

## ORIGINAL ARTICLE

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**HISTOPATHOLOGICAL AND WEIGHT CHANGES OF THE  
RAT FETAL LIVER OF FEMALES FED WITH LOW PROTEIN  
DIET DURING PREGNANCY****Ehab K. Hassan\*and Ahmed M.S. Hegazy\*\***

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**ABSTRACT**

**Objective:** *The study designed to estimate effects of dietary protein reduction, during pregnancy on the liver of rats. Methodology:* Sixty adult Albino rats (30 males and 30 females) were included. At the start of the experiment, all males and females were allowed for mating. Female pregnant rats were divided into 2 equal groups. **Group I** (control group): included rats fed on the standard diet. **Group II** included rats fed on low protein diet. Female rats were weighted just before start and at end of study. Fetuses were extracted, counted and weighted. Liver is extracted and prepared for histopathology and electron microscopy examination. **Results:** At the end of the study, there was significant decrease of female body weight in study when compared to control group ( $238.000 \pm 4.551$  vs  $279.466 \pm 3.335$ g). Also, there was significant decrease of net weight increase in study in comparison to control group. The percentage of increase in body weight was 14.00% in study group and 34.339% in control group. The number of fetuses/ female showed significant decrease in study when compared to control group ( $5.0 \pm 1.25$  vs  $10.66 \pm 1.95$  respectively). Histopathological and electron microcopy examination revealed different changes that agree with changes in weight. **Conclusion:** a protein restricted diet lead to decreased body weight of pregnant females; their offspring body

*and liver weight, when compared to control group. Histopathological and ultrastructure examination revealed different changes (e.g., increased fat droplets, increased glycogen content, cytoplasmic vaculation and cell structure abnormalities). Thus, a well-balanced diet is advocated for pregnant females.*

**Keywords:** *weight, fetal liver, female rat, low protein diet, pregnancy*

## INTRODUCTION

Adequate supply of nutrients across the fetal placental barrier is the most important determinant of fetal growth. Any significant reduction in nutrient transfer from the mother to the fetus has a higher risk to impair fetal growth and development (**Burdge et al., 2004**). Adequate nutrition during pregnancy is very important process. Any deviation or lack of proper nutrients may lead to low birth weight this may be associated with increased risk of future chronic disease development (e.g., hypertension, glucose intolerance, insulin resistance, obesity and type2 diabetes) later in life (**Barker, 1998; Godfrey, 1998; Zambrano et al., 2006**). Mechanisms by which, maternal protein reduction lead to reduction in intrauterine and post-natal growth are not fully understood (**Rosario et al., 2011**). It was suggested to be accomplished through alteration of tissue structure and morphology; changes in the number of cells or type of cells with the tissue can affect organ function to a greater extent (**Langley-Evans, 2006**). The liver played a greatest number of functions, especially in the field of metabolism. Thus, the study of morphological and structural changes in the liver in response to maternal protein deprivation is of paramount importance (**Ramadan et al., 2013**).

### Aim of the work

The present work was designed as an experimental study to estimate the effects of reduction of dietary protein, during pregnancy on the liver of rat fetuses. Histopathological and weight changes of fetal liver will be addressed.

## Methodology

Sixty adult Albino rats (30 males and 30 females) were obtained from Cairo University Animal house (Cairo; Egypt). After arrival to study place (Animal house, Faculty of Pharmacy, Mansoura), they were housed in environmentally controlled cages (25° C, 12-h light/12-h dark cycle). They were allowed to tap water and experimental diet as ad libitum. At the start of the experiment, all males and females were allowed for mating, one male with the one female in a separate cage.

At the morning presence of sperm in the vaginal smear was confirmed and this was taken as day 1 of pregnancy. Female pregnant rats were divided into 2 equal groups (each of 15 females) according to the planned diet during the whole period of pregnancy before dissection on the day 21.

**Group I** (control group): 15 female rats were fed on the standard diet.

**Group II** (study group): 15 female rats were fed on low protein diet.

The standard diet consisted of 68% starch, 4% cellulose, 5% lipid (corn oil) and 20% protein (casein) (g/100 g); while low protein diet consisted of 78% starch, 4% cellulose, 5% lipid (corn oil) and 5% protein (casein) (g/100 g). Both diets were isocaloric and both contained 2 g/100 g yeast, salt 3.5 g/100 g, and vitamin mixture 2.2 g/100 g as described by **Picarel-Blanchot et al. (1995)**. Protein, starch, cellulose and vitamin mixture were purchased from Sigma–Aldrich, Inc (USA). Corn oil, yeast and salt were purchased from local market.

