophoblast, being another sign of abnormal immature villous [19]. [20; 21] found that placental anomalies in pregestational diabetes including increased cytotrophoblastic, therefore this finding in agreement with our study. The barrier between maternal and fetal circulation is reduced by the thinning of the vascular-syncytial membrane. This can negatively affect the transplacental transport, metabolism, and oxygen distributing [22]. Decreased vasculo-syncytial formation can be due to the delayed villous maturation that might be the etiology for the improved risk for intrauterine losses in diabetic women [23].

Extravillous fibrinoid has a lamellar construction and this fibrinous layer can be in connection with the villous trophoblastic edge. This type of fibrinoid either fills spaces in the trophoblastic layer, or contains all chorionic villi or collections of villi [17]. Increased of extravillous fibrinoid deposits are reflected pathological phenomena and it was frequently inconsistent with normal fetal growth [24]. Stromal fibrosis is descripted abnormal when increased in the stem villi. In diabetic women, there is an increased villous stromal oxygen partial pressure, in the side of insufficient uptake by the fetal capillaries, which prompts the synthesis of collagen [25]. At placentae of diabetic women, the fibrinoid material had increased by pushing the basement membrane and pressing the complete villous stroma [14]. Histological pathologies such as the presence of fibrinoid necrosis were detected more repeatedly in DM [16; 26; 27] placentae compared with the control placenta. Regarding stem villi with

depressed margin, continued trophoblastic layer and stroma with suitable cell population, this feature permitted us to recognize villous immaturity was the presence of numerous cell populations in the villous stroma especially in stem villi. Therefore, [11; 28] found to be continued trophoblastic layer and cell population more common in DM group. [11; 29] recorded increased mature intermediate villi with continuous trophoblastic layer and as the current study this feature present more frequent with DM placentae. Noticeable thickening of the syncytiotrophoblast basement membrane was described in numerous pathological conditions; one of them is maternal diabetes. This thickening of syncytiotrophoblast basement membrane was resulted of a higher degree of nonenzymatic glycosylation or an increased quantity of the prominent type of basal lamina collagen, type IV. As well as higher substances of DNA, phospholipids, triglyceride, and of cholesterol are distinguishing features of placenta in diabetes women [14].

Immature intermediate villi are characterised by the existence of a large stroma, loose reticular noticeable channels having Hofbauer cells. These villi dominate during the second trimester of pregnancy, continuing to term only in small amount. The most often reported alteration in the placenta of diabetic women is the relative immaturity of villous, however a closely best metabolic control in these women [22]. This suggestion in agreement with [30; 31], who investigate the changes in morphology immature intermediate villi and density of Hofbauer cells in placentae from normal and diabetic pregnancies. The placental depositions are consisted of calcium phosphate [32]. These depositions are organized mostly near maternal surface in the basal plate, along the septa, perivillous space, sub chorionic space and basement membrane of placental villi, maybe due to its ability to act as placental calcium pump [33]. In many studies, calcifications observed as intracellular as well as extracellular basophilic deposits after stained with haematoxylin and eosin in the placentae of DM group [34; 35]. The increased villous chorangiosis probably a response to the relative hypoxemia due to an increase of VEGF expression [36] and the immaturity of the villi, which considered by centrally placed villous capillaries causing in a greater space for oxygen and nutrients to permit from maternal to fetal exchange [37]. Numerous studies described presence of chorangiosis significantly increased in placentae of pregnancies with DM [16; 21; 37].

Thickness villi vessel walls due to endothelial proliferation and thickening of the basement membrane. As well as it has been observed that increased blood glucose levels prompt oxidative stress (OS) and following variations of the placental architecture [18] principally the vascular properties, which are obvious in diabetic women. Similar results were obtained by [19] in placentae of DM women. The blood vessels in some of the terminal villi showed occulting by thrombus in DM [16] placentae, this finding in line with current study. This feature was diagnosed when a large fetal stem villous vessel was partly or completely obstructed by a thrombus [31] (Bhattacharjee *et al*., 2017).

