

## **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**

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### **ABSTRACT**

Diabetes in pregnancy is associated with a derangement of hormones, cytokines, metabolites and growth factors in the maternal and foetal compartment. These may influence placental and umbilical cord, growth and histological structure that are tightly regulated in time and space.

The blood and placenta and umbilical cord samples were collected in Hawler Maternity Hospital from (70) diabetic (Type-II) pregnant women and (30) healthy pregnant women directly at parturition. The placenta prepared for histo-pathological examination, while the serum used for both leptin and insulin estimation in both diabetic and non diabetic pregnant women.

Our results showed linear significant increase in leptin and insulin levels would all support the increased incidence of placental-related complications observed in diabetic pregnancy witch revealed immaturity of placentae villi, edema of the intima, and intervillous fibrosis in addition of ischemia of the villi. While umbilical cords sections represented thickening parietal layer and vacuolation. So these are found to improve the specificity of diabetic (Type-II) screening tests.

**Keywords:** placenta, diabetes mellitus, vscularisation, invasion, insulin, leptin.

(70)

(30)

## INTRODUCTION

The placenta is a complex fetal organ that fulfills pleiotropic roles during fetal growth. It separates the maternal and fetal circulation, with which it is in contact through different surfaces, i.e., the syncytiotrophoblast exposes the placenta to the maternal circulation and the endothelium is in contact with fetal blood. Because of this unique position, the placenta is exposed to the regulatory influence of hormones, cytokines, growth factors, and substrates present in both circulations and, hence, may be affected by changes in any of these. In turn, it can produce molecules that will affect mother and fetus independently (Dutta, 2004 ; Lindsay *et al.*, 2004 ; Jauniaux and Burton, 2006 and Nelson *et al.*, 2008).

The human placenta expresses virtually all known cytokines including tumor necrosis factor (TNF)- $\alpha$ , resistin, and leptin, which are also produced by the adipose cells. The discovery that some of these adipokines are key players in the regulation of insulin action suggests possible novel interactions between the placenta and adipose tissue in understanding pregnancy-induced insulin resistance. The interplay between the two systems becomes more evident in gestational diabetes mellitus; Placental development is characterized by three distinct periods. At the beginning of gestation, a series of critical proliferation and differentiation processes predominantly of the trophoblast eventually lead to the formation of villous and extravillous structures. The latter anchor the placenta in the uterus and remodel the uterine spiral arteries into low resistance vessels. Then the newly formed villi differentiate through various steps of maturation. The end of gestation is associated with placental mass expansion. During the first half of gestation, the trophoblast is the key tissue that undergoes the most profound alterations, whereas extensive angiogenesis and vascularisation occur in the second half of gestation, i.e., the endothelium is the site of the more prominent processes, although there is overlap. This period is also accompanied by extensive vascular remodeling and stabilization of the vascular bed (Mayhew, 2002a ; Kaufmann *et al.*, 2004).

Gernot and Sylvie, (2004) suggested that the prevalence of diabetes mellitus among women of child bearing age is increasing due to more sedentary lifestyles, changes in diet, and the virtual epidemic of childhood and adolescent obesity.

Classically, histological studies of type 1 diabetic placentas have described grossly abnormal placentas that are enlarged, thick, and plethoric, with abnormalities of villous maturation (Benirschke *et al.*, 2006). These changes would all support the increased

incidence of placental-related complications observed in diabetic pregnancy (Evers *et al.*, 2003; Benirschke *et al.*, 2006 ; Scott *et al.*, 2009).

According to Nelson *et al.*, (2008) and Marilza *et al.*, (2011) the fetal hormonal also response to hyperinsulinemia, fetal hyperinsulinemia has an independent positive association with birth weight and placental weight in offspring of mothers with type 1 diabetes.

Leptin hormone is associated with birth weight, correlated with placental weight and contributes to the matching of fetal and placental growth in control subjects and of diabetes mellitus. It is considered as an in utero signal of nutrient availability (Kadowaki *et al.*, 2006; Nelson *et al.*, 2008 ; Forhead and Fowden, 2009). The aim of this study was to determine the fluctuations in leptin and insulin levels and their correlation with histological structure of placenta and umbilical cord in diabetic pregnant women.