### Weight measurement:

Female rats were weighted just before the start of study (the night of mating) and weight again at the end of the study. On the day 20 late evening, female rats allowed to the food; and then fasted overnight; in early morning of day 21 of pregnancy, rats from both groups were anesthetized with diethyl ether and dissected by abdominal incision. The uterus was identified and opened longitudinally to extract the fetuses by separating the placenta. The fetuses were

counted and weighed using a weighing balance for animals (readability 0.01 g); the mean weight for each female was documented and included in statistical analysis. The fetal livers were removed and weighed after a midline incision, as described by **Bertin et al. (2002)**. Also, mean weight of liver in each female in relation to number of fetuses was calculated and included in the statistical analysis.

### **Histopathological study**

**Light microscopy:** section of fetal liver was fixed in neutral buffered formalin, dehydrated and then embedded in paraffin wax. Paraffin blocks were cut into sections (5 $\mu$ m) using a microtome (Thermo Shandon, UK). The serial sections were mounted on glass slides, hydrated and stained with Hematoxylin and Eosin stain (H&E) as described by **Drury and Wallington (1980)**.

For glycogen staining, periodic acid-Schiff stain (PAS) was used. Sections of the previously prepared paraffin blocks were oxidized for 5 min with aqueous periodic acid, washed, rinsed and then placed for 20 min in Schiff's reagent (**Schiff, 1866**).

**Electron microscopic examination:** The electron microscopic study was done at Ain Shams University. Approximately 0.25 mm thick liver tissue slices were fixed in 5% glutaraldehyde at a pH of 7.25, then post-fixed by 2% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in graded ethanol and embedded in Spur epoxy resin. Ultrathin (pale gold, 50 nm) sections were cut with ultramicrotome using EMCORP diamond knives (USA). Ultrathin sections were collected on copper grids and stained with 4% uranyl acetate in 50% ethanol followed by 0.3% lead citrate (**Reynolds, 1963**). The stained ultrathin sections were examined with a Philips CM 100 (Holland) transmission electron microscope at 60 kV

**Statistical analysis of data:** results were expressed as mean and standard deviation (SD). The statistical analysis was performed using independent

samples Student's (t)-test. All statistics was carried out using statistical package for social science (SPSS), version 13 for windows. P-values less than 0.05 were considered statistically significant.

## RESULTS

As regard to weight of studied female rates; at the start of the study, there was no significant difference between study and control groups ( $208.866 \pm 4.120$  vs  $208.133 \pm 4.627$  g respectively). At the end of the study, there was significant decrease of female body weight in study group when compared to control group ( $238.000 \pm 4.551$  vs  $279.466 \pm 3.335$  g respectively). In addition, there was significant decrease of net weight increase (difference between last and initial weights) in study group in comparison to control group. The percentage of increase in body weight was 14.00% in study group and 34.339% in control group with significant difference (table 1).

