STEREOLOGICAL STUDY OF MICE PLACENTAL BARRIER AFTER PROTEIN MALNUTRITION

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ABSTRACT

Investigations have been carried out on the effect of maternal protein free diet on the placenta of laboratory mice of Balb/C strain. The thickness of placental barrier was measured. Placental weight, fetal weight, fetal numbers were also calculated and histological study was also done. Animal husbandry and the day of conception were arranged. Pregnant mice were dissected at days (12^{th}, 14^{th}, and 18th) of gestation. Thirty placentas from mice on protein free diet and fifteen placentas from mice on control diet, were taken, stained by haematoxylin and eosin and examined under light microscope with screen. The following results are subsequently obtained:

1. ***Reduction in fetal number and weight of mice after protein malnutrition, in comparison to their control at the same stage of gestation.***
2. ***Reduction in placental weight of mice after protein malnutrition, in comparison to their control at the same stage of gestation.***
3. ***Reduction in the thickness of trophoblastic layers of placental barrier in mice after protein malnutrition, which are measured by using light microscope with screen and scientific ruler.***
4. ***Morphological changes showed thin placental barrier, with basophilic cytoplasm and degenerated nuclei. Areas of degeneration infiltrated with phagocytic cells with increase in the number and size of blood vessels were seen.***

These changes may affect the placental function, which may be the main cause of decrease in fetal weight (intra uterine growth retardation), placental weight and fetal number.

INTRODUCTION

The placenta of eutherian mammals is a remarkable biological structure; it mediates the complex interaction between the mother and the fetus that is necessary for fetal growth and development. It comprised of an inner vascular network and covered by an outer epithelium, called trophoblast cells. All are designed to promote the delivery of the nutrients to the fetus. It forms a selective barrier to the transport of nutrients such as protein, fatty acid, glucose, minerals, and immunoglobulin G (IgG)[1]. In mice there are two placentas serving as organ for maternal-embryonic exchange, the discoid chorioallantaoic placenta and the villiary highly vascularized yolk sac. The two placentas are presented together throughout most of pregnancy[2,3]. The chorioallantaoic placenta, attains its full size at day (14^{th}) of gestation in mice, and it consists of labyrinth and junctional zone[4,5]. The placenta fulfils a variety of essential functions during prenatal life, while its global role is to maintain environment that facilitates optimal growth and development of embryo by; transfer of nutrients to the fetus, provision of metabolic byproducts from the fetus, provision of hormonal support to both fetus and mother, and transfer of immunoglobulins[6]. During pregnancy, food restriction is known to produce many structural and functional changes in different organs in man as well as in animals[7]. The offsprings of rat fed on protein deficient diet are not only smaller than control animals but also have a permanent change in the structure and function of their organs in adult life[8]. It is not clear how restricted maternal protein supply affect fetal growth. The embryonic cells are able to respond directly to amino acid deficiency by increasing the expression of a variety of genes, whose products regulate growth and differentiation[9]. Rees, et al[10] observed that a reduction in protein content of maternal diet did not produce a generalized reduction in amino acid supply via serum. Free lysine concentration in mother serum is approximately doubled as a result of pregnancy. This increase is still occurring when the protein content of the diet was reduced. They also found that threonine in both mother and fetus was reduced, when the mother fed on low protein diet[10]. Other studies showed that polyamines (e.g. L-arginine) plays an important role in embryo, and placental development. It was found that maternal protein restriction could lead to decrease in arginine and orthinine concentration[11]. Some observations showed

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that maternal nutrition during pregnancy influences fetal and placental weight by its effect on insulin like growth factor system (IGF)[12]. Rudg et al[13] showed that glycogen stored in placenta was decreased between day (18^{th} - 21^{st}) in control and diet restriction group to 25% of the first day of gestation[13]. Johnson and Greenberg[14] found that both protein and placental ribo nucleic acid (RNA) contents were reduced significantly in protein restricted animals. Observation of Murthy et, al[15] showed that placental weight, birth weight have significantly lesser mean than in pregnancy with low protein diet[15] Lega and co worker[16] observed many morphological alterations like villous atrophy, fibrosis and necrosis in placenta of pregnant mice. Morphological study on mice malnourished placenta by Jbara et al[17], showed a great reduction in trophoblastic barrier in mice than control at the same stages of gestation. They also reported large areas of coagulative degeneration with fibrous edematous areas infiltrated by numerous phagocytic cells.

