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LEVELS OF TOTAL PROTEINS, TRIACYLGLYCEROLS, LIPOPROTEINS (LDL, HDL AND VLDL) AND CHOLESTEROL DURING PREGNANCY IN DIFFERENT SPECIES OF RABBITS

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

* Animal Reproduction Research Institute, Al-Ahram, Giza, Egypt.

** Faculty of Veterinary Medicine, Zagazig University.

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SUMMARY

Four species of rabbits (New Zealand, New Zealand X Balady, Balady X New Zealand and Balady), five adult and healthy female rabbits from each species were chosen to estimate the levels of total proteins, triacylglycerols, lipoproteins {LDL (Low Density Lipoproteins), HDL (High Density Lipoproteins) and VLDL (Very Low Density Lipoproteins)}, and cholesterol. 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 were raised under the balanced levels of nutrition at the experimental farm of the institute. 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 decrease in the total proteins level from the 14th day and during the day 21 of pregnancy, then a significant increase during the 28th day of pregnancy. The level of total proteins returned normal two days after parturition. The behaviour of Triacylglycerols level was the same as total proteins but in the opposite direction. The level of lipoproteins and cholesterol were significant decreased from the 14th to the 28th day of pregnancy, then returns to normal two days after parturition. 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.

INTRODUCTION

Viard-Drouet et al. (1984) determined the changes in biochemical composition of the plasma due to pregnancy in healthy rabbits doe which are total protein, triacylglycerols and cholesterol. The

results showed a fall in total plasma protein and cholesterol levels and a rise followed by a marked fall in plasma triacylglycerols level. During lactation, these levels returned progressively to the values obtained in non-pregnant and non-lactating rabbits.

The changes in blood lipids due to gestation in homozygous and heterozygous Newzealand white rabbits were recorded by Ockert et al. (1985), Shiomi et al. (1987) and Mortensen and Frandsen (1996). They found that the total cholesterol was lower during the gestation while the triacylglycerols were higher when compared to the levels at mating, then returned to the first data after parturition and during lactation.

Montoudis et al. (1999) mentioned that pregnancy is associated with a hyperlipidemic state where the totality of essential fatty acids and 50% of the lipids needed by the fetus are transferred by the placenta from the maternal circulation. They determined the lipid fractions and their lipoproteins in pregnant rabbits before mating and at each trimester of pregnancy. The results showed that at the 10th day of pregnancy the concentration of total cholesterol and lipoproteins decreased and after 20 days of pregnancy, the levels became in continuos decreasing, also, triacylglycerols metabolism was biphasic showing a significant increase followed by a diminution in their concentration.

Wells et al. (1999) studied the serum chemistry parameters in 2 groups of pregnant rabbits to assess changes in these parameters over the course of gestation. These data were used to generate a historical control reference range for embryo-foetal development regulatory toxicology studies. During the 28 days gestation period, the major changes observed were that triacylglycerols increased to their maximum value by day 19 and then steadily decreased until day 28, while, total protein decreased steadily from day 13 onward.

Rabbit livers express two genetically distinct receptors for plasma lipoproteins, the low-density lipoprotein (LDL) receptors (mediates the rapid removal of VLDL and IDL (very low density and intermediate density lipoproteins) from plasma and the chylomicron remnant receptor. During pregnancy in normal rabbits, there was rapid clearance of VLDL and IDL in the liver, which are converted to LDL (low-density lipoproteins). In case of homozygous Watanabe-heritable hyper-lipidemic (WHHL) rabbits, LDL receptors are genetically deficient, but chylomicron remnant receptors are normal. Hence WHHL rabbits clear LDL from the circulation at an abnormal slow rate, but they clear chylomicron remnants at a normal rate leading to massive increase of plasma VLDL levels and atherosclerosis in WHHL rabbits (Attie et al., 1981 and Kita et al., 1981,1982 and 1986).

Blood chemistry of pregnant animals reflects the

nutritional provision for the foetus and the mother and threw some light upon the food requirement of the doe during this critical period of life. Therefore, the present work aimed to study the levels of total proteins, triacylglycerols, lipoproteins {LDL (Low Density Lipoproteins), HDL (High Density Lipoproteins) and VLDL (Very Low Density Lipoproteins)}, and cholesterol 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.