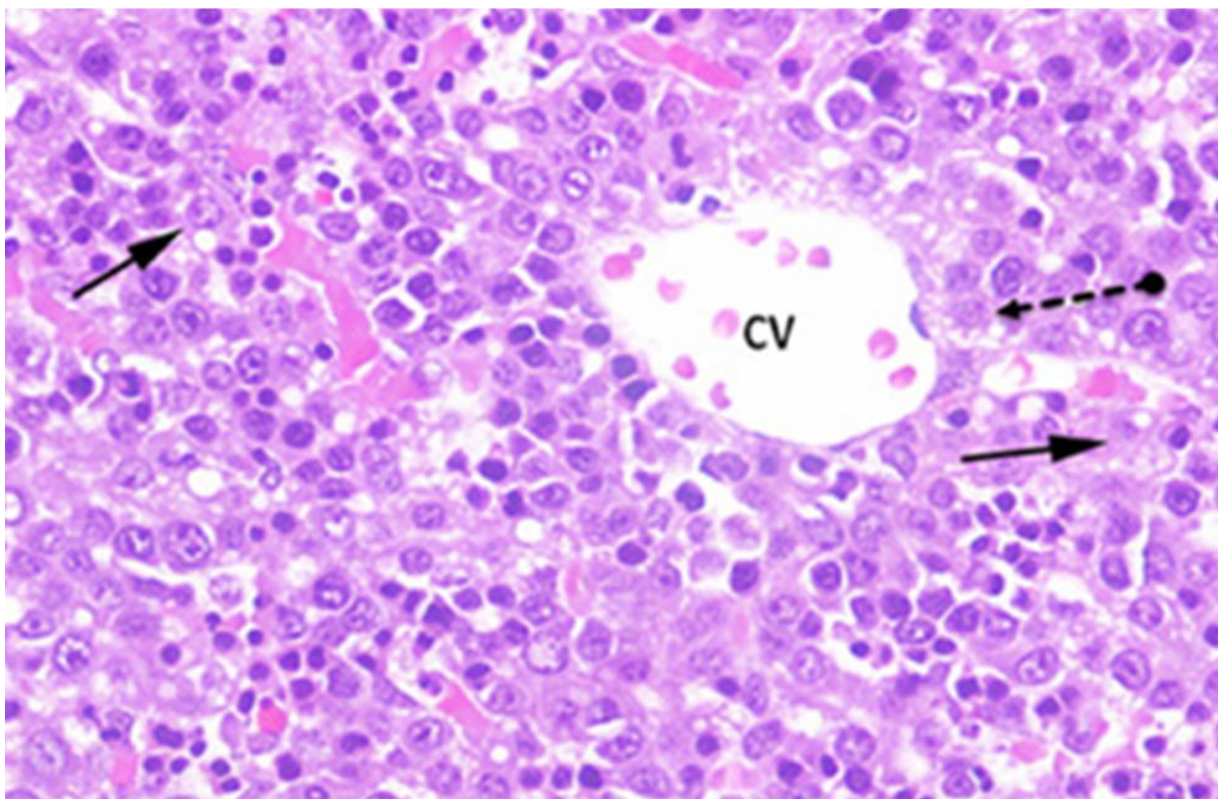
The number of fetuses/ female showed significant decrease in study group when compared to control group ( $5.0 \pm 1.25$  vs  $10.66 \pm 1.95$  respectively). Mean body weight of the fetuses in study group was  $1.872 \pm 0.186$  g, the fetal liver weight was  $0.101 \pm 0.009$ ; while body weight in the control group was  $2.718 \pm 0.496$  g, fetal liver weight was  $0.168 \pm 0.025$  g; and there was significant difference between groups. Finally, percentage of fetal liver weight in relation to fetal body weight in study group was  $5.452 \pm 0.679$  compared to  $6.259 \pm 0.871$  in control group with significant difference (table 1).