Nucleated fetal RBC's are present in the placental vessels during the first-trimester of pregnancy, but are rare later in pregnancy and are generally absent or existing only in small numbers at term [38]. These results were associated with fetal damage and reduced fetal oxygenation in diabetic women [36]. [3; 16] and reported that histological anomalies such as presence of nucleated fetal RBC's, were more frequently observed in diabetic placenta. The accumulation of fluid in the stroma of placental villi called edema. As hyaluronic acid molecules have the particularity to retain water, it was concluded that, the existence of abnormal deposits of mucopolysaccharides in the stroma of villi can lead to the presence of the true villous edema in placentae of diabetic pregnancies [40]. [20; 21] found that increased villous edema have been described with pregestational diabetes.

Lastly, concerning Iraqi studies, one study [41] was conducted in Erbil City showed significant increase complications in diabetic pregnancy revealed immaturity of placentae villi, edema of the villi, and intervillous. As well as [42] indicated in their study at Hilla city that the terminal villi in placentae of diabetes women controlled on insulin appeared the increased number of syncytial knots and the stroma of the villi showed villous edema, fibrinoid necrosis and fetal vessels proliferation.

REFERENCES

1. Lowe, L. P.; Metzger, B. E.; Dyer, A. R.; Lowe, J.; McCance, D. R.; Lappin, T. R.; Trimble, E. R.; Coustan, D. R.; Hadden, D. R.; Hod, M.; Oats, J. J.; Persson, B. and HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcome (HAPO) study: associations of maternal A1C and glucose with pregnancy outcomes. Diabetes Care, 2012, 35: 574-580. doi: 10.2337/dc11-1687.
2. Wang, Y. Vascular biology of the placenta, 2nd (edn.). Morgan & Claypool Life Sciences, Mississippi, USA: 2017, 113 pp. DOI: 10.4199/C00153ED1V01Y201704ISP075.
3. Starikov, R.; Inman, K.; Chen, K.; Lopes, V.; Coviello, E.; Pinar, H. and He M. Comparison of placental findings in type 1 and type 2 diabetic pregnancies. Placenta, 2014, 35(12): 1001-1006. doi: 10.1016/j.placenta.2014.10.008.
4. Gauster, M.; Majali-Martinez, A.; Maninger, S.; Gutschi, E.; Greimel, P.H.; Ivanisevic, M.; Djelmis, J.; Desoye, G. and Hiden, U. Maternal Type 1 diabetes activates stress response in early placenta. Placenta, 2017, 50: 110-116. doi: 10.1016/j.placenta. 2017.01.118.
5. Yung, H. W.; Colleoni, F.; Atkinson, D.; Cook, E.; Murray, A. J.; Burton, G. J. and Charnock-Jones, D. S. Influence of speed of sample processing on placental energetics and signalling pathways: Implications for tissue collection. Placenta 2014, 35: 103e108. https://doi.org/10.1016/j.placenta. 2013.11.016.
6. Feldman, A. T. and Wolfe, D. Tissue Processing and Hematoxylin and Eosin Staining. In: Histopathology Methods and Protocols, Methods in Molecular Biology, vol. 1180. Day, C. E. (Ed.). Springer Science+Business Media New York, USA: 2014, 31-43. DOI 10.1007/978-1-4939-1050-2_3.
7. Bancroft, J. D. and Layton, C. The hematoxylins and eosin. In: Bancroft's Theory and Practice of Histological Techniques, 7th edn. Suvarna, S. K.; Layton, L. and Bancroft, J. D. (Eds.). Churchill Livingstone Elsevier Ltd., Shanghai, China: 2013, 173-186.
8. Tewari, V.; Tewari, A. and Bhardwaj, N. Histological and histochemical changes in placenta of diabetic pregnant females and its comparision with normal placenta. Asian Pacific J. Trop. Dis. 2011, 1(1):1-4. https://doi.org/10.1016/S2222-1808(11) 60001-7.
9. Evers, I. M.; Nikkels, P. G.; Sikkema, J. M. and Visser, G. H. A. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. Placenta, 2003, 24: 819-825. doi:10.1016/S0143-4004(03)00128-0.
10. Stallmach, T.; Hebisch, G.; Meier, K.; Dudenhausen, J. W. and Vogel, M. Rescue by birth: defective placental maturation and late fetal mortality. Obstet. Gynecol., 2001, 97(4): 505-509.
11. Gheorman, L.; Pleşea, I. E. and Gheorman V. Histopathological considerations of placenta in pregnancy with diabetes. Rom. J. Morphol. Embryol., 2012, 53(2): 329-336.
12. Higgins, M.; Felle, P.; Mooney, E. E.; Bannigan, J. and McAuliffe, F. M. Stereology of the placenta in type 1 and type 2 diabetes. Placenta, 2011, 32(8): 564-569. doi: 10.1016/j.placenta. 2011.04.015.
13. Beauharnais, C. C.; Roberts, D. J. and Wexler, D. J. High rate of placental infarcts in type 2 compared with type 1 diabetes. J. Clin. Endocrinol. Metab., 2012, 97(7): E1160-E1164. doi: 10.1210/jc.2011-3326.
14. Benirschke, K.; Burton, G. J. and Baergen R.N. Pathology of the Human Placenta, 6th(edn.). Springer-Verlag, Berlin, Heidelberg, Germany: 2012, DOI: https://doi.org/10.1007/978-3-642-23941-0_8.
15. Mishra, N.; Jamila, A. and Devi, N. S. Pathological Changes in Placentae of Diabetic Mothers & Its Association with Fetal Outcome. J. Dent. Med. Sci., 2017, 16(8): 93-99. DOI: 10.9790/0853-1608069399.