## MATERIALS AND METHODS

### A- Collection of blood samples at term

About 5ml venous blood samples were collected at the time of delivery (predators) from each pregnant participated in the study, by vein puncture using a 5 ml disposal syringe, after 12 hours overnight fasting. Samples were immediately transferred into sterile, plain tubes to obtain the serum by centrifugation at 3000 rpm for 15min. The separated (1.5 ml) serum was stored in 2 sterile, plain tubes to be frozen at -20C<sup>0</sup> (Sony, Wtralow, Japan), in Nanakaly hospital for the latter estimation of hormones: Insulin (Insulin ELISA RE 53171) and leptin (Leptin Sandwich ELISA ELA-2395).

### B- Histological preparation:

Placentae tissues were taken immediately at delivery from one hundred pregnancies at Hawler Maternity Hospital, and classified as follows: Type-II diabetes (n = 70), and normal pregnant (n = 30). Placentae prepared as a paraffin section method, the placental histopathological analysis were performed in a computerized image system coupled to a photomicroscope by a digital camera (Harstad and Klaassen, 2002).

### Statistical analysis

Analysis of data was performed by using SPSS (Version 11.5). Results are expressed as mean  $\pm$  S.E. The correlation analysis was done with Pearsons test. Independent unpaired student t -test was also used.  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

Human pregnancy is characterized by a series of metabolic changes that promote adipose tissue accretion in early gestation, followed by insulin resistance and facilitated lipolysis in late pregnancy. In early pregnancy, insulin secretion increases, while insulin sensitivity is unchanged, decreased, or may even increase (Mayhew and Sisley, 1998; Catalano *et al.*, 1999).

The distinct effects of the diabetic environment depend on the time in gestation when diabetic insult occurs. Because of its establishment in the second half of gestation, diabetes mellitus will influence placental processes in late gestation, whereas pre-gestational diabetes such as Type-I and Type-II diabetes may also affect processes in the first trimester (Benirschke *et al.*, 2006).

Altered placental function in pre-gestational diabetes may include changes in invasion ultimately leading to an enhanced risk of early pregnancy loss, growth restriction as well as a long-term stimulatory effect on placental growth leading to placentomegaly, which is frequently associated with diabetic pregnancies. Diabetes later in gestation affects vascularisation, storage of maternal nutrients in particular glycogen and lipids and may also enhance oxygen transfer. It is still unresolved if the placental alterations in diabetes ultimately contribute to or prevent the foetal phenotype often seen in diabetes i.e., excessive fetal fat accretion (Mayhew and Jairam, 2000 ; Scott, 2009).

**Table 1: the leptin and insulin levels of Type-II diabetes mellitus and non diabetic pregnant women.**

Parameters	Control	Diabetes	Statistical decision
Leptin (ng/ml)	26.244 ±0.566	45.682±0.858	P<0.001
Insulin (ng/ml)	40.9493±1.0	90.270±2.471	P<0.001

Independent unpaired student t -test

**Table 2: The correlation between leptin and insulin hormones of Type-II diabetic pregnant women.**

	r value	P value
<b>Pearson Correlation between Leptin and Insulin</b>	<b>0.939</b>	<b>0.001</b>

