Changes in adiponectin concentration and insulin sensitivity during lactational phases in rabbits

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ABSTRACT

The study was carried out to investigate the changes in plasma adiponectin during the period of lactation in rabbits. It was carried out using 24 New Zealand White rabbits, 20 mature females and 4 mature males weighting approximately 3kg bodyweight. Female animals were divided into 2 groups, one of which contain 5 females (non lactating as control group) and 15 females (lactating group) includes: early lactation phase (from 1^{st} – 10^{th} day of lactation) , mid lactation phase (11^{th} - 20^{th} day of lactation) and late lactation phase (21 – 30 day of lactation). Insulin Sensitivity Test (IST), in which insulin was injected the peak hyperglycemia, then blood glucose level was determined post insulin injection. Blood samples were collected from individual animal in lactating and non lactating (control) females before and after IST. Plasma was separated for measuring adiponectin. The results of the study revealed that: Concerning IST, during insulin injection (peak hyperglycemia), the plasma glucose level was decreased significantly ($P<0.05$) and gradually 30 minutes till 2 hours from insulin injection in both lactating and non lactating groups. Regarding the effect of insulin injection on plasma adiponectin level revealed no significant differences in plasma adiponectin level before and after insulin injection in non lactating group, mid and late lactation phases, While there was a significant increase ($P<0.05$) in plasma adiponectin level after insulin injection in early lactation phase.

INTRODUCTION

Lactation is one of the most important physiological events during post partum period. Milk yield in high yielding dairy cows after parturition is regulated through increase in both biosynthetic activity of mammary tissue and availability of the substances necessary to synthesize milk components. In postpartum period, additional substrate required to support milk synthesis is provided through enhanced mobilization of adipose reserves and skeletal muscle ***(Veerkamp, 1998)***.

Since glucose is the major precursor for lactose synthesis, It is essential, however, that the mammary gland receives a constant and adequate supply of glucose for milk lactose synthesis. ***Annison and Linzell, (1964)*** originally determined that the lactating goat, for example

utilized for milk production, between 60 and 85% of total glucose used by the animal. It is known that the onset of lactation is associated with the demand for utilization of glucose for milk production ***(Chaiybator et al, 1999).*** Large quantities of glucose are removed by the mammary glands for synthesis of lactose, however, dry matter intake (DMI) is insufficient to meet the demands of lactation.

Adiponectin is an adipocyte-derived hormone with multiple biological functions. Moreover, in the last years the role of adiponectin as an important hormone in the regulation of insulin action has been reported ***(Yamauchi et al, 2003). Komatsu et al (2007) and Raddatz Julia (2008)*** reported that, adiponectin concentrations were significantly higher in prepartum than in postpartum cows, and the individual variability between animals was observed which lead to the hypothesis that adiponectin secretion may vary genetically. The large difference between pre and postpartum adiponectin concentrations suggests that, lactation may suppress adiponectin production and that, low adiponectin levels facilitate postpartum insulin resistance which increases the supply of glucose to the mammary gland, and aids in the metabolic support of lactation.

Ohtani et al (2012) also found that serum adiponectin concentrations increased after parturition and there was no significant difference in plasma adiponectin concentrations among lactational stages in Holstein dairy cows. However, plasma adiponectin concentrations decreased as pregnancy and the postpartum period progressed ***(Catalano et al, 2006 and Sato et al, 2006).*** There are some preliminary data indicating that circulating adiponectin concentrations may increase from the first to the fourth week after calving in dairy cows ***(Raddatz Julia, 2008).***

Bauman and Elliot (1983) and McDowell et al (1987) explained that the increase in glucose supply to the mammary gland during lactation may result from an increase in insulin resistance as the decreased insulin-independent glucose uptake in tissues rather than mammary gland.

Insulin concentration tend to decrease in early lactation, particularly in higher yielding cows ***(Baird et al, 1980 and Taylor et al, 2003),*** where low insulin concentration reduce glucose uptake by insulin responsive tissues (adipose and muscle) and facilitate greater uptake by the mammary gland.

