

Dept. of Biochemistry
Fac. Vet. Med., Zagazig University

**STUDIES ON THE PROFILE OF GLUCOSE,
TOTAL LIPIDS, FREE FATTY ACIDS
AND PHOSPHOLIPIDS DURING PREGNANCY
IN DIFFERENT SPECIES OF RABBITS**
(With 2 Tables and 4 Charts)

By

**H.E. EL-BELBASY; SAIEDAT, S. ABDEL-MAGID;
A.A.H. ZAKY*; THANAA, K. HASSANIEN*
and A.A. YOUSSEF**

* Animal Reproduction Research Institute, Al-Ahram, Giza, Egypt.
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دراسة مستوى كل من الجلوكوز، الدهون الكلية، الأحماض الدهنية الحرة
والدهون الفوسفاتية في الفصائل المختلفة من الأرانب أثناء فترة الحمل

حسين إبراهيم البليسي ، سيدات سعد عبد المجيد ، عادل أنيس حنا زكي،
ثناء كامل حسانيين ، عبد الصادق أبو العطا يوسف

تم قياس مستوى كل من الجلوكوز، الدهون الكلية، الأحماض الدهنية الحرة والدهون الفوسفاتية في أربعة فصائل من الأرانب هي النيوزيلاندي النقي، هجين بين أنثى نيوزيلاندي مع ذكر بلدي، هجين بين أنثى بلدي مع ذكر نيوزيلاندي والبلدي النقي. اختيرت عدد خمسة إناث بالغة صحيحة من أرانب كل فصيلة بالإضافة إلى مجموعة إناث أرانب استخدمت كضوابط للتغيرات أثناء الحمل. حيث أن هذه الدراسة ضمن الخطة البحثية الخاصة بمعهد بحوث التسمليات بالهرم - الأهرام - جيزة، لذلك تمت رعاية كل الأرانب وتربيتها بمزرعة المعهد. أخذت عينات بلازما ومصل الدم من أرانب كل المجموعات قبل الحمل (اليوم صفر) وبعد الحمل (أيام ٧، ١٤، ٢١، ٢٨، ٣٢ (يومين بعد الولادة)). أوضحت النتائج وجود زيادة معنوية في مستوى الجلوكوز خلال اليوم ١٤ و ٢١ من الحمل ووجود نقص معنوي خلال اليوم ٢٨ من الحمل. وجدت زيادة معنوية في مستوى كل من الدهون الكلية، الأحماض الدهنية الحرة والدهون الفوسفاتية من اليوم الرابع عشر وحتى اليوم الثامن والعشرون من الحمل. عادت مستويات كل من الجلوكوز، الدهون الكلية، الأحماض الدهنية الحرة والدهون الفوسفاتية إلى مستواها الطبيعي خلال يومين بعد الولادة (اليوم ٣٢ من الحمل). صوما كانت هناك زيادة معنوية في مستوى جميع القياسات بالنسبة للأرانب النيوزيلاندي النقية تلتها النيوزيلاندي الهجين ثم البلدي الهجين أما أقلها فكانت في الأرانب البلدي النقية.

SUMMARY

The levels of glucose, total lipids, free fatty acids and phospholipids were estimated in four species of rabbits (New Zealand, New Zealand X Balady, Balady X New Zealand and Balady). Since, the present study was one of the scientific plan of the Animal Reproduction Research Institute (ARRI), Al-Ahram, Giza, Egypt, so, five adult and healthy female rabbits from each species were raised under the balanced levels of nutrition at the experimental farm of the institute. Both plasma and serum samples were collected from all the rabbits before pregnancy (day 0), during pregnancy (days 7, 14, 21, and 28) and 2 days after parturition (day 32). The results revealed that there was a significant increase in the level of blood glucose during the 14th and 21st days of pregnancy, but it decreased significantly at the 28th day of pregnancy. A significant increase in the levels of total lipids, free fatty acids and phospholipids from the day 14 till the day 28 of pregnancy. After 2 days of parturition, the values of glucose, total lipids, free fatty acids and phospholipids returned back to their normal values. In general the figures of all the parameters, in this study, were significantly higher in the New Zealand pure (NP) species followed by New Zealand X Balady (NC) then Balady X New Zealand (BC) species. The lowest parameters were noticed in the Balady pure (BP) species.