MATERIALS AND METHODS

Four species of rabbits (New Zealand pure, New Zealand X O Balady, Balady X O 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 group. Two male rabbits (one New Zealand pure and one Balady pure) were kept for breeding. 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).

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 total proteins: -

Serum total proteins were determined by protein kits of Bio Merieux after the method of Henry (1964) and Gornall (1968).

Determination of triacylglycerols: -

Serum triacylglycerols were determined by triacylglycerols kits of Bio Merieux according to the method of Fossati and Prencipel (1982).

Determination of LDL cholesterol: -

Serum low-density lipoprotein (LDL cholesterol) was determined by using Bio Merieux reagent kits according to the method of (Steinberg, 1981).

Determination of HDL cholesterol: -

Serum high-density lipoprotein (HDL cholesterol) was determined by using Bio Merieux kits according to the method of (Burstein, 1970).

Determination VLDL (Very Low Density Lipoprotein): -

VLDL was calculated from triacylglycerols according to Friedwald et al. (1972) who reported that VLDL is present in a concentration equal to

Table (1): Serum total protein, triacylglycerols and cholesterol in rabbits at different time intervals of pregnancy.

Parameters	Group	0	7	14	21	28	32
		Time intervals (days)					
Total protein (g/100 ml)	Control	5.50±0.88	5.55±0.09	5.65±0.15	5.85±0.02	5.75±0.06	5.72±0.90
	NZ P.	5.55±0.30	5.15±0.22	*5.05±0.17	*5.00±0.25	*7.05±0.30^{C}	5.10±0.77
	NZ Cr.	5.45±0.16	5.09±0.18	*5.02±0.21	*4.95±0.19	**6.75±0.16	5.05±0.68
	Balady Cr.	5.12±0.44	5.06±0.11	*4.69±0.21	**4.10±0.13	**6.40±0.02	5.00±0.55
	Balady P.	5.10±0.55	5.04±0.13	*4.50±0.06	**4.05±0.03	**6.10±0.25	5.00±0.64
Triacylglycerols (mg/100)	Control	104.48±5.22	104.99±5.45	103.54±5.60	104.72±3.43	104.05±1.22	103.06±4.13
	NZ P.	106.48±1.21	112.18±1.46	121.24±2.12*	^{CC}134.92±1.38**	100.52±0.22*	102.39±2.53
	NZ Cr.	104.57±2.09	110.62±1.82	118.07±0.68*	127.31±1.98**	98.23±1.17*	101.80±1.42
	Balady Cr.	101.42±0.27	107.80±0.25	115.23±1.35*	122.39±1.71**	^{C}97.61±108*	100.91±0.52
	Balady P.	100.08±0.65	105.10±0.44	113.55±0.36*	119.72±1.02**	93.40±1.04*	98.23±0.57
Cholesterol (mg/100 ml)	Control	131.14±4.87	131.86±5.73	132.21±2.80	133.57±2.43	130.39±3.18	130.35±4.80
	NZ P.	132.17±2.52	130.43±2.46	*125.32±1.07^{C}	**120.28±2.15	***107.18±1.72	127.10±2.58
	NZ Cr.	130.93±1.01	127.60±1.44	*121.51±1.70	**116.55±2.50	***105.75±1.60	126.60±1.95
	Balady Cr.	129.85±2.55	126.40±1.60	*119.40±2.40	**113.60±3.30	**99.65±2.66	123.72±1.43
	Balady P.	127.78±1.47	124.62±1.50	*117.66±3.64	**107.10±4.25	***95.67±2.76	123.67±2.45

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). No. of rabbits in each group =5 & No. 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= $P < 0.05$ & CC= $P < 0.01$.

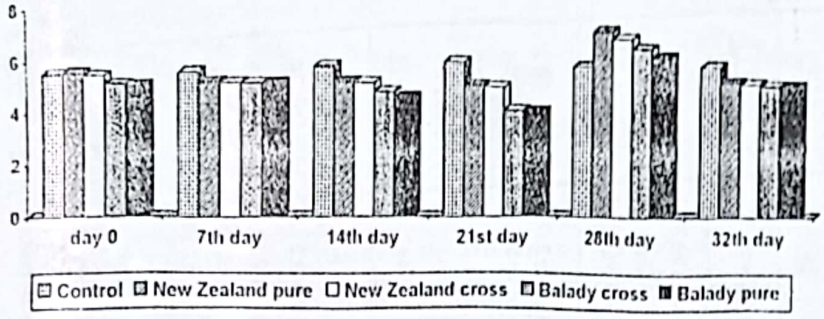

Chart (1): Serum total protein (g/100 ml) in rabbits at different time intervals of pregnancy.