**Table (1): Comparison between study and control groups as regard to weight measurements and number of fetuses/female**

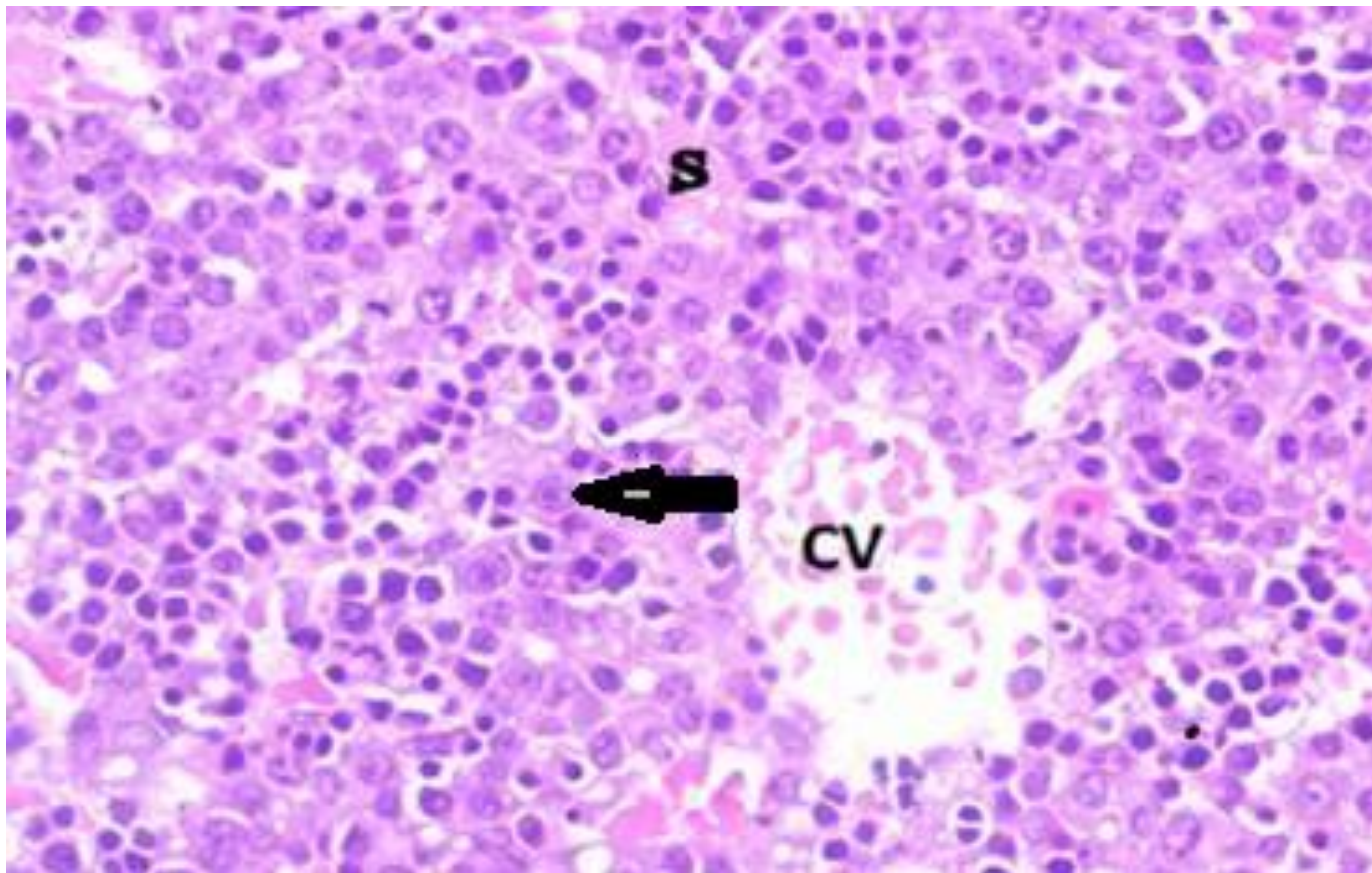
	Study group		Control group		t	p
	Mean	S. D	Mean	S. D		
Initial female weight (g)	208.866	4.120	208.133	4.627	0.45	0.65(NS)
Female weight at the end (g)	238.000	4.551	279.466	3.335	28.46	<0.001*
Weight increase (g)	29.133	7.199	71.333	6.102	17.31	<0.001*
Percentage of increase in FBW	14.004	3.660	34.339	3.572	15.39	<0.001*
Number of fetuses/mother	5.000	1.253	10.666	1.951	9.46	<0.001*
Body weight of fetuses (g)	1.872	0.186	2.718	0.496	6.17	<0.001*
Weight of fetal liver (g)	0.101	0.009	0.168	0.025	9.29	<0.001*
Fetal liver weight/fetal body weight	5.452	0.679	6.259	0.871	2.83	0.009*

Results of histological examination On examining sections of fetal livers from study using H&E stain, some of the hepatocytes were pale with cytoplasmic vacuolization, and microvesicles were presented in some cells. Others were swollen and ballooned with larger vacuoles. The nucleus is centered and surrounded by a clear halo (figure 1).



**Figure (1): Picture of study group, showed central vein (CV), microvesicles in some cells (dashed arrow); others show fat droplets (black non-dashed arrow)**