Key words: Glucose-Lipids-Pregnancy-Rabbits

INTRODUCTION

During labor free fatty acids are the principal fuel. This increase in maternal free fatty acids may serve to spare glucose as a metabolic fuel in the fetus. Free fatty acids and blood glucose levels were significantly higher in the maternal than in the fetal side during the course of pregnancy (Kashyap *et al.*, 1976 and Gilbert *et al.*, 1991 and 1993). Also, Hay *et al.* (1984) mentioned that in the pregnant rabbits, blood glucose concentration fell by the end of gestation to an average value of 74.6 ± 2.7 mg/dl, significantly less than the glucose concentration in the same animals before pregnancy (88.2 ± 2.4 mg/dl).

Gilbert *et al.* (1984) and Bouisset *et al.* (1986) studied the metabolic demands of the uterus in rabbits at days 24 and 30 of gestation, they found that during these two periods, the amount of glucose directed to the gravid uterus represented 13 and 36% of the maternal glucose turnover rate, respectively. The maximum

contributions of glucose and free fatty acids to the uterine oxygen consumption on day 24 were 80 and 30%, respectively. They confirmed that at term, the gravid uterus is a site of high glucose consumption and they demonstrated that in a non ruminant species, free fatty acids, would be a substantial source of carbon and the glucose concentration decreased with pregnancy to a value at term equal to 85% of the pregnancy value but there were rise significantly in the second half of pregnancy.

Elphick *et al.* (1975) and Elphick and Hull (1977) studied the passage of fatty acids across the placenta in 28 day pregnant rabbits, by comparing the fatty acid distribution in plasma free fatty acids (FFA) of umbilical cord artery and vein with that in maternal plasma. They found that, all the major fatty acids present in fetal adipose tissue cross the placenta and the net transport of each fatty acid depends in part on maternal concentration.

Stammers *et al.* (1983) measured the serum lipid profiles in pregnant rabbits at the three trimesters of pregnancy, after parturition and during lactation. They indicated a significant increase in the serum concentrations of free fatty acids, total lipids and phospholipids in the second trimester of pregnancy. Very highly significant increase demonstrated in the level of free fatty acids, total lipids and phospholipids at third trimester of pregnancy.

Stammers *et al.* (1995) and Montoudis *et al.* (1999) stated that there was a significant increase in the maternal concentration of phospholipids and free fatty acids (FFA) at the third trimester of pregnancy in rabbits. However, the composition of FFA crossing the placenta is modulated to become more similar to that of the exogenous lipid.

Viard-Drouet *et al.* (1984) determined the changes in biochemical composition of the plasma due to pregnancy in healthy rabbits doe which are total lipids and phospholipids. The results showed a significant increase in total lipids and phospholipids levels at the second week of pregnancy. These levels still highly increased till the day 28 of pregnancy and return progressively, after parturition, to the values obtained in non-pregnant and non-lactating rabbits.

With a few exceptions, changes that occur in the serum chemistry parameters during pregnancy in the rabbits are similar to those observed in pregnant women. So, the rabbit can be considered a suitable species for embryo-fetal development toxicity studies (Wells *et al.*, 1999). Therefore the present work aimed to study the levels of glucose,

total lipids, free fatty acids and phospholipids during pregnancy in rabbits. The obtained data will be necessary to record at any time the levels of these parameters will be affected, and to determine, the daily need of these parameters all over the gestation period and after parturition.

