

# RAT EMBRYO'S ORGAN DEVELOPMENT UNDER SOME NUTRITIONAL SYSTEMS

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## ABSTRACT

***The maturation of rat fetus organ in connection to mother fed some special food pattern was examined histopathologically. These organs histology did not fit with that of basal diet or even those fit mothers. Organs' data, e.g., liver, kidneys, hearts, brain, as well as uterus have supported the idea that mother or fetus requirement is not actually exactly the same. With exception of control, all other organ with different histological health degree was negatively affected. This might be due to different demined to different running hormonal statuses in which hormonal balance is connected to genetic activity and diet composition. Date (D) in diet with special rats of each of skemmilk (SM) and rich plant protein antioxidants mixture (AC) or sweet potatoes (SP) was examined. The overall biological view, based on the ranking numbers used herein for fetus' organs, has asserted a group as the nearest one to the control. The best AC, SM, SP and D ratio in diet structure for mothers to fetus were 10 to 20, 30 to 10, 25 to 35 and 25 to the both, respectively. It seems that food function is not only to keep alive, but also to provides mother and embryo with the most suitable health agent that coordinates their hormonal situation. Concerning dietary balance, there is an assumption that all diet groups (G1 to G4), comparing to the health level shown for control, did not facilitate liver development except G4. This may due to the appropriate both level and source of energy in that diet. Specifically, date in diet at an optimal level is important in pregnancy for optimal embryonic growth, giving an evidence that excess energy intake may alter expression of some gene constructs within the developing embryo. Moreover, lower plasma levels of vitamins may result in hyperhomocysteinemia, a known risk factor in pregnancy. Accordingly, challenge remains to modify nutritional and management strategies in such way of wide rang of variation. The nanotechnology interpretation of nutrints distribution between fotues and mother is an important theme.***

## INTRODUCTION

**One of the main areas where nutrition influences reproductive efficiency is at the level of embryo production. Pregnancy is a special physiological important time for nutrition to come into focus producing a healthy baby. It is not just a matter of luck that nutritional factors affect the baby's health and future (Mosby, 1993).**

**Human pregnancy imposes remarkably small demands on the mother's nutrition because the fetus grows so slowly and the extra nutrient needs can be obtained from a healthy balanced diet consumed in very slight excess, only about 10–15% extra (Prenticea, 2004). During pregnancy, body weight and particularly adiposity increases due to hyperplasia rather than decreased energy metabolism. These physiological adaptations provide the growing fetus with nutrition and prepare the mother for the metabolically-demanding lactation period following birth. However, hormonal mechanisms' underlying the hyperphagia is still poorly understood. Several biological adaptations in hypothalamic neuroendocrine systems may converge to increase ingestive behavior. Oxytocin is one of the anorectic hypothalamic neuropeptides. Oxytocin neurons, both centrally projecting parvocellular oxytocin neurons and central dendritic release of oxytocin from magnocellular neurons, may play a key role in regulating energy intake. The most recent data suggest that lack of central oxytocin action may partly contribute to maternal hyperphagia. Although opioid inhibition is a major factor in oxytocin neuron restraint during pregnancy and options enhance food intake, increases in opioid orexigenic actions was not observed. While changes in several central input pathways to oxytocin neurons are likely to be involved, the high level of progesterone secretion during pregnancy is probably the ultimate trigger for the adaptations. Although the peripheral signals that drive appetite and satiety centers of the brain are increased in pregnancy, the brain may become insensitive to their effects. For example, leptin secretion increases but hypothalamic resistance to leptin actions develops (Douglas, et al, 2007). In addition, fetus growth studies on fetal programming of adult diseases have highlighted the importance of maternal nutrition during pregnancy (Raoa et al 2006). Folic acid and long-chain essential polyunsaturated fatty acids (LC-PUFAs) have independent effects on fetal growth. However, folic acid effects may also involve alteration of LC-PUFA metabolism. Because marginal deficiency of LC-PUF as during critical periods of brain growth and development is associated with risks for adult diseases, it is highly relevant to investigate how maternal supplementation of such nutrients can alter brain fatty acid levels (Raoa et al 2006). They examined the impact of folic acid supplementation, conventionally used in maternal intervention, on brain essential fatty acid levels and plasma corticosterone concentrations in adult offspring at 11 months of age. Results suggest that maternal folic acid supplementation at marginal in protein intake decreased brain docosahexaenoic acid levels probably involving corticosterone increase (Raoa et al 2006). Moreover, lower plasma levels of vitamins may result in hyperhomocysteinemia, a known risk factor in pregnancy (Wersch, et al 2002).**