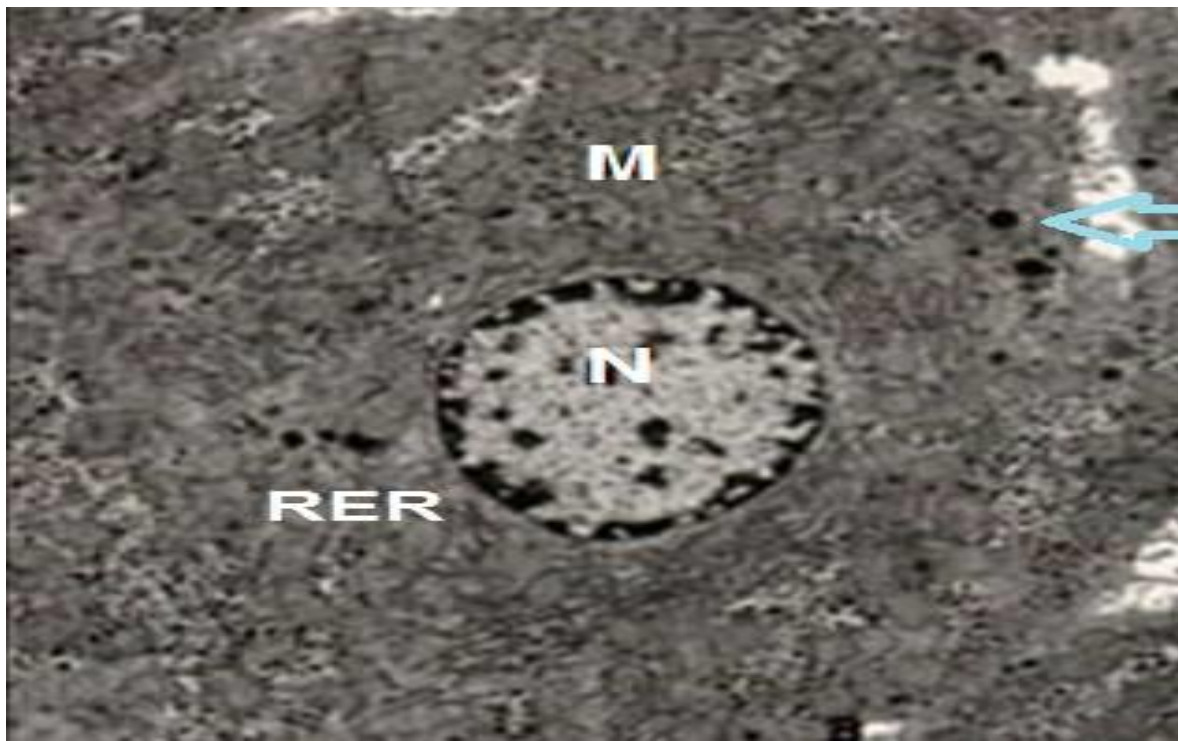
Light microscopic examination of sections of the fetal livers of control group using H&E stain showed ill-defined demarcation of hepatic lobules and the interlobular connective tissue was poorly developed. In each hepatic lobule, hepatocytes were arranged as irregular, branching and interconnected cords originating from a central vein and goes peripherally. Blood sinusoids were seen between the hepatic cords. The cytoplasm was abundant, granular and stained acidophilic. The nucleus was euchromatic, located centrally, rounded, and contained one or more nucleoli. (Figure 2).



**Figure (2): A picture from control group showing central vein (CV); hepatocytes with central nuclei and abundant cytoplasm (black arrow) sinusoids (S); and cords radiating from central vein to periphery**