MATERIAL and METHODS

Four species of rabbits (New Zealand pure, New Zealand X Balady, Balady X New Zealand and Balady pure), five adult and healthy female rabbits from each species were submitted for the experimental procedures, another four female rabbits (one from each species) were kept as a control. Two male rabbits (one New Zealand pure and one Balady pure) were kept for fertilizing the experimental females only. Since, the present study was one of the scientific plane of the Animal Reproduction Research Institute (ARRI) Al-Ahram, Giza, Egypt, so, all the rabbits (6-8 months old, 2.1 - 3.2±0.20 Kg body weight and non pregnant) were raised under the balanced levels of nutrition at the experimental farm of the institute. Pregnancy was diagnosed by abdominal palpation 10 days after breeding and by vaginal smears examination of the female rabbits (Ciro and First, 1976).

Both plasma and serum samples were collected from all the female rabbits before pregnancy (day 0), during pregnancy (days 7, 14, 21, and 28) and 2 days after parturition (day 32).

Determination of glucose: Plasma glucose was determined by glucose kits of Bio Merieux according to the method of Trinder (1969).

Determination of total lipids: Serum total lipids were colorimetrically determined by lipid kits of Bio Merieux according to the methods of Chabrol and Charonnat (1937) using sulfo-phospho-vanillic mixture.

Determination of free fatty acids: Serum free fatty acids were colorimetrically determined according to the method described by Schuster (1979) using methyl red indicator and heptan-isopropanol mixture.

Determination of phospholipids: Serum phospholipids were determined by kits of Bio Merieux after the method of Takayama et al. (1977).

RESULTS

The data recorded in Tables 1 and 2 and Figures 1 - 4 showed the differences in the levels of plasma glucose, serum total lipids, free fatty acids and phospholipids between the non pregnant (control group) and the pregnant females, also, between the pure and the cross bred species of rabbits.

Plasma glucose: A significant increase ($p < 0.05$) in the values of blood glucose was recorded at the 14th day of pregnancy in New Zealand pure (NP), New Zealand \times Balady (NC), Balady \times New Zealand (BC) and Balady pure (BP) (83.65 ± 1.25 , 82.24 ± 1.32 , 81.94 ± 1.33 and 80.46 ± 1.48 mg/dl, respectively) than the control group (76.45 ± 1.47 mg/dl). A highly significant increase ($p < 0.01$) was detected at day 21 of pregnancy but, at day 28 of pregnancy there was a significant decrease in the level of blood glucose than the control group. At day 32 (2 days after parturition) the values of the pregnant groups returned near the values recorded in both the control group and the day before mating (day 0).

Serum total lipids: A significant increase ($p < 0.05$) in the serum total lipids at day 14 of pregnancy (479.73 ± 1.10 , 476.61 ± 2.25 , 473.59 ± 1.23 and 468.4 ± 1.40 mg/100ml in NP, NC, BC and BP, respectively) than the control group (462.12 ± 5.59 mg/100ml). At day 21 of pregnancy a highly significant increase ($p < 0.01$) was detected. At day 28 of pregnancy there was a very highly significant increase ($p < 0.001$) in total lipids value in pregnant rabbits than the control group. At day 32 (2 days after parturition) the values of the pregnant groups returned near the values recorded in both the control group and the day before mating (day 0).

Serum free fatty acids: There was a significant increase ($p < 0.05$) in the values of free fatty acids of NP, NC, BC and BP rabbits (58.15 ± 2.99 , 57.55 ± 2.43 , 55.17 ± 1.56 and 52.92 ± 0.26 mg/100ml, respectively) than the control group (47.76 ± 2.41 mg/100ml) at the day 14 of pregnancy. At day 21 of pregnancy there was a highly significant increase ($p < 0.01$) in the four groups of rabbits than the control group. At day 28 of pregnancy there was a very highly significant increase ($p < 0.001$) in free fatty acids value in pregnant rabbits than the control group. At day 32, (2 days after parturition), the values of the pregnant groups returned near the values recorded in both the control group and the day before mating (day 0).