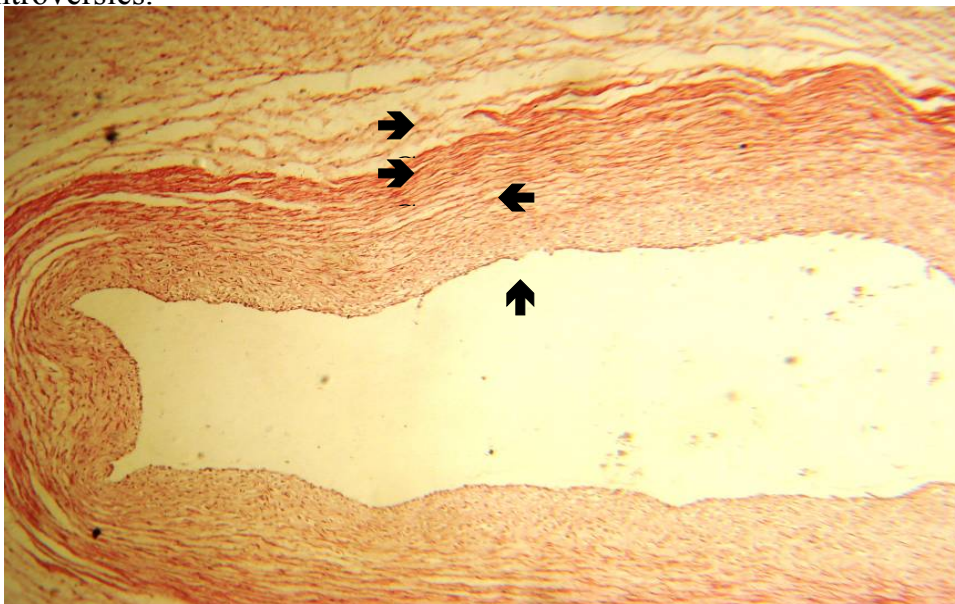
In present study insulin and leptin showed significant differences between diabetic and non diabetic patients during parturitions at ( $p<0.01$ ) respectively, (Table 1). As well as, (Table 2) showed positive and linear correlation between the two hormones. Since the diabetic environment can be regarded as a network of substances (hormones, nutrients, cytokines) with altered concentrations. The abnormal maternal metabolic environment may generate stimuli within the adipose tissue and the placental cells resulting in the increased production of inflammatory cytokines, whose expression is minimal under normal pregnancy, changes in circulating TNF- $\alpha$ , adiponectin, leptin, and resistin link inflammation to metabolic changes by enhancing insulin resistance in the mother. Those leads to elevated levels of insulin, leptin, and other cytokines have been well documented (Desoye and Mouzon, 2007). These changes would all support the increased incidence of placental-related complications observed in diabetic pregnancy (Mayhew, 2002b and Evers *et al.*, 2003).

Cross sections obtained from umbilical cords obtained from diabetic women showed thickening in the wall of umbilical's artery (Fig. 1) and thickening in the arteries wall, due to accumulation of vacuole and foam cells in intima (Fig. 2), in comparison with normal umbilical cord section (Fig. 12) that duo to observed diabetes associated with endothelial dysfunction which plays an important role in progression of vascular complications Horio *et al.*, (2002).

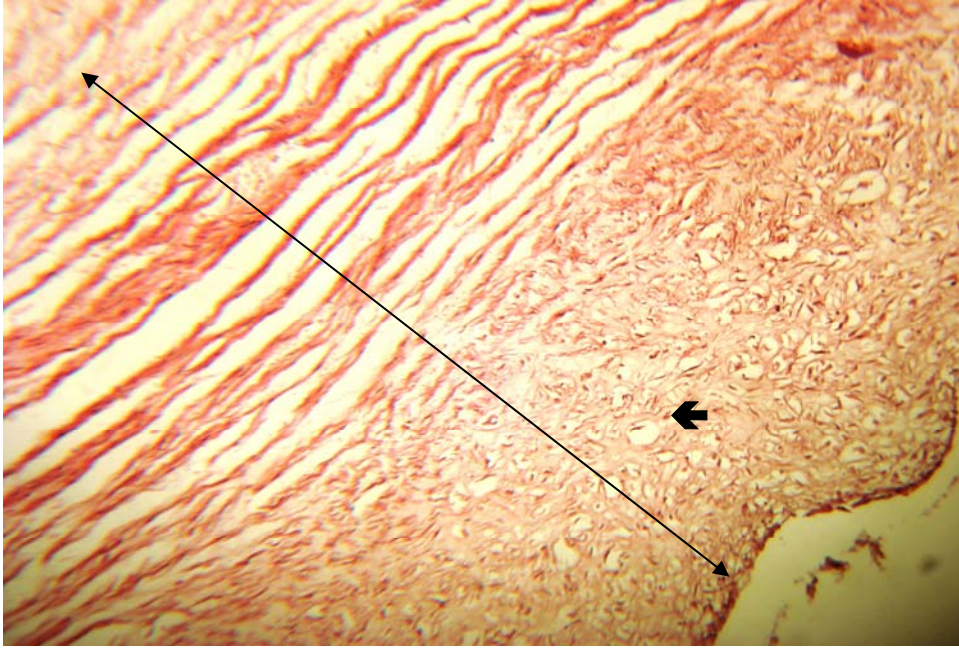
All histopathological changes were present in the placentae of the over diabetes mellitus of which were already observed in preterm and at term period. The placentas of the diabetes mellitus presented histopathological changes were characterized by ischemic villi