**The research on embryos growth, either in human or domestic animal, is, for different purposes, rapidly developed. Over the past few decades there has**

been resurgence of interest in the culture media used in clinical in vitro fertilization. Unfortunately, during this time more confusion than consensus appears to have developed regarding the composition of these media. In order to facilitate a clearer understanding of this field, it is important to understand how specific medium components are regulated by the embryo. The roles of the key nutrients glucose, pyruvate, lactate, and amino acids during the preimplantation period have therefore been presented. Analysis of how the embryo regulates the utilization of such nutrients has led to a clearer understanding of the embryo's requirements during the dynamic period of preimplantation development. From such information, sequential culture media have been developed along with novel noninvasive tests of embryonic viability (*Gardner, et al, 2000*). Some animal indicate that ad libitum diets are highly detrimental for superovulatory programmes when compared with low and control diets. In addition, the results from this study indicated that a low energy diet during early embryo development increases the uterine production (Lozano et al, 2003). Earlier, Dunn (1980) studied the relationship of nutrition to successful embryo transplantation. Again, Dunn (2007) reviewed a new perspective on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows that connected to the relationship of nutrition to successful embryo transplantation. Yet, nutrition, synchronization, and management of beef embryo transfer recipients was investigated (Jones and Lamb, 2007). Hormonal diversity of mother during pregnancy is quite important. Excess energy intake in sheep, for instance, leads to significant reductions in progesterone concentrations; the effects in cattle are not so clear. Nutrition, unless radically changed, will have little effect on gonadotropin concentrations in ruminants, and this is in contrast to the situation for pigs and for primates, where very short-term nutritional changes manifest themselves in altered gonadotropin secretion. Cattle with reduced energy intake have smaller dominant follicles and more three-wave cycles, compared with animals on higher feed intakes. Several studies indicate that excess energy intake reduces the response to superovulation and also decrease the yield of embryos and alters expression of some gene constructs within the developing embryo. The mechanism of this effect is not clear but indications are that the quality of the oocytes may be compromised. Indeed recent data indicate that nutritional changes around the time of mating may have detrimental effects on the establishment of pregnancy in heifers. Thus, nutritional balancing is critical for high-yielding dairy cows, in particular. The challenge remains to modify nutritional and management strategies in such cows to maintain the levels of production made possible by genetic selection and still maintain an acceptable level of fertility (Boland et al, 2001). López de Torre et al (1993) biologically examined the effects of experimental prenatal intestinal obstruction on the growth and blood composition of chick embryos. Intestinal atresia (IA) found haematocrit was

