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INVESTIGATION OF OF GLYCOGEN STORAGE AND THE AMOUNT OF DNA APPEARANCE IN PLACENTA OF PREGNANT EWES

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ABSTRACT : The study aimed to investigate the role of glycogen and DNA in placental normal and complicated pregnancies, using animal models (ewes). Ten placentas from pregnant ewes were used at 50 and120 days of gestation. A histochemical study was conducted to demonstrate the storage glycogen deposited using Periodic Acid Schiff s (PAS) and Methyl Green Pyronin to demonstrate the storage deoxyribonucleic acid (DNA). Results of the glycogen by PAS reaction (Purple colour) in histochemical determination at day (50) showed a moderate density, but in day (120) compared to day (50) of gestation showed a high density of glycogen. The DNA amount (blue green colour) by Methyl Green Pyronin reaction at day (50) showed the histochemical investigation of the amount of DNA appears no evidence different in maternal layer, also in day (120) the amount of DNA as compared to day (50), which appeared no evidence different in maternal layer while in villous (fetal layer) showed very high distribution. In conclusion, Glycogen and genetic material (DNA) storage in placenta increase with the progression of the gestation days as a result of the need the fetal to provide it the necessary energy for the fetal development.

Key words : Glycogen, DNA, placenta.

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INTRODUCTION

The placenta acts important role to mediate the supply of oxygen and nutrients from the mother to fetus, only during pregnancy the placenta is a unique organ that exists to perform a diverse range of functions that collectively support optimal fetal growth while maintaining maternal well-being (Banister *et al*, 2011). The placenta removes waste products from the fetal circulation and forms a physical barrier as affords some protection against certain pathogens and toxins (Kulkarni *et al*, 2011). Furthermore, adapt maternal physiology and behavior ultimately to ensure that the pregnancy is sustained and fetal growth is supported the placenta also synthesizes and secretes hormones into the mother's blood (Hogg *et al*, 2013).

The purpose of placental glycogen stores in normal pregnancy remains unclear, when the placenta metabolizes glucose for its own use and stores it in the form of the multi-branched polysaccharide glycogen by facilitating glucose transport (Lambertini *et al*, 2011). During late gestation the placental glycogen stores ensure that fetal glucose supply is maintained according to the most widely accepted theory (Schroeder *et al*, 2013). Some studies support the idea that placental glycogen serves as a source of energy for its own needs, in the fetal liver is used during the neonatal period to maintain levels of glycaemia (Yuen *et al*, 2010).

Many recent studies suggest that demonstrated the existence of a specific G6Pase gene present in the placenta, this gene is unrelated to the hepatic G6Pase gene, for the placenta could generate glucose that potentially play a role in maintaining fetal blood glucose concentrations (Leonce *et al*, 2006). According to recent studies suggests that placental methylation in several placental disease phenotypes, including pre-eclampsia plays a critical functional role and targeted assays have implicated aberrant DNA methylene (Esquiliano *et al*, 2009; Burton and Fowden, 2015; Simon *et al*, 2020) and in growth restriction (Gude *et al*, 2004; Coan *et al*, 2006). In the placenta primarily hyper methylated state with whole genome bisulfite sequencing (WGBS) has provided higher resolution maps of DNA methylation that have

confirmed this lower methylation and detected the presence of partially methylated domains (PMDs), long stretches of the genome where methylation levels drop below the background (Napso *et al*, 2018).

MATERIALS AND METHODS

This study used ten placenta from pregnant animals they collected from local slaughter of Basra city at ages of 50, 120 days. A histochemical study was conducted to demonstrate of storage glycogen deposited by used Periodic Acid Schiff s (PAS) that prepared according to Kiernan, (2008) also the Methyl Green Pyronin was used to demonstrate the storage of deoxyribonucleic acid (DNA) according to Luna (1968).Then a light microscope (1000X) magnification was used with a camera (0.5X) as well as a (10X) computerized magnification to provide a high-resolution image.

The histological technique

This study was used ten placentomes of pregnant ewes at 50 and 120 days of gestation. After slaughtered the animal, the placentomes were removed and then washed to remove blood from it in normal saline and immediately fixed in (10%) formalin for 48hours. The formalin solution is prepared by the addition (10ml) from (38%) formalin to (90ml) tap water (15-16). The histological sections were processed according to the following steps which include: Dehydration, Clearing, Infiltration, Embedding, Trimming, Sectioning, Mounting, and Staining (Sumner, 1969).

Histochemical study methods

Methyl Green- Pyronin stain : According to method of Luna (1968), we used the special stains to the demonstration of nucleic acid (DNA) and fetal blood vessels. Where methyl green –pyronin solution: consist from Methyl green, purified (0.52 gm), Pyronin Y (0.1 gm) and distilled water (100 ml). Differentiating solution formation from Tertiary butyl alcohol (30 ml) and 100% alcohol (10 ml).