with increased syncytial sprouts and fibrin (Fig. 3), in comparison with normal placenta section (Fig. 11) this result in agreement with (Al-Okail and Al-Attas, 1994), they represent that fibrin thrombi, villous oedema, hyperplasia and thickening of basement membrane in the placenta and various changes in syncytiotrophoblast structure were marked in women with gestational diabetes or diabetes mellitus. While Ranjana *et al.*, (2010) showed that the present of large number of syncytial knots, fibrinoid necrosis, villous edema, villous fibrosis and proliferation of the capillaries which associated with diabetic can be controlled by insulin injection.

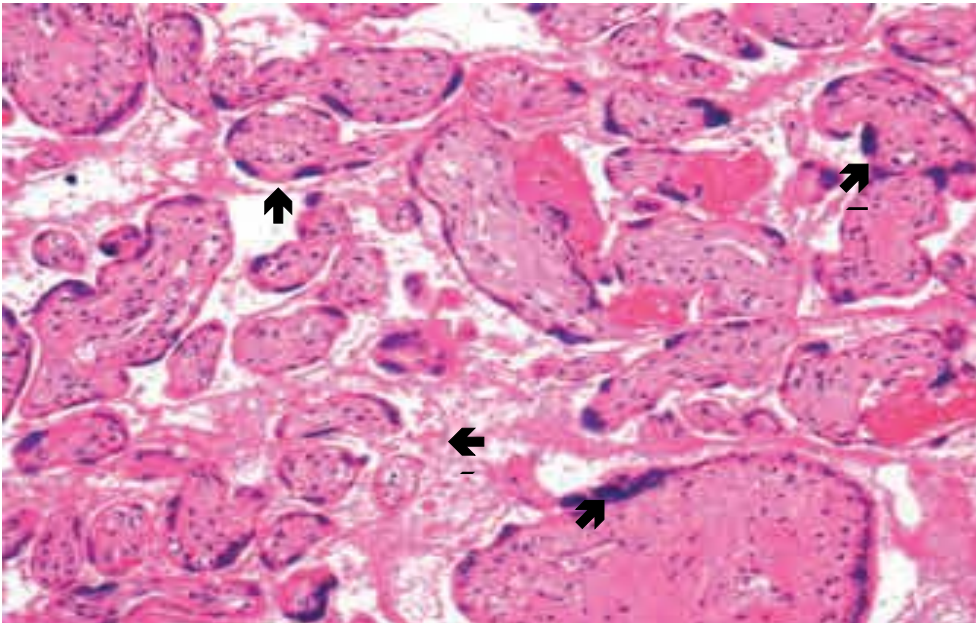
Dysmature villi with persistence of central vessels and increases nucleated red blood cell were seen in (Fig. 4), coagulation of placental villi (Fig. 5), coagulation of placenta associated with infiltration of inflammatory cells (Fig. 6), some of placental villi atrophied with sclerosis of placenta artery (Fig. 7), others showing cellular thrombus in the lumen of blood vessels (Fig. 8), with thickening in the wall of blood vessels and narrowing in the lumen in addition to atrophied of some villi (Fig. 9 and 10). According to Scott *et al.*, (2009) and Marilza *et al.*, (2011). the placentas of diabetic mothers at term shows a number of variations in comparison with the non-diabetic women placentas, the placentas of diabetic once tend to be heavier and exhibit immaturity of villi, identified the lesions of circulatory pathology included chorial edema, edema of the intima, interstitial hemorrhage, congestion and infarction. Lesions indicative of degeneration included villus edema, villus or intervillus fibrosis, calcification and focal degeneration. Lesions of proliferation included dysmaturity, Hofbauer cell hyperplasia, and syncytial nodes. Lesions of inflammation included villitis endarteritis and cord hemorrhage. However, the morphological and histopathological changes in diabetes mellitus placentas are inconsistent and even some what controversies.