slightly lower, and total protein increased. Pre-albumin was absent in their sera and albumin, alpha and beta globulins were significantly decreased whereas gamma-globulin was greatly increased. Sodium, potassium chloride, urea and glucose remained within normal limits. The lack of placenta in the avian embryo precludes any supply of nutrients by this route and the ingestion of amniotic fluid, which is protein-rich after the 13th day of incubation becomes the main source of nutrients until hatching. Obstruction of the main incoming avenue by IA induces severe malnutrition in this model which relies on this route to a greater extent than the human foetus. In spite of the obvious biological differences between the avian embryo and the human foetus, evidence supports the hypothesis that prenatal interruption of the amniotic fluid transit contributes to fetal undergrowth in IA. More recently, the effects of dietary factors on the development and viability of avian embryos have been extensively documented. A good nutritional status of the parent birds is crucial to the transfer to the egg of an adequate, balanced supply of nutrients required for normal development of the embryo. The consequences to the embryo may be lethal if the egg contains inadequate, excessive, or imbalanced levels of nutrients (Wilson, 1997). Moran (2007) stated that a portion of the albumen is absorbed by the small intestine to expand body glycogen reserves. Mobilization of body fat complements contributions from the yolk sac to provide fatty acids for generating energy, heat, and water while also combining with hepatic cholesterol for membrane expansion and continued development. Continued studies on the human embryo will lead to further improvements in embryo culture conditions and the optimization of viability assays, culminating in the ability to transfer single embryos for the majority of, if not all patients (Gardner et al, 2000). Correlation of foetus health and nutrition has been studied very early (Nikitiuk and Vysheslavova, 1964). Recently, a little higher ratio of proteins, i.e. 18%, most of which are plant, with a plenty amount of vegetable CHO and less than 10% fat in oily form with an adequate amount of minerals and vitamins that are particularly found in dates and sweet potatoes, are important food toleration for pregnancy (Ahmed *et al*, 2007). In this study, the overall data have got the assumption that productivity and healthy rat offspring were reached when a special plant protein source with a unique antioxidant structure was incorporated at a level of 20%. The other food component of the best diet that met the highest productivity and healthy both mothers and kids were the skimmed milk of 10% plus another source for CHO such as sweet potatoes at a high ratio of 35%.

Further research is needed in this area of nutritional biochemistry. Therefore, special diet or dietary supporting is proposed here to meet this particular hormonal balance to proper metabolic situation associated with pregnancy.

## MATERIALS AND METHODS

**Materials:** All food materials, e.g., dates, as semisolid, sweet potatoes and skimmed milk powders, as well as honey, apple, and spices used in preparation of special additives were obtained from local market in Cairo. The ACES is an antioxidant formula first prepared by Ahmed et al (1999).

**Rationales and Animals:** Female albino rats of western strains were housed in the Ophthalmology Research Institute, Giza, Egypt. The groups (5 x 6 = 30 animals) were fed on basal diet for 10 days for adaptation then divided into those groups taking Bowman et al, (1990) basal diet as control (C), where salt and vitamins mixtures have followed that of AOAC (1984).

**Table 1, Groups of dietary formulations.**

<i>Group</i>	<i>SM</i>	<i>SP</i>	<i>S</i>	<i>D</i>	<i>AC</i>	<i>VM</i>	<i>MM</i>	<i>AD</i>	<i>FAT</i>
<b>C</b>	50	-	40	-	-	1	4	-	5
<b>G1</b>	10	10	-	50	20	1	4	1	4
<b>G2</b>	30	-	-	50	10	1	4	1	4
<b>G3</b>	10	35	-	25	20	1	4	1	4
<b>G4</b>	30	25	-	25	10	1	4	1	4

Where: C, control diet, SM skimmed milk, SP sweet potato, S starch, D date, AC protein antioxidant semi modified food VM and MM vitamin and mineral mixtures. AD is a mixture of special extract.

The thirty adult female albino rats were of average weight of  $222\pm 5$  g. Those females that almost detected pregnant have been prepared for pregnancy according to UFAW (2000), whereby a more elaborated way of determination of the female's fertile state was by monitoring the oestrous cycle by means of daily vaginal smears. These smears are taken by flushing the vagina with a drop of saline solution using a small drip pipette or by gently scarping the vagina with a small flexible spatula. The sample was subsequently put on a glass slide, dried and stained with crystal violet. The phase of the oestrous cycle can be determined under the microscope on the basis of the presence and quantity of confirmed epithelial cells and leucocytes. The male was mounted an oestrous female frequently and after several intromissions and ejaculation occurred. This sequence of events was repeated two or three times with postejaculators interval of 5-10 minutes. After a while, the female arises courtship behavior and defends herself from the male. The vaginal fluid and ejaculate was coagulated to form a plug. According to UFAW (2000), the time of conception has to be timed accurately, either the behavior of the male and female can be observed directly or a series of vaginal smears can be