Serum phospholipids: The serum phospholipids parameters were significantly increased ($p < 0.05$) at the 14th day of pregnancy in the

NP, NC, BC and BP rabbits (122.30 ± 3.22 , 119.31 ± 3.29 , 117.12 ± 2.21 and 114.06 ± 0.15 mg/100ml, respectively) than the control group (108.77 ± 2.13 mg/100ml). At days 21 and 28 of pregnancy there was a highly significant increase ($p < 0.01$) in the phospholipids value in pregnant rabbits than the control group. At day 32, (2 days after parturition), the values of the pregnant groups returned back near the values recorded in both the control group and the day before mating (day 0).

In general the figures of all the parameters, in this study, were significantly higher in the New Zealand pure species followed by New Zealand X Balady then Balady X New Zealand species. The lowest parameters were noticed in the Balady pure species.

DISCUSSION

The present study revealed that, the normal plasma glucose averaged 78.11 ± 1.49 mg/dl in healthy control rabbits. This finding was on line with Coles (1980) who reported values of 55-75 mg/dl, Hay et al. (1984) who mentioned a value of 88.2 ± 2.4 mg/dl, and Kancko (1989) who reported that blood glucose in healthy rabbits was 50-80.2 mg/dl. The increase in values of glucose during the time intervals of pregnancy was in agreement with Gilbert et al. (1984), while the decrease in its level at the day 28 of pregnancy was agree with Hay et al. (1984) who reported values of 88.2 ± 2.4 mg/dl before mating then became significantly less by the end of gestation to reach values of 74.6 ± 2.7 mg/dl.

In regard to the normal lipidogram of healthy rabbits examined in the present study, it is evident that the serum total lipids was averaged 460.21 ± 7.20 mg/100ml in the control rabbits under examination. This result was near the results mentioned by Yanny (1989) who reported values of 483.59 ± 77.88 mg% in New Zealand white rabbits. Lower levels of total lipids were recorded by Boyd (1942) (243.0 ± 89.0 mg%) and Garbus et al. (1967) (32.8 ± 8.0 mg%) in the serum of New Zealand white rabbits, while rather higher values were recorded by Westerman et al. (1970). The increase of levels of total lipids, in the present study, during the 14th, 21st, and 28th days of pregnancy, then its values returned near normal as before mating was on line with data recorded by Stammers et al. (1983) and Viard-Drout et al. (1984) who stated that there was an increase in total lipids during time of pregnancy and then return to normal values before pregnancy after parturition.

This work revealed that, the value of serum free fatty acids averaged 47.60 ± 1.85 mg%. Yanny (1995) reported that serum free fatty acids in two groups of healthy New Zealand rabbits were 50.82 ± 13.88 and 48.22 ± 10.4 mg% but Coles (1967) reported a value of 45.8 mg% in healthy New Zealand rabbits. The increase in levels of free fatty acids during the time intervals of pregnancy was in accord with Stammers *et al.* (1995) and Montoudis *et al.* (1999).

In the present study, the average level of phospholipids in control healthy rabbits was 106.01 ± 2.49 mg%. Our data were in agreement with the values reported by Coles (1974) who reported phospholipids value of 105.2 ± 3.0 mg% and Kaneko (1989), who reported a value of 104.0 ± 8.0 mg%. On the other hand, lower values were recorded by Boyd (1942) (78.0 ± 33.0 mg%), but higher values (113.0 ± 29.0 and 116.22 ± 33.4 mg%) were tabulated in New Zealand rabbits by Westerman *et al.* (1970) and Yanny (1988), respectively.

The use of four different species (pure and cross breed), in this study, is to find the differences in the biochemical parameters of their serum during pregnancy. The results revealed that these differences were minute, so, the cross breed species of rabbits could be used in case of the difficulty in obtaining the pure species. Also we found that the free fatty acids (which highly significantly increased) were the main source of fuel to the foetus than the glucose (which decreased) at term.

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Table (1): Plasma glucose and total lipids in rabbits at different time intervals of pregnancy.