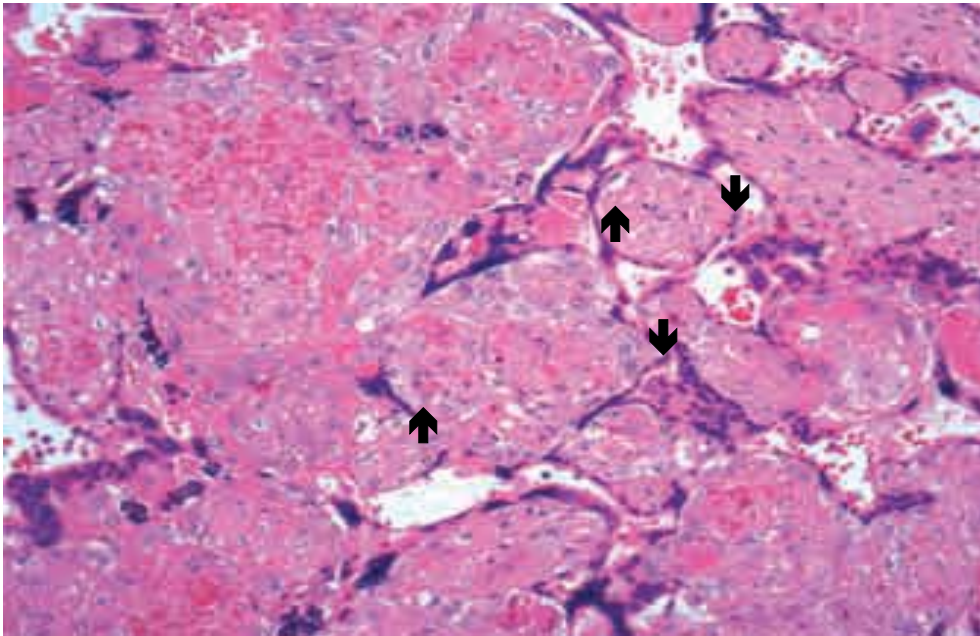
**Fig. 1: Histological section of diabetic women's umbilical cord showing thickening in the wall of umbilical artery [tunica intima (↑), tunica media (←) and tunica adventitia (→)] (H&E 115X).**



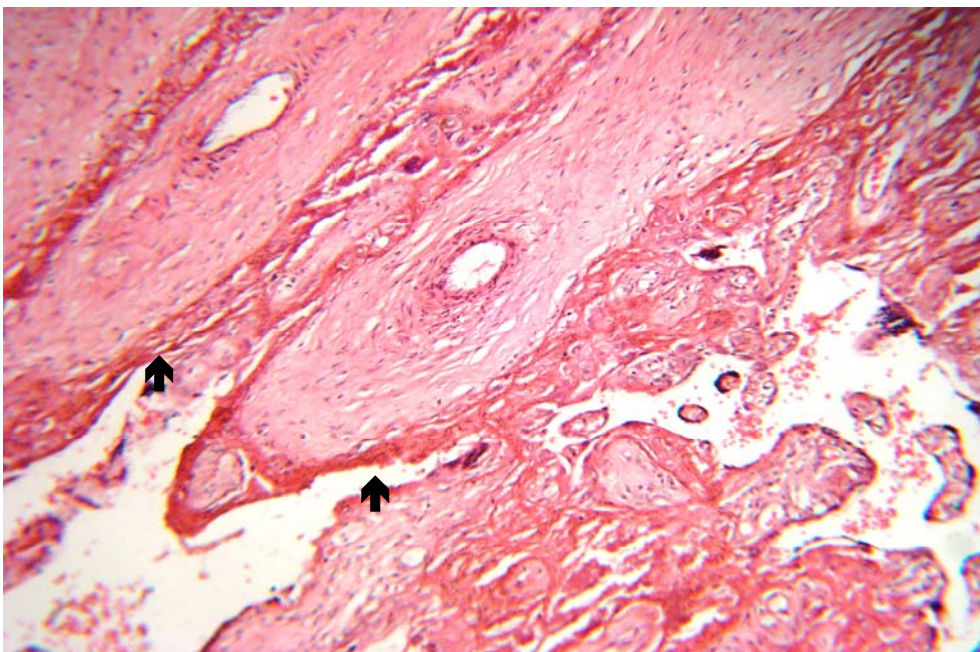
**Fig. 2:** Histological section of diabetic women's umbilical cord showing thickening in the wall, due to accumulation of vacuole and foam cells in intima (↔) (H&E 450X).