taken and monitored for the presence of sperm. Sperm cells were detected microscopically in the vaginal smear for up to 12 hours after conception. It is difficult to detect pregnancy before the fifteenth day post conception. The thirty females of very near timing of gestation were optionally selected. Those animals were divided into that five groups and fed on the diets outlined in Table 1. The food and water were given ad libitum daily. Foetuse have been surgically delivered the expected two days before delivery.

**Histopathological examination:** *Organs such as liver, kidney, brain, heart, as well as uterus* were collected then the post-mortal examination was done as soon as possible. Fixation was done in 10% of natural formalin, dehydrated, cleared, and ended paraffin then sectioned at (4-6 mm), stained with Harris hematoxylin and casein for histopathological examination (*Frankel and Reitman, 1963*). Data has been expressed in mean of almost six measurements.

## **RESULTS AND DISCUSION**

At pregnant stage, the nutrition of the embryo is solely depending on its mother's health, nutrition, and metabolism. Data listed in Tables 2 to 5 showed the health of foetuse organ after feeding mothers with some dietary systems earlier shown in Table 1. Despite the defects of the control itself, food additives used to help pregnancy may reversely affected fetus organ development in most cases. In some details, liver histopathological examination of fetus from control rats revealed congestion of central veins and hepatic sinusoids as well as sinusoidal leucocytosis, meanwhile, sections of fetus rats from groups 1 and 2 showed necrosis of hepatocytes, leucocytic cells infiltration and presence of megacaryocytes. On the other side, liver of foetus from group 3 revealed vaculation of some hepatocytes, dissociation of some hepatocytes, leucocytes and megalearyocytes in the hepatic sinusoids. Group 4 liver showed some vaculation of hepatocytes and presence of multiple megakariocytes. The later most probably gave relatively best feeding condition for liver growth even over findings presented for the control.

Contrary, kidneys histopathological findings of control foetus showed no changes in tissues, meanwhile, a marked necrosis of renal parenchyma was noticed in kidneys from group 1. However, kidneys of foetus rat from group 2 revealed necrosis of epithelial lining some renal tubules. Apparent normal renal parenchyma was observed in kidneys of foetus from group 3 in comparison to a development for renal parenchyma in kidneys of foetus from group 4. It seems that there is an association between that organ health and feeding on a balanced animal and plant food resources.

*Table 2, Histopathological examination of foatus' livers from control and dietary treated rats.*

<i>Liver histopathological picture</i>	<i>Control</i>	<i>G1</i>	<i>G2</i>	<i>G3</i>	<i>G4</i>
<b>Necrosis of hepatocytes</b>		+	+		
<b>Leucocytic cells infiltration</b>		+	+		
<b>Presence of megalocaryocytes.</b>		+			
<b>Vacuolation of some hepatocytes</b>				+	
<b>Dissociation of some hepatocytes, and leucocytes</b>				+	
<b>Megalearyocytes in the hepatic sinusoids.</b>				+	
<b>Marked vacuolation of hepatic cell</b>					+
<b>Presence of multiple megalocaryocytes.</b>					+
<b>Congestion of central veins</b>	+				
<b>Hepatic sinusoids</b>	+				
<b>Sinusoidal leucocytosis</b>	+				
<b>Ranking number</b>	<b>4</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>2</b>

**Table 3, Histopathological examination of foetus' Kidneys from control and dietary treated rats.**

<i>Kidneys histopathological picture</i>	<i>Control</i>	<i>G1</i>	<i>G2</i>	<i>G3</i>
<b>No changes in tissues.</b>	+			
<b>Marked necrosis of renal parenchyma</b>		++		
<b>Necrosis of epithelial lining some renal tubules.</b>			+	
<b>Developed renal parenchyma</b>		+	+	
<b>Ranking number</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>1</b>