Periodic Acid Schiff's (PAS) : According to method of Kiernan (2008), we used the special stains to the demonstration of glycogen deposit, it is prepared consist from 1% Periodic acid solution, Schiff's reagent solution and (0.5ml) sodium met bisulfite solution.

Examination and photography of tissue section

The slides were examined by using a light microscope and photography by microscope connects with camera.

RESULTS

Demonstration of glycogen : The histochemical determination of glycogen by PAS reaction (Purple color) at day (50) showed moderate density, but at day (120) showed high density of glycogen compared to day (50) of gestation (Figs. 1, 2, 3 and 4).

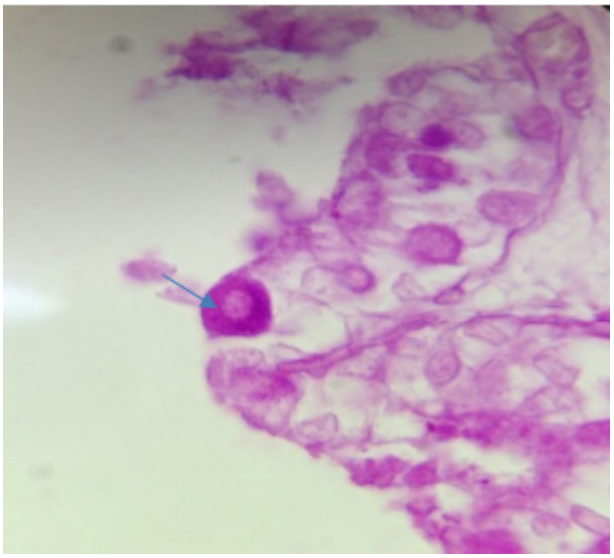

Fig. 1 : Cross section of placentom show: Glycogen (moderate density) (arrow) at day 50 (PAS 400x).

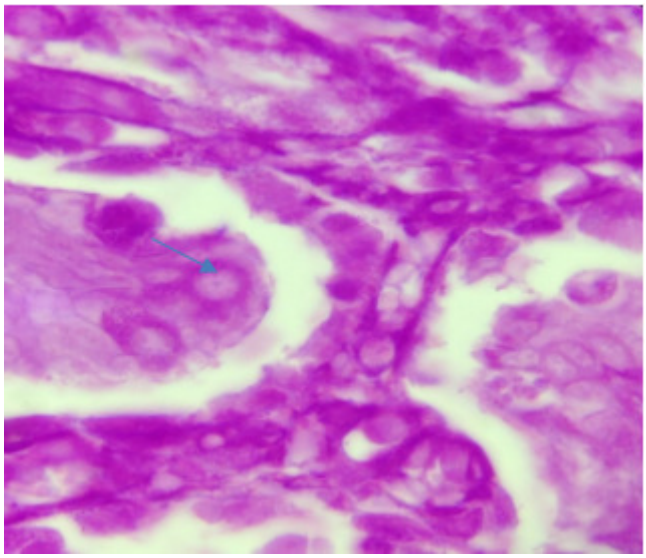

Fig. 2 : Cross section of placentom show: Glycogen (high density) (arrow) at day 120 (PAS 400x).

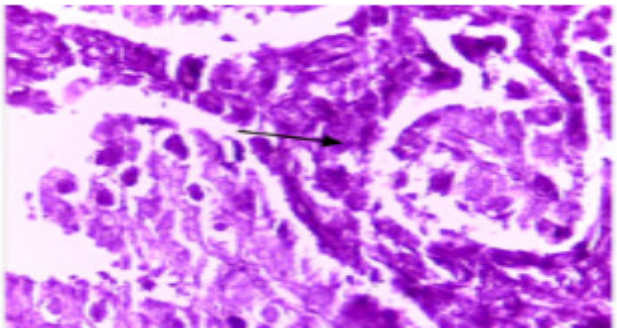

Fig. 3 : Cross section of placentom show: Glycogen (high density) (arrow) at day120 (PAS 400x).

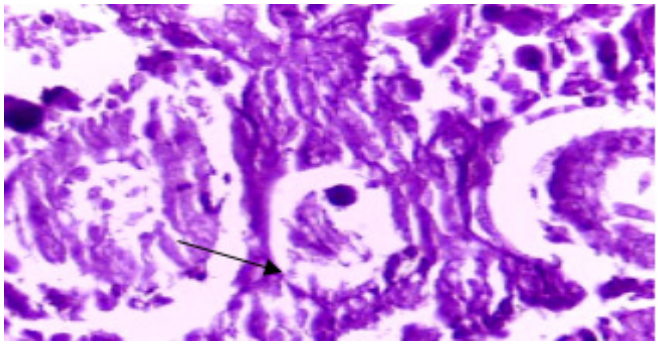

Fig. 4 : Cross section of placentom show: Glycogen (moderate density) (arrow) at day 50 (PAS 400x).

villous (fetal layer) showed very high distribution (Figs. 5, 6, 7 and 8).