Parameters	Group	Time intervals (days)						
		0	7	14	21	28	32	
Glucose (mg/dl)	Control	78.11±1.49	77.99±2.60	76.45±1.47	77.13±2.75	79.67±1.42	76.16±2.82	
	NZ P.	79.69±1.26	81.31±1.29	*83.65±1.25	**89.71±1.28 ^c	*75.44±0.45	74.37±1.85	
	NZ Cr.	79.19±1.37	80.63±1.29	*82.24±1.32	**86.38±0.32	*75.24±0.31	74.26±1.45	
	Balady Cr.	76.55±1.51	78.29±1.39	*81.94±1.33	**85.75±0.43 ^c	*74.71±1.22	72.83±2.26	
	Balady P.	75.78±2.25	77.50±1.60	*80.46±1.48	**82.38±0.30	*74.13±1.45	72.41±2.24	
	Control	460.21±7.20	463.84±4.17	462.12±5.59	465.84±3.42	464.81±5.69	461.35±2.40	
Total lipids (mg/100ml)	NZ P.	463.35±2.60	470.45±0.53	*479.75±1.10	**489.94±2.25	**500.50±2.26 ^c	460.32±2.31	
	NZ Cr.	462.37±2.74	468.54±1.20	*476.61±2.25	**485.08±2.41	**494.25±1.10	457.48±2.88	
	Balady Cr.	459.38±2.55	465.54±0.46	*473.59±1.25	**481.47±1.29 ^c	**489.63±0.52 ^c	457.48±2.71	
	Balady P.	457.58±0.67	462.81±2.32	*468.40±1.40	**475.17±1.30	**480.71±0.63	454.15±2.65	

Mean ± standard deviation. NZ P. = New Zealand pure group. NZ Cr. = New Zealand cross-breed group (female New Zealand x male Balady). Balady P. = Balady pure group. Balady Cr. = Balady cross-breed (female Balady x male New Zealand). Number of rabbits in each group = 5 & Number of rabbits in control group = 4. Significant comparison between control and each group are represented by: * p < 0.05 (significant), ** p < 0.01 (highly significant), *** p < 0.001 (very highly significant). Significant comparison between NZ P. and NZ Cr., also between Balady P. and Balady Cr. are represented by: C < P. = 0.05

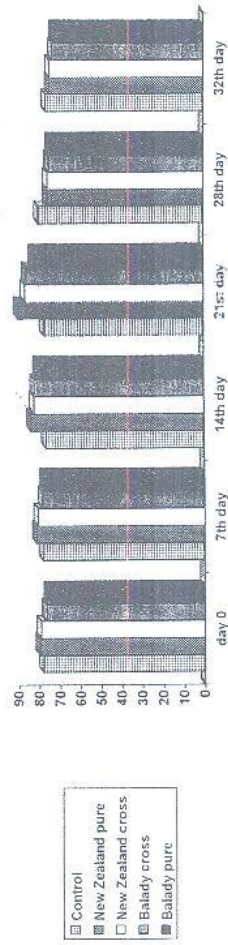


Chart (1): Plasma glucose (mg/dl) in rabbits at different time intervals of pregnancy.

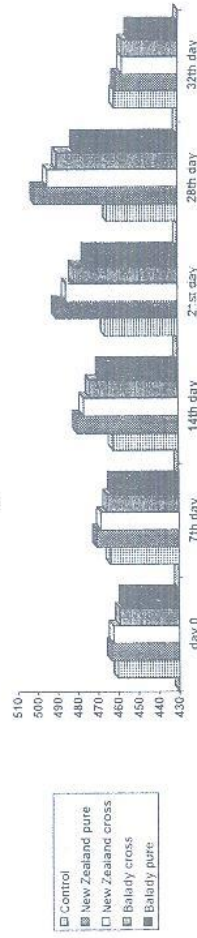


Chart (2): Serum total lipids (mg/100 ml) in rabbits at different time intervals of pregnancy.

Table (2): Serum free fatty acids and phospholipids in rabbits at different time intervals of pregnancy (mg/100ml).