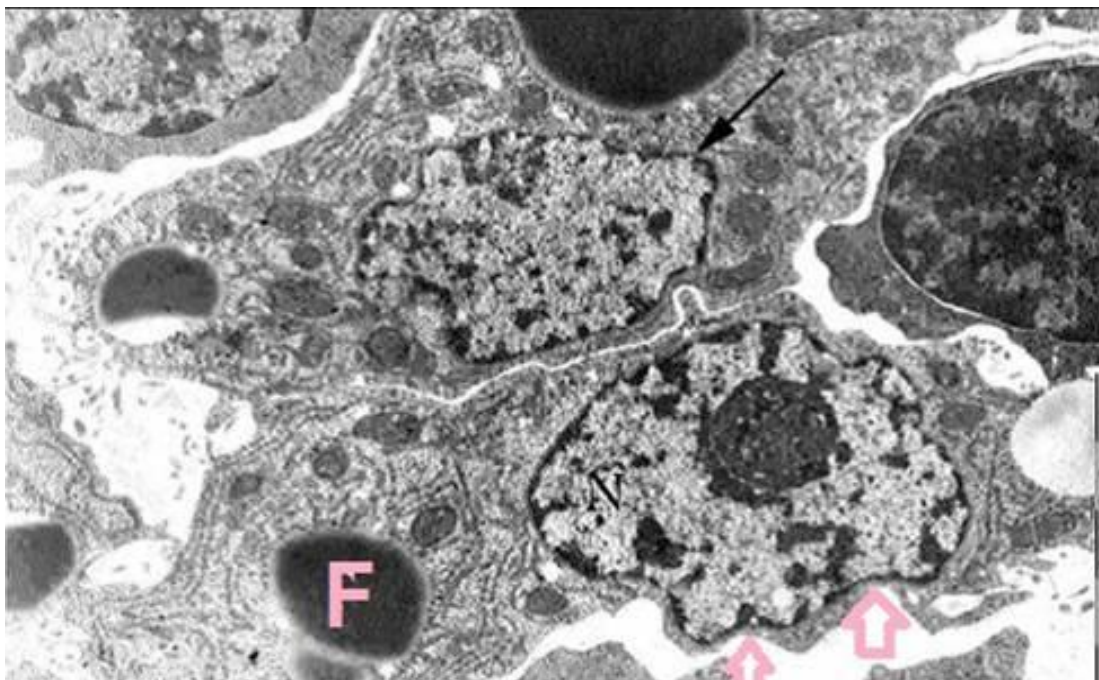
**Ultrastructure study:** Sections of fetal livers from mothers of control group showed that hepatic cells had smooth cell surfaces; the nucleus had an easily identified, regular nuclear membrane; nucleus contains euchromatin with scattered areas of heterochromatin; cell had granular cytoplasm; rosette shaped glycogen granules were scattered in the cytoplasm. Rough endoplasmic reticulum was profuse; and cisternae of it, were studded with numerous adherent ribosomes (Figure 3)





**Figure (3):** Uranyl acetate and lead citrate stained ultrathin section of fetal liver of in control group. It showed smooth cell surface, regular well-identified nuclear membrane; granular cytoplasm, glycogen granules (arrow); profuse amount of rough endoplasmic reticulum (RER) around the nuclear envelope and between the mitochondria (M).N= nucleus

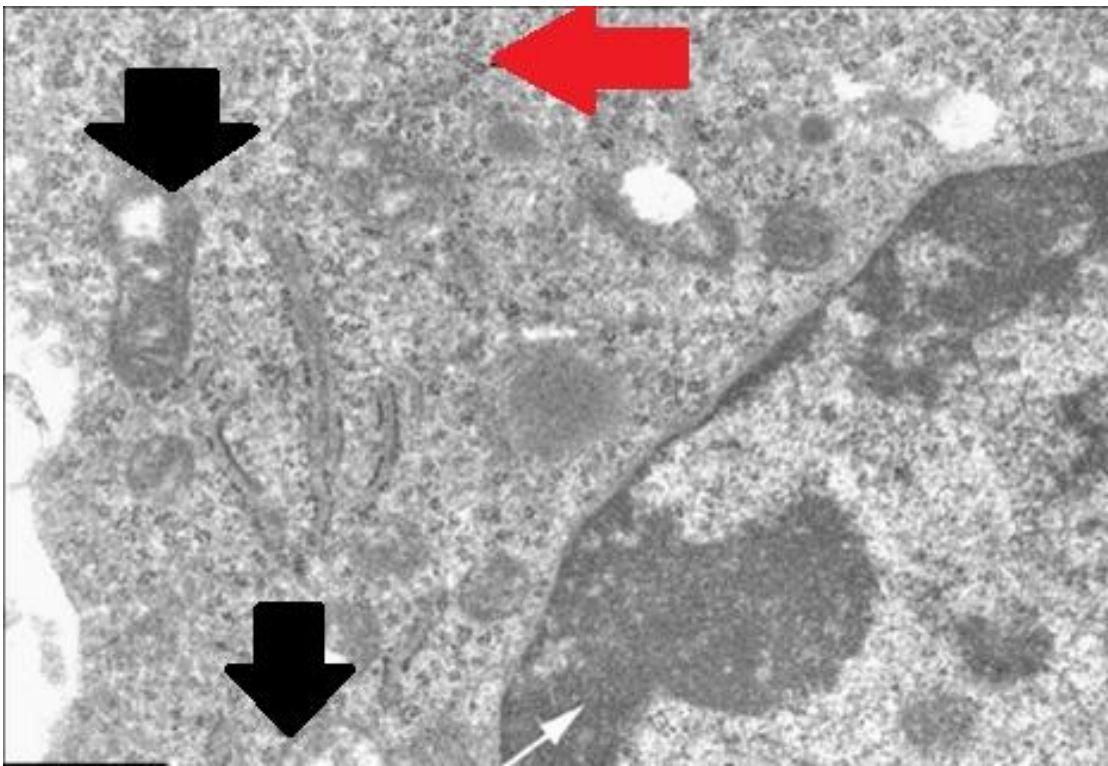
The examined sections of fetal livers from mothers of study group revealed irregularity in the cell membranes of some hepatocytes. The nucleus had an irregular nuclear envelope. The cytoplasm contained fat droplets of variable sizes (figure 4).