DISCUSSION

The DNA density in the present histochemical study showed that DNA density is higher in the late gestation as compared to the early gestation. That some have required sophisticated equipment, for example Ferrell (1991). The protein concentrations in to cotyledons and caruncle, and concluded that this changed in relation to cell size and cell number, in cow measured the RNA, in protein – depleted pregnant rats. Also, Hastings-Roberts

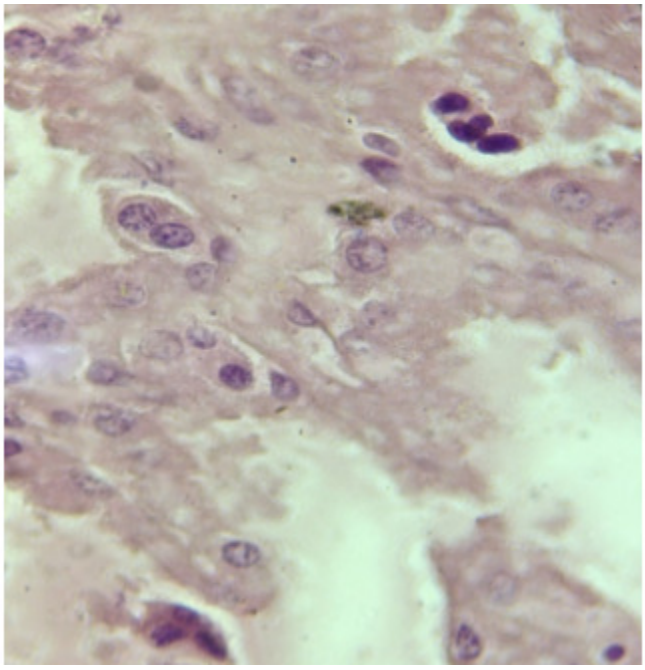

Fig. 5 : Cross section of placentom show: DNA distribution (arrow) at day 50 (Methyl green pyronin 400x).

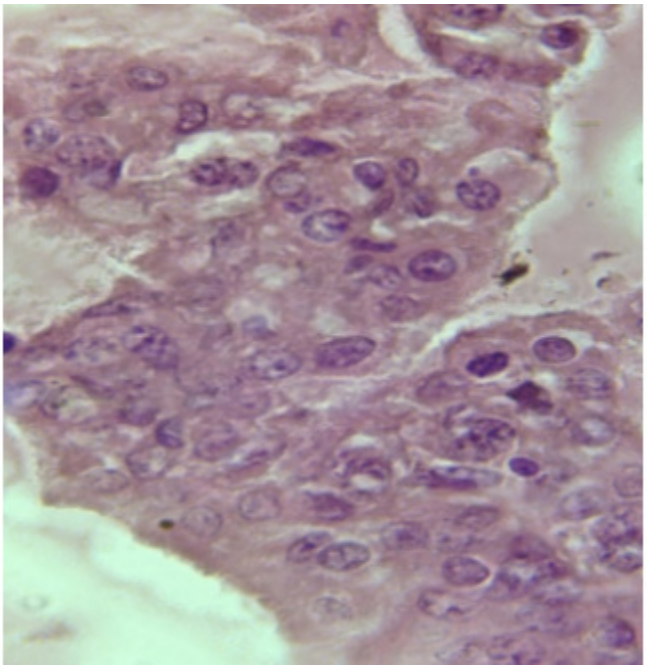

Fig. 6 : Cross section of placentom show: DNA distribution (arrow) at day 120 (Methyl green pyronin 400x).

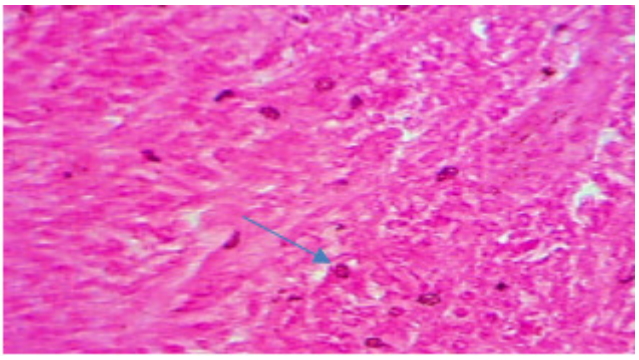

Fig. 7 : Cross section of placentom show: DNA distribution (arrow →) at day 50(Methyl green pyronin 400x).