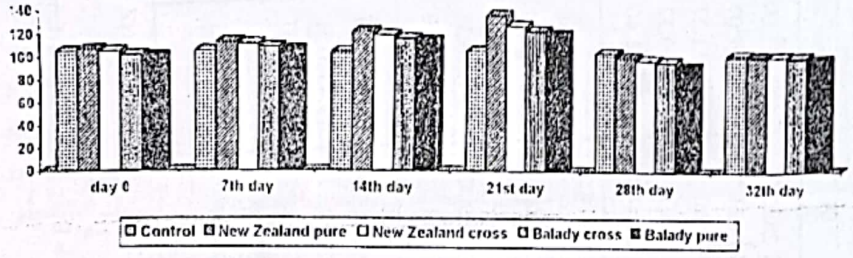

Chart (2): Serum triacylglycerols (mg/100 ml) in rabbits at different time intervals of pregnancy.

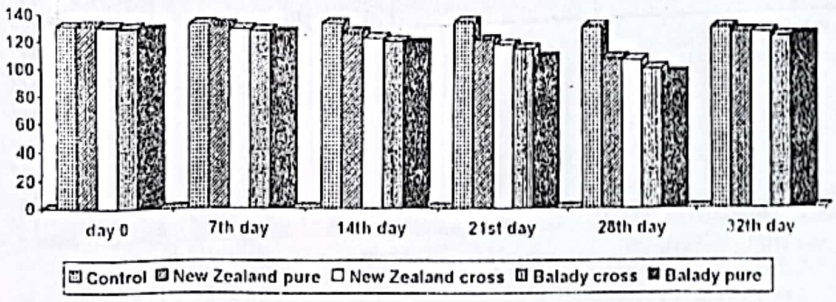

Chart (3): Serum total cholesterol (mg/100 ml) in rabbits at different time intervals of pregnancy.

Table (2) Serum lipoproteins (HDL, LDL, and VLDL) in rabbits at different time intervals of pregnancy (mg/100ml).

Parameters	Group	Time intervals (days) 0	7	14	21	28	32
LDL	Control	61.28±2.61	60.63±3.09	59.22±2.47	59.46±2.16	60.74±1.15	59.56±3.13
	NZ P.	61.66±1.70	59.97±1.07	57.26±0.85^c	*53.62±1.09	**50.70±2.02	56.99±1.34
	NZ Cr.	60.15±0.71	56.86±1.37	*54.77±0.23	**51.78±1.06	**49.19±2.32	54.75±1.01
	Balady Cr.	59.49±2.36	56.98±0.32	*53.57±0.71^c	**50.40±1.25	***47.08±1.61	52.02±2.19
	Balady P.	58.14±0.27	55.98±1.40	50.21±1.40*	**48.71±2.22	***45.51±2.88	50.48±2.16
HDL	Control	39.03±2.43	37.66±1.58	38.59±2.73	39.34±1.66	38.54±2.09	37.72±2.72
	NZ P.	39.21±0.44	37.39±1.84	35.30±1.54	*33.88±1.59	**30.62±1.42	35.21±1.65
	NZ Cr.	39.01±0.24	37.70±0.21	35.70±1.22	*33.51±1.32	**30.78±0.40	34.41±1.60
	Balady Cr.	37.24±0.43	35.42±0.38	*31.40±1.43	**29.36±2.37	***27.35±2.31	32.96±1.47
	Balady P.	36.15±1.36	34.33±1.39	*30.06±1.39	**28.83±2.48	***26.04±2.49	31.87±2.51
VLDL	Control	20.92±1.16	20.83±1.19	20.64±1.24	20.83±1.06	20.17±1.13	20.01±1.24
	NZ P.	21.29±0.39	19.43±0.51	17.24±1.40	*15.63±2.34	**13.04±2.30	18.94±1.24
	NZ Cr.	20.01±0.42	18.08±0.37	16.92±1.40	*14.88±2.47	**12.80±1.37	18.88±1.07
	Balady Cr.	19.87±0.06	18.37±0.07	17.07±2.07	*15.93±2.08	**12.60±2.27	19.74±1.16
	Balady P.	19.62±0.13	17.92±0.15	15.69±2.10	*13.67±2.04	**11.80±2.22	19.42±1.10