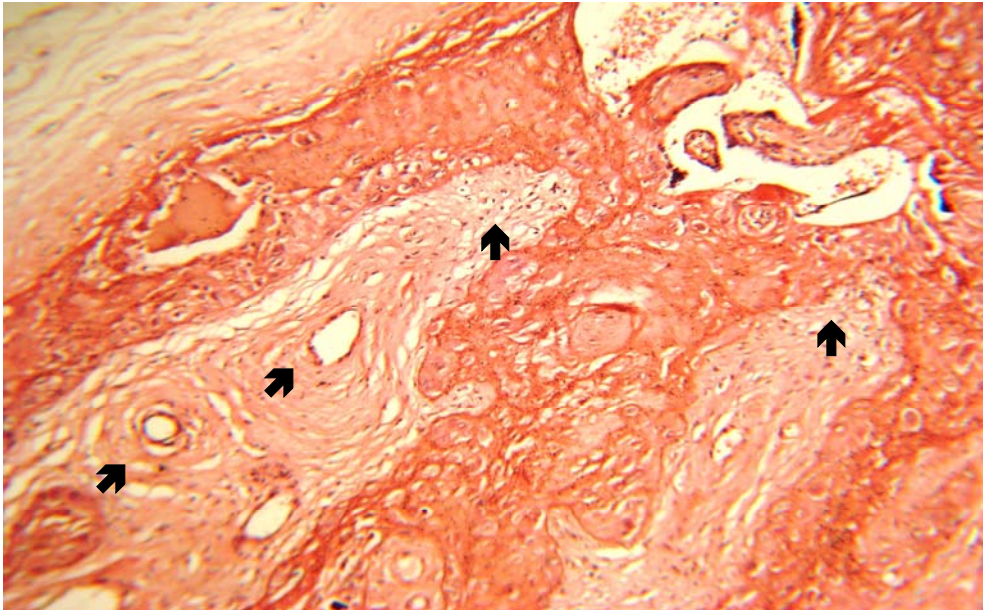
**Fig. 3:** Histological section of diabetic women's placenta showed ischemic villi (↑) with increased syncytial sprouts (↗) and fibrin (↖) (H&E 115 X).



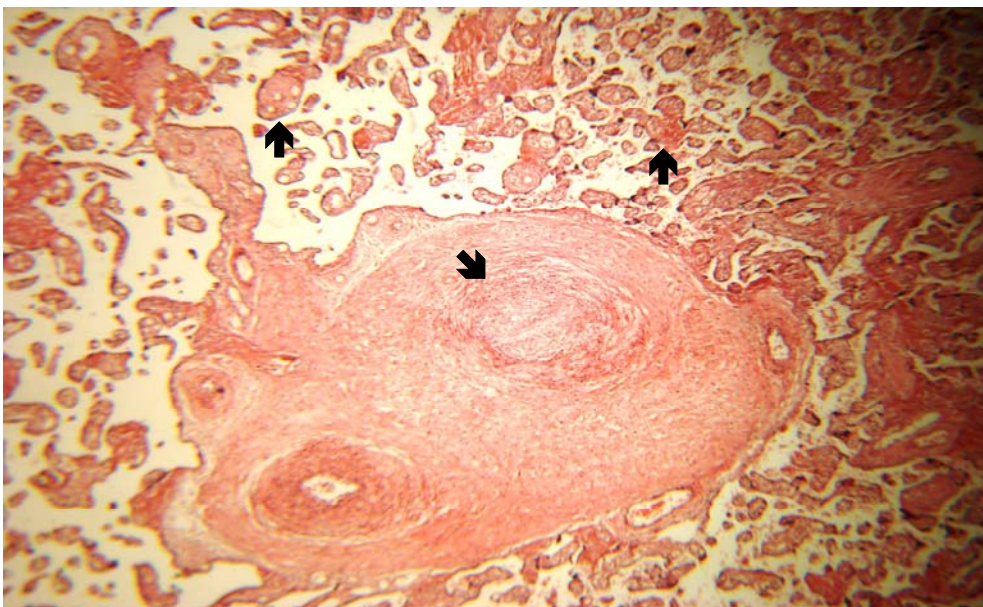
**Fig. 4: Histological section of diabetic women's placenta showed dysmature villi (↑↓) with persistence of central vessels and increases nucleated red blood cell (↓) (H&E 115 X).**