Regarding heart, control foetus rats showed vacuolation of some myocardial muscle fiber. Meanwhile, heart of foetus from group 1 showed atrophy of myocardial muscle fibers associated with intramuscular edema. Heart of foetus rat from group 2 showed vacuolation of cardiac muscle fibers and granularity of sarcoplasm of some muscle fibers. On the other hand, heart of foetus from group 3 revealed thin atrophied cardiac muscle fibers and intramuscular edema. Necrosis of cardiac muscle fibers was noticed in heart of rat foetus from group 4.

Similarly, microscopy of brain tissues from control foetus as in Table 5, gave apparent normal structures. In brain of foetus rat from G1 there were demyelination of nerve fibers and focal gliosis. Moreover, the most prominent changes in foetus from G2 were focal gliosis. Examined brain of rat from G3 revealed necrosis of neurons and diffuse gliosis, meanwhile, diffuse gliosis was the most conspicuous histopathological changes observed in brain of foetus rat from G4.

**Table 4, Histopathological examination of foetus' hearts from control and dietary treated rats.**

<i>Hearts histopathological picture</i>	<i>control</i>	<i>G1</i>
<b>Vacuolation of some myocardial muscle fiber.</b>	+	
<b>Atrophy of myocardial muscle fibers associated with intramuscular edema.</b>		+++

Vacuolation of cardiac muscle fibers and granularity of sarcoplasm of some muscle fibers.		
Thin atrophied cardiac muscle fibers and intramuscular edema.		
Marked necrosis of cardiac muscle fibers.		
Ranking number	1	3

**Table 5, Histopathological examination of foetus' brain from control and dietary treated rats.**

Brains histopathological picture	Control	G1	G2	G3	G4
Apparent normal structures.	+				
Demyelination of nerve fibers and focal gliosis.		++			
Focal gliosis.			+++		
Necrosis of neurons.				+	
Diffuse gliosis.				+	++
Ranking number	1	2	3	2	2

**Table 6, Histopathological examination of foetus' uterus from control and dietary treated rats.**

Uterus histopathological picture	Control	G1	G2	G3
No histopathological changes.	+			
Thin mucosa, congestion of submucosal blood vessels and submucosal edema.		+++		
Marked dilatation and congestion of blood vessels and submucosal edema.			+++	
Necrosis and hemorrhage in the mucosal surface				++
Ranking number	1	3	3	2

Lastly, the uterus microscopically examination of control rat, as in Table 6, showed no histopathological changes, where uterus of rat G1 revealed thin mucosa, conjugated edema. However, uterus of rat from G3 and G4 showed necrosis and hemorrhage in the mucosal surface.

In an overall biological view based on the ranking numbers used herein for foetus' organs, shown in Table 7, it is clear that the nearest group to the control is G4 then G3, meanwhile; G1 is the most propounded health risk one. Accordingly, the best AC, SM, SP and D diet structure for mothers to foetus were 10 to 20, 30 to 10, 25 to 35 and 25 to each, respectively. It seems that date level at 25% in diet did not make any nutritional differences, but skemilk and rich plant protein antioxidants mixture significantly do. However, mother liver function as well as organs such as liver, heart and kidney histology was kept at most acceptable parameters under this feeding condition mostly controlling glycemia which is important role of dieting during pregnancy. Gestational diabetes, however, is associated with significant pregnancy complications such as macrosomia, perinatal mortality and prematurity (Ahmed *et al*, 2007). For mothers, a little higher dietary ratio of proteins, i.e. 18%, most of which are plant, with plenty amount of vegetable CHO and less than 10% fat in oily form with an adequate amount of minerals and vitamins found in D and SP, are important food toleration. Concurrently, animal protein ratio in diet for fetus is critical. Lozano *et al* (2003) concluded that changes in diet can affect the quality of the oocyte and embryo in superovulated sheep. Likewise, a lower superovulation response and a