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). No. of rabbits in each group =5 & No. 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= $P. < 0.05$

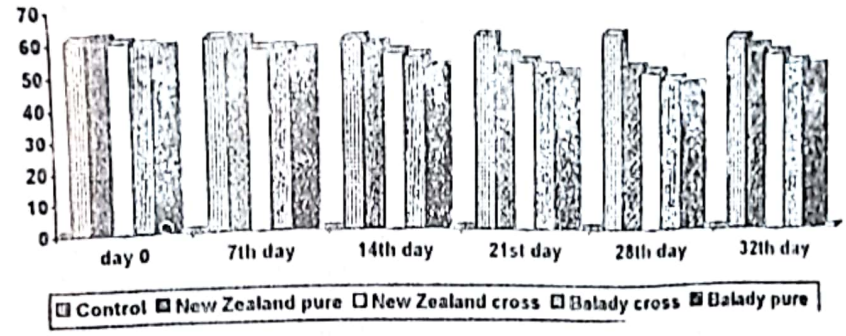

Chart (4): Serum LDL (g/100 ml) in rabbits at different time intervals of pregnancy.

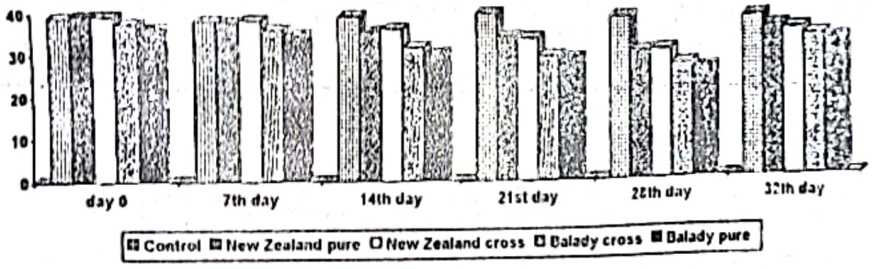

Chart (5): Serum LDL (mg/100 ml) in rabbits at different time intervals of pregnancy.

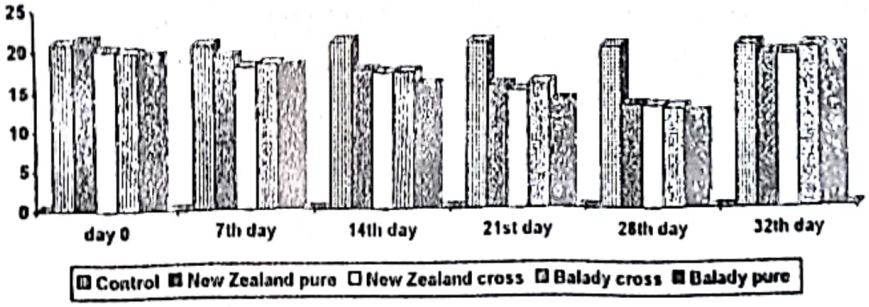

Chart (6): Serum VLDL (mg/100 ml) in rabbits at different time intervals of pregnancy.

one fifth of triacylglycerols concentration. So, VLDL= triacylglycerols ÷ 5, then it is calculated by statistical analysis according to Hill (1977).

This is usually valid for triacylglycerols concentrations<400 mg/dl.

Determination of total cholesterol: -

Serum total cholesterol was determined by total cholesterol kits of Bio Merieux according to the method of Richmond (1973) and Allain et al. (1974).

RESULTS

The results recorded in tables 1 and 2 and figures 1 - 6 showed the differences in the levels of serum total proteins, triacylglycerols, lipoproteins {LDL (Low Density Lipoproteins), HDL (High Density Lipoproteins) and VLDL (Very Low Density Lipoproteins)}, and cholesterol between the non pregnant (control group) and the pregnant females, also, between the pure and the cross bred species of rabbits.

Serum total proteins: A significant decrease (P<0.05) in the values of total proteins was recorded at the 14th day of pregnancy; followed by a highly significant decrease (P<0.01) during the 21st day of pregnancy. A highly significant increase (P<0.01) during the 28th day of pregnancy till parturition, the level of total protein returned normal two days after parturition.