The aim of this study is to determine whether maternal protein malnutrition has an effect on the thickness of trophoblastic layers of labyrinthine placenta *(by using stereological study)*, fetal weight, fetal number, and placental weight.

MATERIALS AND METHODS

Forty five (45) female albino mice of Balb/C strain aged (8-9) weeks used, they were kept in a room at (22-25) C° 10/14 hrs, light/dark. Mice were grouped into two groups:

Group 1: 15 mice, the control, fed on normal diet as in (Table-1).

Group 2: 30 mice, the experimental, fed on protein free diet as in (Table-1).

Females of both groups were matted over night with males of the same strain and the day on which vaginal plug was seen was taken as day of gestation.

Pregnant females were killed by cervical dislocation after anesthesia; caesarian sections were done at day (12^{th}, 14^{th}, and 18^{th}) of gestation. Placental tissues were taken. Placental weights were calculated. The fetal numbers and weights were also calculated.

Preparation of tissues for microscopically examination

The placental tissue samples were placed in (10%) formalin as a fixative solution. Dehydration was done by using ethanol. Then the tissues were embedded in paraffin wax.5µ paraffin section were stained by haematoxylin and eosin and obtained for examination by light microscope with screen (Reichert Austria Nr. 381 116), and scientific plastic ruler as follow:

1. Transparency paper was placed over the microscope.
2. By using rotting variant pen (0.1) the maternal sinuses and fetal capillaries were drawn.
3. The area in between which was the trophoblastic barrier, thickness was measured by scientific ruler.

Data were analyzed statistically by using student t-test of significant and P-value. The test of significance was done between the two groups regarding trophoblastic layers thickness, the placental weight, the fetal weight and fetal number at (12^{th}, 14^{th}, and 18^{th}) of gestation.

RESULTS

The main results are detailed in tables (2, 3, 4, and 5). These tables show comparisons between the experimental and the control mice at days 12^{th}, 14^{th}, and 18^{th} of gestation:

1. Fetal number: the main value of fetal numbers in experimental mice was less than the control ($P<0.01$) (Table-1)
2. Fetal weight: The results show that there is a decrease in the mean fetal weight of the protein malnourished mice than that of the control at day (12^{th}, 14^{th}, and 18^{th}) of gestation. The difference between the fetal weight of the two groups was not significant at day (14^{th}) of gestation ($P>0.05$) (Table-3). The significant decrease is obtained at days 12^{th} and 18^{th} of gestation ($P<0.01$) (Table-2)
3. Placental weight: In the two groups of mice, the mean placental weight is significantly greater in the control than the experimental mice at days 12^{th}, 14th, and 18^{th} of gestation ($P<0.01$) (Table-3)
4. Histological changes:

   a. Trophoblastic thickness:

   There is a decrease in the mean of trophoblastic thickness of the experimental mice at day 12^{th}, 14^{th}, and 18^{th} of gestation than that of the control mice at the same stage of gestation. There is no significant difference at day 12^{th} and 18^{th} of gestation ($P>0.05$) The only significant difference is obtained in the thickness of trophoblastic barrier at day 14^{th} of gestation ($P<0.01$) (Table-5)

Table 1. ***Feeding schedule of control and experimental mice.***

	Control mice	Experimental mice
Protein	14.3%	0
Soya bean	28.6%	0
Wheat and wheat residue	57%	0
Wheat residue	0	100%

Table 2.*The effect of protein malnutrition on fetal number*

Day of gestation	Protein free diet		Normal diet		
	Number of mice	Mean ± SE of fetal number	Number of mice	Mean ±SE of fetal number	P -value
12^{th}	10	5.9±2.07	5	9.6±1.51	P<0.01
14^{th}	10	6.5±2.63	5	9.4±1.14	P<0.01
18^{th}	10	5.7±1.49	5	10.2±3.28	P<0.01

Table 3. ***The effect of protein malnutrition on fetal weight***

Day of gestation	Protein free diet		Normal diet		*P- value*
	Number of mice	Mean ± SE fetal weight (g)	Number of mice	Mean ± SE fetal weight(g)	
12^{th}	10	0.19±0.15	5	0.03±0.12	P < 0.01
14^{th}	10	0.59±0.48	5	0.06±0.007	P > 0.05
18^{th}	10	1.211±0.07	5	1.4±0.15	P > 0.01