decrease in the quality of oocytes and embryos indicated that ad libitum diets are highly detrimental for superovulatory programmes when compared with low and control diets. Data indicated that pregnancy is very sensitive time where mother nutrition is playing a critical role on embryonic health. Concerning diet balance, Table 7 gave an assumption that all working diet groups did not fit organs development except G4 for a relative extent. Note that group 3 was seen to be more useful for mothers with exception of control. All other organ health, with different degree, was negatively affected.

**Table 7, Biological evaluation based on the histopathological examination of foetus' organs from control and dietary treated rats.**

<b>Organs</b>	<b>Control</b>	<b>G1</b>	<b>G2</b>	<b>G3</b>	<b>G4</b>
Liver	4	4	2	4	2
Kidney	1	3	3	1	1
Hearts	1	3	3	2	2
Brains	1	2	3	2	2
Uterus	1	3	3	2	2
<b>Total ranking number</b>	<b>8</b>	<b>15</b>	<b>14</b>	<b>11</b>	<b>9</b>

This is highlighted the importance of maternal nutrition during pregnancy, as nutritional deficiencies or excesses occur; it is common for the effects on the embryo to also become more severe and to occur at earlier stages of development. We can conclude that diet in pregnancy should be prepared dense with a wide rang of varieties depending on a recognizable food item selection at an optimal level of energy content. A wide rang contains modest amount of vitamins and minerals should be calculated. Data interpretation from the nanotechnology point of view may declare critical information about the facts of the food fragment distribution between foetues and mother as an important theme. In fact, fetus brain regulates its development, it directs the utilization of available nutrients none simultaneously with mother. Take in account that maternal folic acid supplementation at marginal in protein intake decreases brain docosahexaenoic acid levels that probably involving corticosterone increase. Moreover, low dietary folate and deficiency of methylenetetrahydrofolate reductase (MTHFR) were reported to increase the risk for congenital heart defects, but contributory mechanisms have not been elucidated (Li and Rozen, 2006) Again, our data showed that even slight poor balanced diet consequences to the embryo may be lethal where type of nutritional stress signs visible in the embryonic organ maturation often depend upon the severity of the maternal nutritional stress. It means that feeding here should be concentrated of wide variation with energy controlled. Accordingly, challenge remains to modify nutritional and management strategies in this area of nutritional biochemistry.

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## الملخص العربي

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### التطور الصحي للأجنة مع بعض نظم تغذية الحوامل في الفئران

فتحية ابوزيد و اميرة شتيوي\* وامل عبد الباقي و داليا النحال

قسم الأغذية الخاصة و التغذية-معهد تكنولوجيا الأغذية وكليات-التربية النوعية بجامعة الاسكندرية\*

اعتمد البحث في التقييم الغذائي للام الحامل علي الصفات التشريحية للأجنة. لقد أكد البحث ان عملية

التغذية اثناء الحمل تعتبر من العمليات المعقدة حيث ثبت حساسية الاجنة لمكونات الغذاء مهما كانت

متقاربة التكوين كما ثبت – وهذا هو المهم- ان للاجنة شخصية غذائية تتباين مع احتياجات الام. ان افضل ما يقدمه بحث كهذا هو التذكير بأهمية استخدام اغذية كثيفة المحتوى كثيرة التنوع مع التحكم في مستوى الطاقة التي تتناولها الام. لقد وجد ان الاسراف في تناول غذاء بعينة حتي و ان كان غذاءا حاويا كالبلح علي حساب غيره من الاغذية المكملة لة ضار بالجنين و معطل لتكوين اعضاؤه بالشكل الكامل وان التوازن الغذائي في هذا الظرف الفسيولوجي الخاص يساهم في انتاج اجنة كاملة الاعضاء و جنين خال من المرض وخاصة امراض الكبد.

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