**Figure (4):** ultra-structure picture of fetal liver of study group showing lateral irregular hepatic cell surfaces (colored arrows). The nucleus had irregular nuclear envelope. The cytoplasm contained fat droplets (F).



In other hepatocytes, the nucleus appeared more regular and the cytoplasm revealed vacuolization showing an irregular cloudy appearance. Different shapes of the mitochondria were apparent. Loss of mitochondrial cristae was observed in other examined grids. Occasionally, the nucleus showed compaction and margination of heterochromatin resulting in formation of sharply circumscribed masses at its periphery. Cytoplasm of the hepatocytes was granular. There was also an increase in glycogen granules in comparison to control group (figure 5).



**Figure (5): Fetal liver of group II (LP) showing loss of crestea in some mitochondria (black arrow). The nucleus showed compaction and margination of heterochromatin (white arrow) and increase in glycogen granules in the cytoplasm (Red arrow).**

## DISCUSSION

Various aspects of adult anatomy, physiology and metabolism are programmed through exposure in utero to decreased nutritional environment (Bellinger and Langley-Evans, 2005).

Fetal growth depends mostly on the amount and type of nutrients obtained from the mother. A number of previous studies have shown that maternal protein restriction during pregnancy and lactation leads to reduction in birth

weight of the offspring and produces long-term decrease in the body weight and absolute liver weight (**Zambrano et al., 2006; Woods et al., 2001**).

The present work was designed to estimate the effects of reduction of dietary protein, during pregnancy on the liver of rat fetuses. Histopathological and weight changes of fetal liver were addressed. Results of the present study revealed that, females in the restricted protein group had a decreased weight at the end of the study; weight increase of the females at the end of the study was significantly decreased in the restricted protein group. In addition, restricted protein group had significantly lower number of fetuses, decreased body weight of fetuses and decreased liver weight. The reduction in body weight of maternal rats **Jansson et al. (2006)** was ascertained to the catabolic state and a breakdown of maternal proteins and fat stores.

In the present study, there was a lower number of fetuses in protein restricted group when compared to normally-fed group. **Ballen et al. (2009)** attributed this decrease to the reduction in hormones caused by protein restriction during pregnancy.

Decreased body and liver weights of fetuses in restricted protein group when compared to the control group is in line with **Minana-Solis and Escobar (2007)** who reported that, the body weights of pups from dams fed a low protein diet during gestation, weight decreased by 45% than pups from dams fed a standard diet during gestation. In addition, and to explain mechanism of decreased body weights in offspring, **Rosario et al. (2011)** found that the placental leptin and insulin/insulin-like growth factor (IGF) were decreased in maternal protein restriction. These hormones stimulate the activity of placental amino acid transporters, with subsequent decrease of body weight. Furthermore, results of the present study are in accordance with **El-Khattabi et al. (2003) and Zhang and Byrne (2000)** who reported that the liver weights in fetuses of rat dams fed a low protein diet was significantly reduced compared to those fed

on normal protein diet. In addition, **Gruppuso et al. (2005)** reported that, restricted nutrient availability sends signals toward the hepatocyte cell cycle in fetuses of fasted mothers. It accounts for decreased hepatocyte proliferation and liver mass (decreased liver weight).

Histological and ultrastructure examination of sections of fetal livers of protein-restricted group showed several degrees of cytoplasmic vacuolization (hydropic degeneration). Comparable results were reported by **Filho (2007)** and **Kumar et al. (2007)**. However, they reported a contradictory explanation for this vacuolization. Filho (2007) reported that cause to be increased permeability of cell membranes to water, with subsequent increase of intracellular water. On the other hand, Kumar et al. (2007) stated that the cytoplasmic vacuolization is due to distended and pinched-off segments of the endoplasmic reticulum. In addition, Kumar et al. (2007) reported that, protein restriction leads to cell injury with increased eosinophilia, nuclear shrinkage, fragmentation with small masses of the condensed heterochromatin distributed throughout the nucleus. This was in agreement with the present study.