Table 4.*The effect of protein malnutrition on placental weight*

Day of gestation	*Protein free diet*		*Normal diet*		*P - value*
	Number of mice	*Mean ± SE placental weight(g)*	*Number of mice*	*Mean ± SE placental weight(g)*	
12^{th}	10	0.09±0.07	5	0.12±0.002	P < 0.01
14^{th}	10	0.09±0.12	5	0.137±0.006	P < 0.01
18^{th}	10	0.13±0.01	5	0.15±0.003	P < 0.01

Table 5. ***The effect of protein malnutrition on thickness of trophoblasts***

Day of gestation	Protein free diet		Normal diet		P- value
	Number of mice	Mean ± SE thickness of trophoblast(μ)	Number of mice	Mean ± SE thickness of trophoblast(μ)	
12th	10	2.71 ± 0.33	5	2.91± 0.05	P > 0.05
14th	10	2.77 ± 0.36	5	3.38 ± 0.22	P < 0.01
18th	10	1.59 ± 0.27	5	1.75 ± 0.21	P > 0.05

b. Morphological changes:

In the placenta of control mice, the fetal capillaries are separated from maternal blood space by trophoblasts (Fig-1).

In comparison to the control mice there are several histological changes in the villi and intervillous space were seen in experimental mice at the same stage of gestation. These changes are:

- ***Histological changes at 12th day of gestation:***
  Morphological and histological studies show thin placental barrier after protein malnutrition in placenta with basophilic cytoplasm. The nuclei of the cytotrophoblastic cells look pyknotic and unhealthy there is also areas of degeneration infiltrated with phagocytic cells, broken blood vessels, areas of hemorrhage are also detected (Fig-2).
- ***Placental histological changes at 14th day of gestation:***
  At this day of gestation, there is a great reduction in the thickness of placental barrier. The nuclei look pyknotic and unhealthy. Large areas of labyrinthine placenta show, coagulative degeneration which are infiltrated with phagocytic cells. Fetal and maternal blood vessels appeared broken, areas of hemorrhage. The number and the size of blood vessels are also increased (Fig-3).
- ***Placental histological changes at 18th day of gestation:***
  The results at this stage show a great reduction in the thickness of the placental barrier in most areas examined. Large areas of coagulative degeneration, which are infiltrated with phagocytic cells between placental barriers, are also noticed. The nucleus looks degenerated and pyknotic, broken blood vessels, hemorrhagic areas with marked increase in the size and number of blood vessels are also seen (Fig-4).

Fig 1. ***Section of placenta of control mice at 12th day of gestation shows placental barrier (arrows) X742.5.***