**Fig. 5: Histological section of diabetic women's placenta revealed coagulation of placental villi (↑) (H&E 115X).**

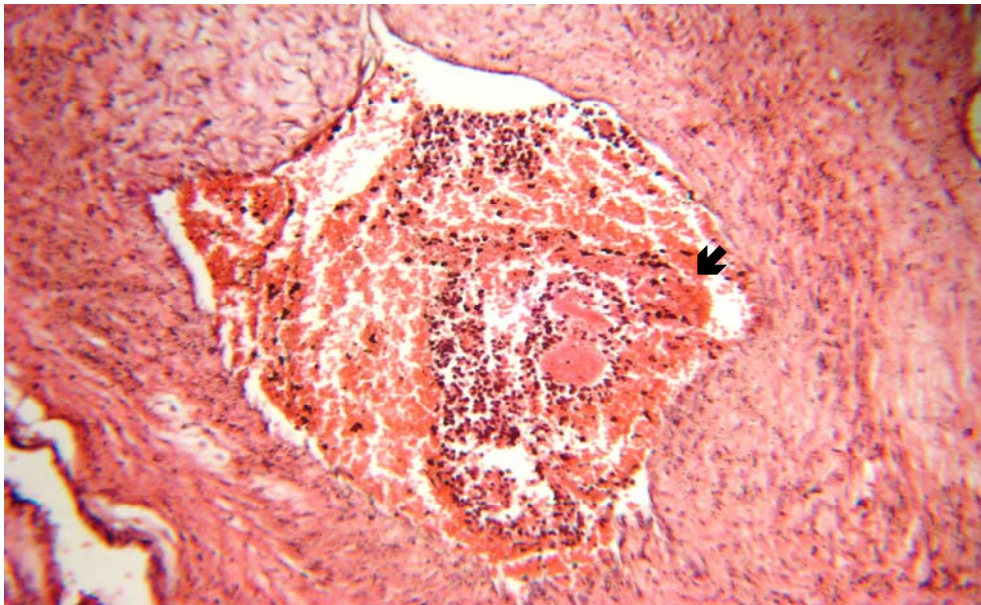


**Fig. 6:** Histological sections of diabetic women's placenta shown coagulation of placenta (↗) associated with infiltration of inflammatory cells (↑) (H&E 115X).



**Fig. 7:** Histological sections of diabetic women's placenta shown some of placental villi atrophied (↑) sclerotic of placenta artery (↘) (H&E 145X).