Parameters	Group	Time intervals (days)					
		0	7	14	21	28	32
Free fatty acids	Control	47.60±1.85	46.36±2.91	47.76±2.41	47.79±2.34	48.04±2.34	49.85±2.51
	NZ P.	47.84±0.74	50.52±1.97	*58.15±2.99	**62.8±0.28 ^c	**66.18±1.74	48.78±2.60
	NZ Cr.	47.49±1.20	48.71±1.16	*57.55±2.43	**59.11±1.19	**63.79±1.45	48.36±1.20
	Balady Cr.	45.59±1.44	47.45±1.64	*55.17±1.56 ^c	**57.13±1.28	**62.65±1.29	47.08±0.24
	Balady P.	44.75±1.48	46.20±1.15	*52.92±0.26	**56.37±1.24	**60.04±1.24	46.05±1.51
	Control	106.01±2.49	108.77±2.13	107.30±3.01	107.69±4.39	106.66±5.30	105.72±3.56
phospholipids	NZ P.	109.49±2.74	114.28±2.15	*122.30±5.22	**132.25±2.99	**140.40±1.28 ^c	108.61±2.74
	NZ Cr.	107.41±1.48	112.64±1.53	*119.31±3.29	**130.31±2.64	**137.96±1.22	107.32±1.23
	Balady Cr.	106.39±1.84	111.01±1.48	*117.12±2.21	**127.39±1.21 ^c	**131.84±1.21 ^c	106.13±1.32
	Balady P.	104.98±2.56	109.48±0.36	*114.06±0.15	**123.51±1.16	**127.18±0.98	104.95±0.38
	Control	106.01±2.49	108.77±2.13	107.30±3.01	107.69±4.39	106.66±5.30	105.72±3.56
	NZ P.	109.49±2.74	114.28±2.15	*122.30±5.22	**132.25±2.99	**140.40±1.28 ^c	108.61±2.74

Mean ± standard deviation. NZ P. = New Zealand pure group. NZ Cr. = New Zealand cross-breed group (female New Zealand x male Balady).
 Balady P. = Balady pure group. Balady Cr. = Balady cross-breed (female Balady x male New Zealand). Number of rabbits in each group = 5 & Number of rabbits in control group = 2.
 Significant comparison between control and each group are represented by: * p < 0.05 (significant), ** p < 0.01 (highly significant) *** p < 0.001 (very highly significant). Significant comparison between NZ P. and NZ Cr., also between Balady P. and Balady Cr. are represented by: C = p < 0.05

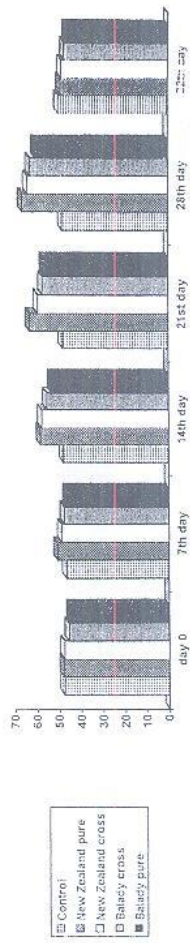


Chart (3): Serum free fatty acids (mg/100 ml) in rabbits at different time intervals of pregnancy.

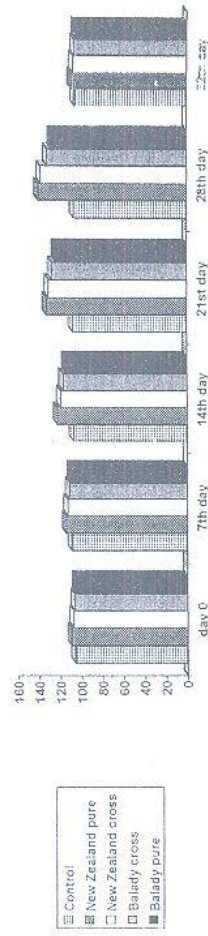


Chart (4): Serum phospholipids (mg/100 ml) in rabbits at different time intervals of pregnancy.