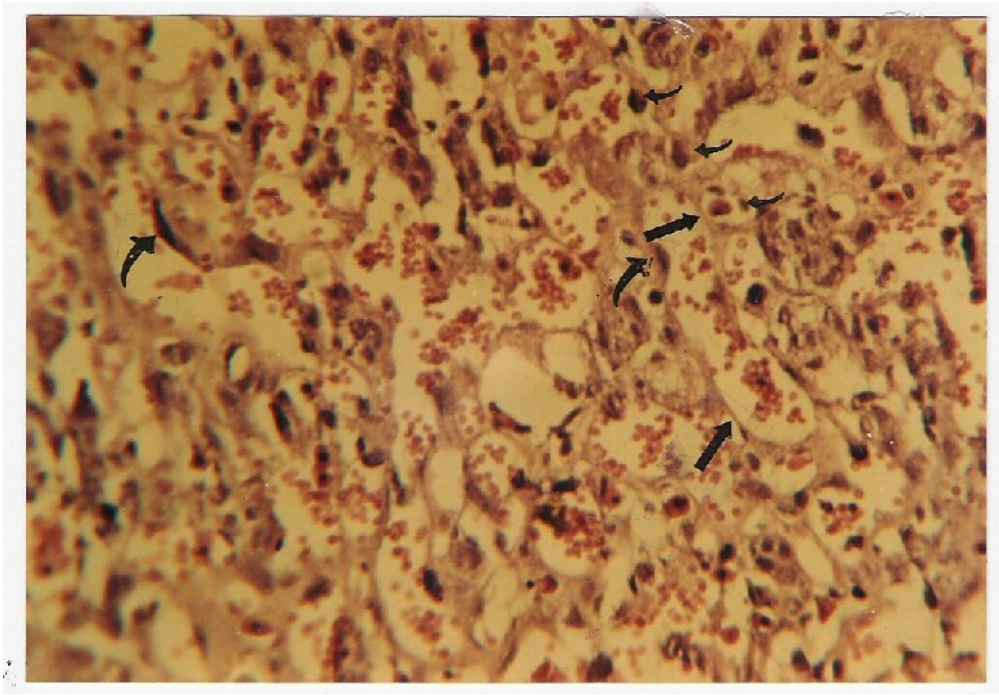

Fig 2. ***Section of protein malnourished placenta at 12th day of gestation shows thin placental barrier (straight arrows) degenerated nuclei (bend arrows) degenerated area (small bend arrows) X742.5.***

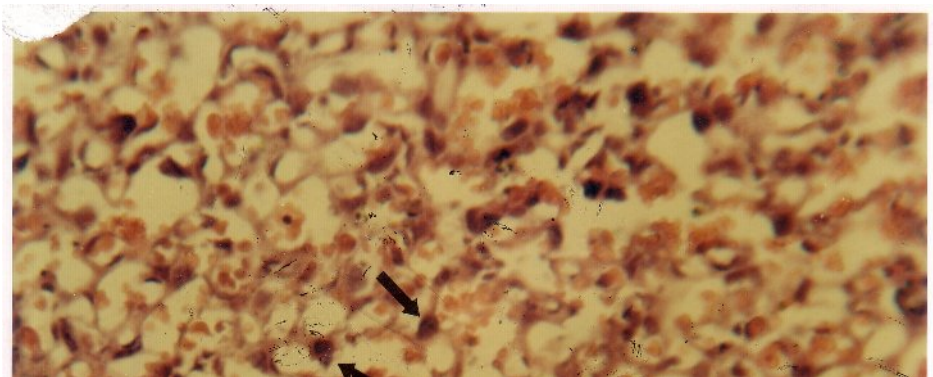

Fig 3. ***Section of protein malnourished placenta at 14th day of gestation shows thin placental barrier (straight arrows), degenerated area (small bend arrows), degenerated nuclei (bend arrows), X742.5 .***

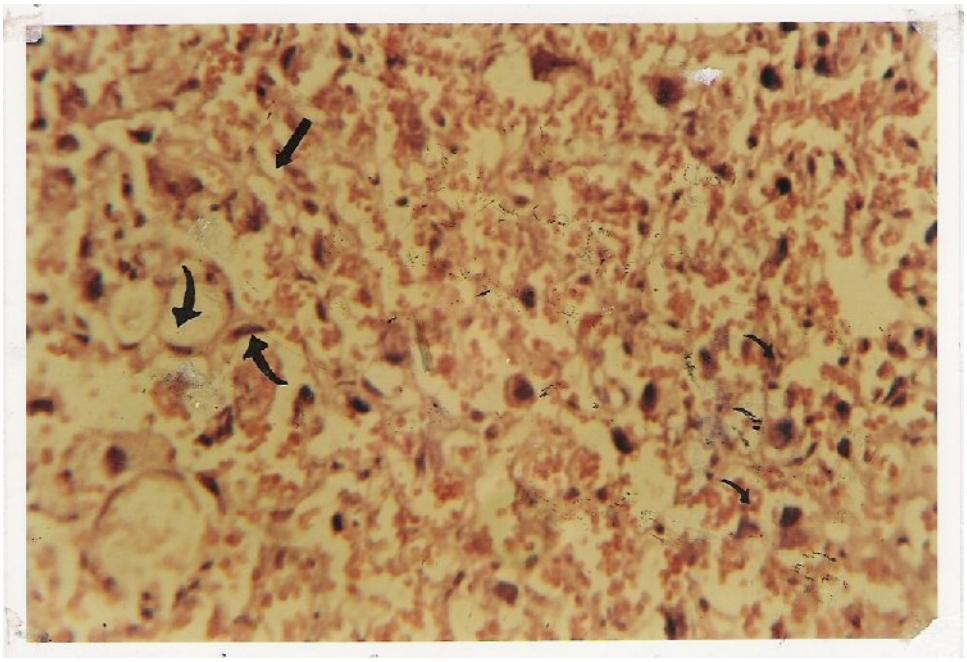

Fig 4. ***Section of protein malnourished placenta at 18th day of gestation shows degenerated area (small bend arrows), degenerated nuclei (bend arrows), and thin placental barrier (straight arrows), X742.5***