In the present work, fat droplets were increased in protein-restricted group. These results are in line with **Cheng et al. (2009)** who reported that, lipid droplets were significantly increased in protein-restricted group. In addition, **Erhuma et al. (2006)** found that, maternal low protein diet causes macrovesicular steatosis in livers by appearance of fat droplets. Also, **Park et al. (2003)** hypothesized that as lipid homeostasis is mainly dependent on the liver, the underlying lipid deregulation in nutritionally restricted rats would be mediated partly through alterations of liver structure.

In the present study, the hepatocytes of fetal liver of restricted protein diet had increased levels of glycogen granules. In agreement with these results **Gosby et al. (2003)** reported that, offspring of rats fed on a restricted-protein diet had increased hepatic glycogen. They explained that the increase in



glycogen storage was not because of an increase in glycogen synthesis but due to increased sensitivity to insulin despite reduction in its secretion. In addition, **Hilakivi-Clarke et al. (1999)** reported that, the increase in glycogen content is attributed to an increase in insulin receptors in the liver.

The irregularity of both cell and nuclear membranes found in protein restricted group by ultrastructure examination in the present study, were signs of necrosis according to **Kumar et al. (2007)**. In addition, and in agreement with results of the present study, **Magwere et al. (2006)** reported that, dietary restriction alters mitochondrial morphology (size, shape, and number). **Wei et al. (2006)** also reported that protein restriction in early life causes long-lasting changes in mitochondria, and this change is more evident in the liver and skeletal muscle.

In short, results of the present study revealed that, a protein restricted diet lead to decreased body weight of pregnant females; their offspring body and liver weight, when compared to control group. Histopathological and ultrastructure examination revealed different changes (e.g., increased fat droplets, increased glycogen content, cytoplasmic vaculation and cell structure abnormalities). Thus, a well-balanced diet is advocated for pregnant females.

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## التغيرات النسيجية وتغيرات الوزن لدي إناث الفئران البيضاء اللاتي تعرضن للتغذية بوجبات ناقصة البروتين أثناء الحمل

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قسم التشريح، كلية طب الأزهر وكلية طب بنها

صممت الدراسة الحالية بهدف تقييم تأثير نقص البروتين لإناث الفئران البيضاء أثناء الحمل علي وزن الجسم ووزن الكبد، وعلي عدد الأجنة ووزنها وتقييم التغيرات النسيجية بالكبد. وقد اشتملت الدراسة علي ٦٠ من الفئران البيضاء، ٣٠ من الذكور و ٣٠ من الإناث، وقد تم وضع كل ذكر مع أنثي بقفص واحد وسمح بالتزاوج لمدة ليلة واحدة، وتم تأكيد الحمل في الصباح التالي، ثم تم تقسيم الإناث إلي مجموعتين متساويتين طبقاً لنوع التغذية، الأولى تم تغذيتها بالتغذية القياسية والثانية تم تغذيتها بوجبات ناقصة البروتين . وقد تم وزن الفئران قبل بدء الدراسة مباشرة، وعند الانتهاء من الدراسة. كم تم ذبح الإناث بعد تخديرها، واستخراج الأجنة وقياس وزنها وعددها، كم تم استخراج الكبد، وإعداده للفحص النسيجي بالميكروسكوب الضوئي والميكروسكوب الإلكتروني. وقد أسفرت نتائج الدراسة عن وجود انخفاض يعتد به إحصائياً في وزن الأم، الجنين، ونقص في عدد الأجنة في المجموعة التي تم تغذيتها بوجبة ناقصة البروتين مقارنة بالمجموعة التي تم تغذيتها بالوجبة القياسية. كذلك وجدت تغيرات بالفحص الميكروسكوبي الضوئي والإلكتروني للكبد، ما يعني ارتباط الكبد بالتغيرات في الوزن. وباختصار فقد أسفرت نتائج الدراسة عن أن نقص البروتين في الغذاء يؤدي إلي نقص الوزن الكلي للجسم ووزن الأعضاء الداخلية وخصوصاً الكبد، كما وجدت تغيرات نسيجية بالكبد منها زيادة الدهون، زيادة محتوى الجليكوجين، وتغيرات في تركيب الخلايا.