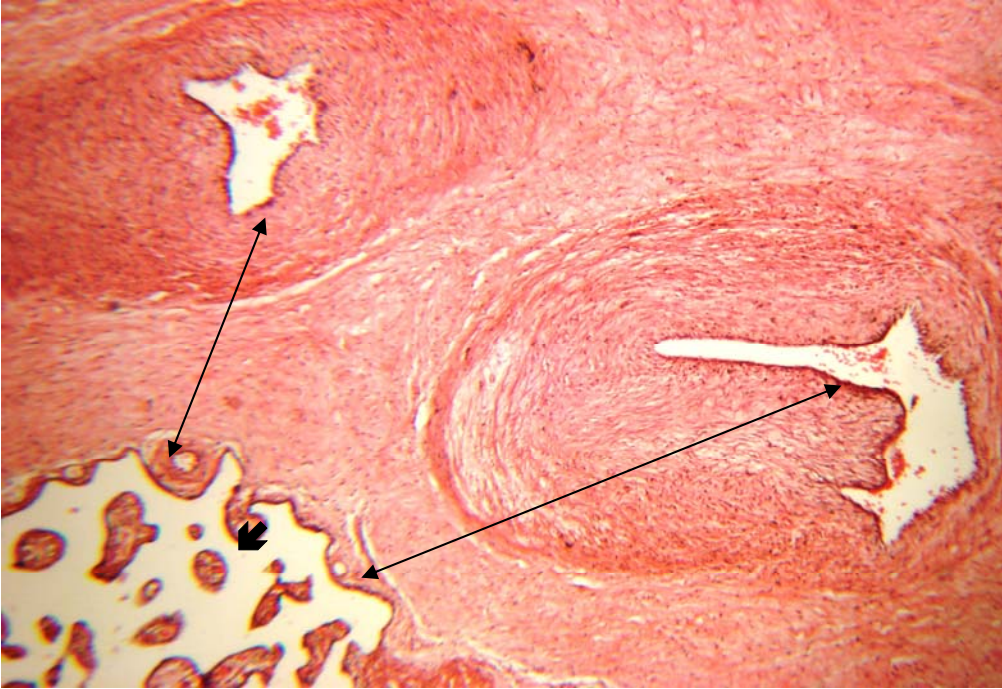




**Fig. 8: Histological sections of diabetic women's placenta revealed cellular thrombus in the lumen of blood vessels (↙) (H&E 370X).**



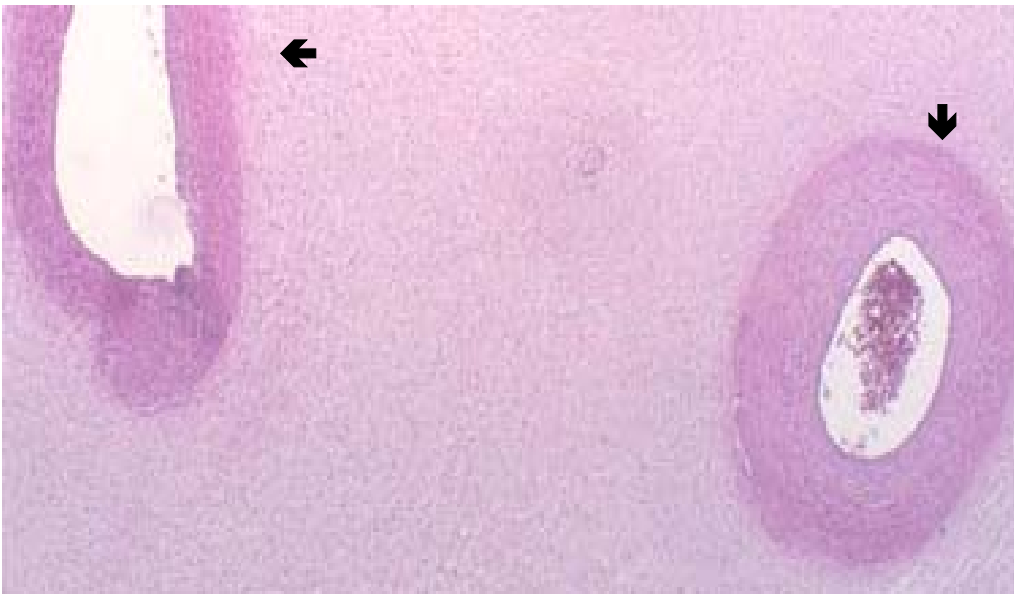
**Fig. 9: Histological sections of diabetic women's placenta shown thickening in the wall of blood vessels, narrowing in the lumen (↙) in addition to atrophied of some placental villi (➔) (H&E 145X).**



**Fig. 10:** High magnification of in the wall of blood vessels in a diabetic woman shown thickening in the wall of blood vessels in addition to atrophied of some placental villi (↙) (H&E 370X).



**Fig. 11:** Histological section of normal women's placenta revealed normal villi of placental (↓) (H&E 115X).



**Fig. 12: Histological section of normal women's umbilical cord revealed normal artery (↓) and vein (←) (H&E 115X).**

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