Serum triacylglycerols: A significant increase (P<0.05) in the values of triacylglycerols was recorded at the 14th day of pregnancy; followed by a highly significant increase (P<0.01) during the 21st day of pregnancy. A significant decrease (P<0.05) during the 28th day of pregnancy till parturition. The level of triacylglycerols returned near normal two days after parturition.

Serum lipoproteins:

LDL-cholesterol and HDL-cholesterol: There was a significant decrease (P<0.05) in LDL cholesterol and HDL cholesterol levels from the 14th day, during the day 21 of pregnancy (P<0.01), and during the 28th day of pregnancy (P<0.001) till parturition. Its level returned around normal two days after parturition.

VLDL-cholesterol: There was a significant decrease (P<0.05) in the VLDL cholesterol level from the 21st day and during the 28th day (P<0.01) of pregnancy till parturition, Its level returned near normal two days after parturition.

Serum total cholesterol: There was a significant decrease (P<0.05) in serum cholesterol at the 14th day of pregnancy. At day 21 of pregnancy there was a highly significant decrease (P<0.01) in its levels, which changed to a very highly significant decrease (P<0.001) at day 28 of pregnancy till the day of parturition, then returns near to the normal values two days after parturition.

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

DISCUSSION

In the present study, the values of serum total proteins in healthy rabbits averaged 5.50±0.88 gm%. Our data agreed with those reported by Carlos et al. (1967) and Hegazy (1989) who found that the amount of total protein in the serum of normal female New Zealand rabbits was 5.7±0.27 and 5.8±0.29 gm%, respectively. The observed decrease in values of total proteins at the day intervals of pregnancy was in accord with Wells et al. (1999).

In the present study, the average serum triacylglycerols was 104.48±5.22 mg% in control healthy rabbits. This data was near the values recorded by Yanny (1988) who reported that the serum triacylglycerols of healthy New Zealand rabbits was 108.22±17.4 mg% and by Kaneko (1989) who reported that the level of triacylglycerols equal to 90-122 mg% as a normal range. The biphasic reaction of triacylglycerols recorded during the stages of pregnancy by increase at the 14th day of pregnancy, then steadily decrease near the term (28th day) was in agreement with the data reported by Harlow et al. (1980) and Montoudis et al. (1999).

The average serum cholesterol was 131.14±4.87 mg% in the present study. These results were on line with Kaneko (1989) who reported that, the normal values of total cholesterol were 95 - 135±5.3 mg%. Extremely lower values of cholesterol were reported during different time intervals of pregnancy in New Zealand white rabbits as reported by Boyd (1942), Burns et al. (1966) and Burroughs (1971) who gave values of 45.0±18.0, 26.7±1.3 and 76.0±3.0 mg%, respectively. The decrease in values of cholesterol during the day intervals of pregnancy, in our results, were in agreement with Harlow et al. (1980) and Ockert et al. (1985) who reported that, during the time of pregnancy there was a decrease in values of cholesterol and return normal after parturition.

In this study, the values of LDL, HDL and VLDL (serum lipoproteins) averaged 61.28±2.61, 39.03±2.43 and 20.92±1.16 mg%, respectively. These data were in agreement with the data recorded by Kaneko (1989) who reported that the serum HDL-cholesterol in healthy rabbits was 35-60 mg%, LDL-cholesterol was 55-70 mg% and VLDL was 18-22 mg%. Also, our data were on line with the results of Yanny (1995) who reported that the normal values of lipoproteins in healthy rabbits were 62.4±12.4, 72.8±11.9 and 23.7±10.8 mg% in HDL, LDL and VLDL, respectively. The decrease in values of serum lipoproteins during the time intervals of pregnancy was in agreement with Ockert et al. (1985).

It is obvious, from the present study, that there was a decrease in the values of total proteins at the first and second trimesters of pregnancy which was referred to the great need of proteins to form the foetal membranes, foetal fluids and helps the foetus to grow up and increase in its weight. The increase in values of total proteins and the highly degradation of the maternal triacyloglycerols, lipoproteins and cholesterol during the third trimester of pregnancy provide the foetus, at this critical stage of life, with energy to form its body, adipose tissues and for its movement specially at term. Two days after parturition, the levels of total proteins, triacyloglycerol, lipoproteins and cholesterol were found to return back near their normal levels before pregnancy and of control group. This was referred to the dependence of the doe on its stored energy in the adipose tissues in the form of triacyloglycerols.

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