16. Khaskhelli L.; Memon, S.; Goswami, P. and Bano, S. Change in Normal Morphology of Placenta and Its Possible Effects on Fetal Outcome in Diabetic Mothers as Compared to Non-Diabetic Mothers. JLUMHS 2013, 12(1): 49-54.
17. Treesh, S. A. and Khair, N. S. Histological changes of the human placenta in pregnancies complicated with diabetes. J. Cytol. Histol., 2015, 6(2): 307. doi:10.4172/2157-7099.1000307.
18. Bhanu, S. P.; Sankar, D. K.; Swetha, M.; Kiran, S. and Devi, S. V. Morphological and micrometrical changes of the placental terminal villi in normal and pregnancies complicated with gestational diabetes mellitus. J. Evid. Based Med. Healthc., 2016, 3(68): 3676-3680. DOI: 10.18410/jebmh/2016/789.
19. Gheorman, V.; Gheorman, L.; Ivănuş, C.; Pană, R. C.; Gogănău, A. M. and Pătraşcu A. Comparative study of placenta acute fetal distress and diabetes associated with pregnancy. Rom. J. Morphol. Embryol., 2013, 54(3):505-511.
20. Gauster, M.; Desoye, G.; Tötsch, M. and Hiden, U. The placenta and gestational diabetes mellitus. Curr. Diab. Rep., 2012,12: 16-23. DOI 10.1007/s11892-011-0244-5.
21. Bentley-Lewis, R.; Dawson, D. L.; Wenger, J. B.; Thadhani, R. I. and Roberts, D. J. Placental Histomorphometry in Gestational Diabetes Mellitus: the relationship between subsequent type 2 diabetes mellitus and race/ethnicity. Amer. J. Clin. Pathol., 2014, 141(4): 587-592. http://doi.org/10.1309 /AJCPX81AUNFPOTLL.
22. Gersell, D. J. and Krau, F. T. Diseases of the Placenta. In: Blaustein's Pathology of the Female Genital Tract (6th ed.). Kurman, R. J.; Ellenson, L. H. and Ronnett, B. M. (Eds.). Springer Science+Business Media LLC., New York, USA: 2011, 1000-173. DOI 10.1007/978-1-4419-0489-8_19.
23. Augustine, G.; Pulikkathodi, M.; Renjith, S. and Jithesh, T. K. A study of placental histological changes in gestational diabetes mellitus on account of fetal hypoxia. Int. J. Med. Sci. Public, 2016, 5(12):2457-2460. DOI: 10.5455/ijmsph.2016.29042016494.
24. Castellucci, M. and Kaufmann, P. Basic Structure of the Villous Trees. In: Pathology of the Human Placenta, 6th (edn.). Benirschke, K. and Kaufmann, P. (Eds.). Springer Science+Business Media, LLC, New York, USA: 2006, 50-120. https://doi.org/10.1007/978-1-4757-4199-5_6.
25. Faye-Petersen, O. M; Heller, D. S. and Joshi, v. V. Handbook of Placental Pathology, 2nd(edn.). Taylor & Francis, an imprint of the Taylor & Francis Group, Oxon, UK: 2006, 328 pp.
26. Verma, R.; Mishra, S. and Kaul, J. M. Cellular changes in the placenta in pregnancies complicated with diabetes. Int. J. Morphol., 2010, 28(1): 259-264.
27. Shams, F.; Rafique, M.; Samoo, N. A. and Irfan, R. Fibrinoid necrosis and hyalinization observed in normal, diabetic and hypertensive placentae. J. Coll. Physicians Surg. Pak., 2012, 22(12): 769-72. doi: 12.2012/JCPSP.769772.