DISCUSSION

Despite that, the role of the placenta in fetal nutrition and fetal growth have been well established by many investigators, few workers concentrated on the histological changes that occurred in the placental labyrinth after protein malnutrition during pregnancy[17]. It is well established that in normally developed placenta, the 12th day of gestation in mice is considered the beginning of placental development. The placenta reaches the peak of development at the day 14th of gestation, and ceases at the day 18th of gestation[4]. The results showed that there is a decrease in trophoblastic layers thickness at day 12th of gestation, which is started at the same time of placental development and continues at days 14th, and 18th of gestation of the experimental mice in comparison to the control mice. The results have been reported previously by Jbara et al[17], depending on morphological observation. They suggest that the great reduction in the thickness of trophoblastic layers is to compensate for the deficiency in the

amount of nutrition, which is in turn allows more blood coming in to the maternal blood spaces[17]. This hypothesis is supported by the present finding, which can also be explained by the fact that protein restricted diet starts its effect on the trophoblastic barrier at the beginning of placental development, and continues with the placental development at day 14th, and 18th of gestation. Usually placental development ceases at day 18th of gestation, so the effect of protein restriction appears greater. As a result of protein restricted diet, the blood vessels are found to be fused, swollen, and broken which can be related to the decrease in the blood supply leading to cells death. These changes are also observed by Jbara et al[17] in studying the histological changes of mice placenta after protein mal-nutrition, hence the changes that occurred to the tissues are caused by an in adequate supply of nutrition to the fetus during different stages of placental development. The features of placenta are also altered by maternal malnutrition in comparison to control at the same stages of gestation indicated, that maternal malnutrition has regular effect on the delivery of nutrient via the placenta, and the labyrinth zone is the main barrier for tran placental transport. The placental barrier appears thin after protein malnutrition to compensate for the deficiency in the amount of nourishment. A large area of coagulative necrosis, which is infiltrated by numerous phagocytic cells to engulf, the dead degenerated cell and cellular debris. It had been suggested that the protein malnutrition can lead to the decrease of feto-maternal amino acid ratio in experimental mice and this will lead again to reduce a nutrient transfer to the fetus by down regulation of specific amino acid transport protein[19]. This reduction can be related to the fact, that fetal growth is a complex dynamic process, which is dependant on continuous supply of nutrient from the mother. A reduction in fetal weight and number was also observed in this study. The decrease in maternal protein intake leads to a decrease in protein and amino acid synthesis, which are required for growth both functionally and structurally like *(collagen, hemoglobin, and others).* This causes failure of growth and development and also a decrease in the number of embryos. The results of this study in turn, support previous findings of Malandro et al[19] and Wodall et al[19]. They suggested that maternal malnutrition during pregnancy causes intra uterine growth restriction (IUGR). They also observed that the mean body weight of the fetuses in protein malnourished mice was significantly lower in comparison with fetuses from control mice. Rees et al[10] showed their that, the fetus of rat carried by mother fed on normal diet was heavier than that carried by rat fed on protein malnourished diet. The present study also shows that there is a decrease in the placental weight of experimental mice in comparison to the control at the same stages of gestation. The decrease in the placental weight could be due to the reduction in protein synthesis and in turn, this will lead to a decrease in protein in placenta and failure of placental development, which causes placental dysfunction. These changes were also observed by Rudg et al[34] and Wodall et al[19]. They observed that placental weight was also reduced in maternal protein malnutrition. Some investigators had suggested that placental ribo nucleic acid (RNA) content was significantly reduced in protein restricted animals. Fractional protein synthesis was also reduced in placenta. This will affect the nutrient transport to the fetus by down regulation of specific amino acids[20]. Others however had suggested that these changes cause inhibition of the reaction controlling adenosine triphosphate (ATP) consuming process such as micromolecular synthesis pathway and active transport of substrate near term[21].

In conclusion, it is clear that placenta plays a global role for growth and healthy development. Future studies might be directed towards ultra structural study of placental barrier by electron microscope, after protein malnutrition.

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