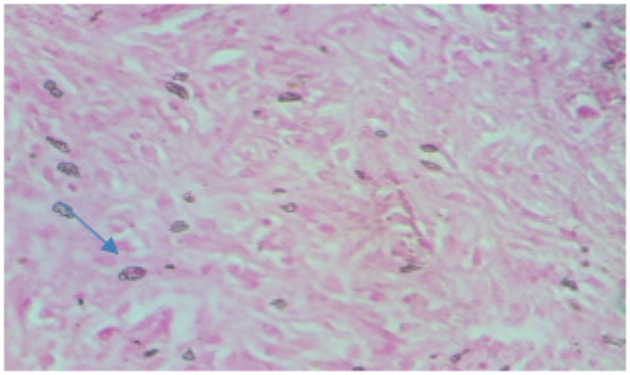

Fig. 8 : Cross section of placentom show: DNA distribution (arrow →) at day120 (Methyl green pyronin 400x).

Demonstration of DNA : The histochemical investigation of DNA amount (blue green color) by Methyl Green Pyronin reaction shown at day (50) the amount of DNA appears no evidence different in maternal layer at day (120) the quantity of DNA as compared to day (50) appear no evidence different in maternal layer while in

and Zeman (1977) observed smaller placentas containing less RNA and DNA. Especially when the protein-deficient diet was fed during the second half of pregnancy and they found in rat no reduction in total DNA content although weight and protein content were reduced (Van Marthens and Shimomaye, 1978).

In the human, the DNA content of the placenta ceases to increase later, about the 36th week. Consequently, the cell population of the placenta is vulnerable to stunted growth if malnutrition occurs during the long period of hyperplasia, while in the placenta of the rat, which has a 21-day period of gestation, undergoes its most rapid growth during the third week, but DNA content does not increase beyond day 17 of pregnancy in both species (Ilekis *et al*, 2016). And no change or little in cell size (protein/DNA ratio)· the placentas of malnourished women show a reduced DNA content (Napso *et al*, 2019).

Some investigators finding that DNA content is reduced, in rat that it may even exceed that of controls on an adequate diet according to studies on rats receiving protein –deficient diets are contradictory (Tellechea *et al*, 2017).

In the red blood cells of the fetal blood vessels in human showed the strong reaction for methyl green (bluish green color) and pyrone in reaction (red color) in chorional epithelium (Treesh and Khair, 2015). The DNA is self- replicating and determines the genetic characters, while RNA is particularly concerned with protein synthesis, in methyl green pyronin reaction demonstrates two nucleic acids, namely DNA which is the main nuclear component and RNA which present mainly in the cytoplasm and to less extent in the nucleus, methyl green pyronin stain of the chorional epithelium showed extremely week reaction of the karyoplasm of cytotrophoblasts compared with control cases, denoting a decrease in DNA and RNA of cytotrophoblasts in human (Treesh and Khair 2015).

Placental DNA in the diabetics continued to increase until day 18-19 of gestation, whereas DNA content in control placentas remained constant after day 16. Thus, the diabetic placentas apparently continue the process of DNA replication after DNA synthesis is complete in the controls in rats, the placentas in this model are markedly increased in size late in gestation. No difference in protein concentration or protein / DNA ratio was noted, total DNA content per placenta was significantly increased in the diabetic placentas after day 16 when compared to controls (Prieto *et al*, 2015).

The present study in histochemical of glycogen showed elevation of glycogen density by PAS reaction during pregnancy. This result was agreed with Ira *et al* (1983), who reported that the placenta of rodent is able to accumulate glycogen in glycogen trophoblast cell, while in mice glycogen accumulation is limited to the latter half of pregnancy and specially occur in a subset of cells in the spongiotrophoblast layer after embryonic day (12) and after later invade the wall of the uterus (Simmons and Cross, 2005). Also has been identified noticed that glycogen in amniotic epithelial cell cytoplasm in early pregnancy but this accumulation revealed to quantitative changes related to the presence of maternal diabetes in human (Adamson *et al*, 2002; Benirschke *et al*, 2006; Lewis and Benirschke, 2006). A potential substrate and energy source for both placental and fetus are importance of placental glycogen (Madazli, 2008).

The result from increase glucose utilization and increasing metabolic a rate as pregnancy progresses contributed the decrease of glycogen (Esquiliano *et al*, 2009). In common pathological finding indifferent tissue, the glycogen deposit, but in placenta occur in all pregnancy so it appears as normal physiological function (Plows *et al*, 2018).

Thickened basement membranes and especially in the terminal villous extra cellular in human, while in animal (PAS) material with sub-syncytial location as show above and on the other hand, focal deposits located both in (James, 2006). Also, in human, they observed positive PAS reaction in the villous core and basement membrane of the trophoblast (Treesh and Khair, 2015).

CONCLUSION

Glycogen and genetic material (DNA) storage in placenta increase with the progression of the gestation days as a result of the need the fetal to provide it the necessary energy for the fetal development.

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