28. Dubova, E. A.; Pavlov, K. A.; Yesayan, R. M.; Nagovitsyna, M. N.; Tkacheva, O. N.; Shestakova, M. V. and Shchegolev, A. I. Morphometric characteristics of placental villi in pregnant women with diabetes. Bull. Exp. Biol. Med., 2011, 151(5): 650-654.
29. Maly, A.; Goshen, G.; Sela, J.; Pinelis, A.; Stark, M. and Maly, B. Histomorphometric study of placental villi vascular volume in toxemia and diabetes. Hum. Pathol., 2005, 36(10):1074-1079. DOI: 10.1016/j.humpath.2005.07.021.
30. Grigoriadis, C.; Tympa, A.; Creatsa, M.; Bakas, P.; Liapis, A.; Kondi-Pafiti, A. and Creatsas, G. Hofbauer cells morphology and density in placentae from normal and pathological gestations. Rev. Bras. Ginecol. Obstet. 2013, 35(9): 407-412.
31. Bhattacharjee, D.; Mondal, S. K.; Garain, P.; Mandal, P.; Ray, R. N. and Dey, G. Histopathological study with immunohistochemical expression of vascular endothelial growth factor in placentae of hyperglycemic and diabetic women. J. Lab., Physicians, 2017, 9(4): 227-233. http://doi.org/10.4103/JLP.JLP_148_16.
32. Poggi, S. H.; Bostrom, K. I.; Demer, L. L.; Skinner, H. C. and Koos, B. J. Placental calcification: A metastatic process? Placenta, 2001, 22(6): 591-596. DOI: 10.1053/plac.2001.0688.
33. Goswami, P.; Memon, S. and Pardeep, K. Morphological, histological and radiological study of calcified placenta and its relation with fetal outcome. J. Dent. Med. Sci., 2013, 7(6): 82-88.
34. Moran, M.; Mulcahy, C.; Daly, L.; Zombori, G.; Downey, P. and McAuliffe, F. M. Novel placental ultrasound assessment: potential role in pre-gestational diabetic pregnancy. Placenta, 2014, 35(8): 639-644. doi: 10.1016/j.placenta.2014.03.007.
35. Tandon, A; Singh, D.; Mishra, P. P. and Mishra, A. A morphology and histological study of placenta in normal and diabetic pregnancies. Int. J. Res. Med. Sci., 2018, 6: 1778-1781. DOI: http://dx.doi.org/10.18203/ 2320-6012.ijrms20181778.
36. Akarsu, S.; Bagirzade, M.; Omeroglu, S. and Büke, B. Placental vascularization and apoptosis in Type-1 and gestational DM. The J. Matern-Fetal Neonat. Med., 2016, 30(9): 16. https://doi.org/10.1080/ 14767058.2016.1199676.
37. Stanek, J. Chorangiosis of Chorionic Villi: What Does It Really Mean?. Arch. Pathol. Lab. Med., 2016, 140(6): 588-593. https://doi.org/10.5858/ arpa.2015-0160-OA.
38. Hermansen, M. C. Nucleated red blood cells in the fetus and newborn. Arch. Dis. Child Fetal Neonatal Ed., 2001, 84(3): F211-F215. doi: 10.1136/fn.84.3.F211.
40. Olejnik, A.; Goscianska, J.; Zielinska, A. and Nowak, I. Stability determination of the formulations containing hyaluronic acid. Int. J. Cosm. Sci., 2015, 37: 401-407. doi: 10.1111/ics.12210.
41. Ali, K. A.; Salih, L. S. and Almarzany, Z. S. The Role of Leptin and Insulin Hormones in the Pregnant Women Serum Infected of Diabetic Mellitus and its Histological Structure Effects on the Placenta and Umbilical Cord in Erbil City. Raf. J. Sci., 2012, 23(3): 38-49.
42. Al-Mamori, R. H. L. Morphopathology of human placenta in diabetic pregnancy. J. Biol. Agricult. Healthcare, 2014, 4(3): 99-104.