From the concept that adiponectin has insulin sensitizing effects in some species. It should valuable to know if adiponectin plays a role during lactation in conjugation of the period of negative energy balance. This study aims to the changes in concentration and

insulin sensitivity during different phases of lactationin rabbits.

MATERIAL & METHODS

This study was carried out in the Department of Physiology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

During the period from November through February, A total of 24 New Zealand White rabbits of either sex, (20 females and 4 males used for mating), weighting approximately 3kg body weight purchased from a private rabbit farm, Sarabyom, Ismailia. The animals were housed in an animal room in individual metallic cages and maintained at ambient temperature (20 – 24°C) with relative humidity (55 – 65 %) and natural daylight rhythm.

Rabbits were fed on a standard commercial food pellets (Atmida company, Dakahleia, Egypt). The food and water were allowed *ad libitum*. Rabbits were kept two weeks for acclimatization before starting the experiment and received oxyvet antibiotic (pharma swede company, Egypt) in water at a dose of (1gm / litre) as prophylactic and provided by vit AD_3E in water (Dream vit company, 10^{th} of Ramadan, Egypt). All animals were treated and sampled in accordance with the guideline for care and use of animals which is approved by the research ethics committee in the Faculty of Veterinary Medicine, Suez Canal University.

After two weeks of acclimatization, female animals were divided into 2 groups, first group contained 5 females rebbits kept without mating (non lactating group) and represented as control group. The second group contained 15 females (lactating group) were naturally mated twice daily one hour a part ***(El-Darawany and Siam, 1995)*** and examined afterwards for pregnancy via abdominal palpation at week to 10 days post mating. The pregnant females were observed and examined daily till delivery, they usually littered in the night and the first day the young were seen was considered day 1 of lactation. The lactating rabbits were subdivided into 3 subgroups according to lactation phase into: early lactation phase (from 1^{st} – 10^{th} day of lactation), mid lactation phase (11^{th} - 20^{th} day of lactation) and late lactation phase (21 – 30 day of lactation). Oral glucose tolerance test (OGTT) and Insulin Sensitivity Test (IST) were carried out in both non lactating and lactating groups.

Insulin Sensitivity Test (IST):

Rabbits were deprived of food but not water for 12 hour (overnight) before the start of the test. Fasting blood glucose levels were measured, then a 50% glucose solution was orally administered to the animals at a dose of 1.5 g/kg body weight. During the glucose stabilizing period (peak hyperglycemia (30 minutes after oral glucose administration) insulin was injected at dose of 0.75 units/kg body weight intraperitoneally (i.p) after dilution in PBS in a rate of

1:100 ***(Yin et al, 2003)***. Blood glucose level was determined at 0, 30, 60, 90 and 120 min post insulin injection from the marginal ear vein using digital accu-check glucometer **(*Georgiev et al, 2006)***.

Blood samples were collected from individual animal in non lactating and lactating females before insulin sensitivity test and after insulin injection and during all over early, mid and late phases of lactation. Samples were collected via ear vain using small sized cannula in suited EDTA tubes (Meus sri piove di sacco company, Italy). Blood samples were centrifuged at 3000 rpm for 20 minutes to obtain plasma. Plasma were separated and kept at -20 °C till analysis of adiponectin. Plasma adiponectin levels were measured using ELISA kits specific for rabbit adiponectin (WKEA med supplies, New York, USA) according to ***Tschritter et al (2003) and Sato et al (2006).***

Statistical analysis:

The obtained data are subjected to statistical analysis as outlined by IBM SPSS Statistics program version 20 (SPSS, USA) to determine ANOVA, LSD and correlation. A value of $P<0.05$ is considered significant.

RESULTS

Table 1 and figure 1 (A & B) demonstrate plasma glucose level before and after IST in lactating and non lactating group. Concerning non lactating group, plasma glucose level was increased significantly ($P<0.05$) 30 minutes after glucose administration (289.8±1.15 mg/dl) (peak hyperglycemia) at which insulin was injected. The plasma glucose level was decreased significantly ($P<0.05$) and gradually and reached 60.2%, 44.7%, 75.9% and 87.8% from original values at 30, 60, 90 and 120 minutes respectively after insulin injection. The same results were observed in all phases of lactation.

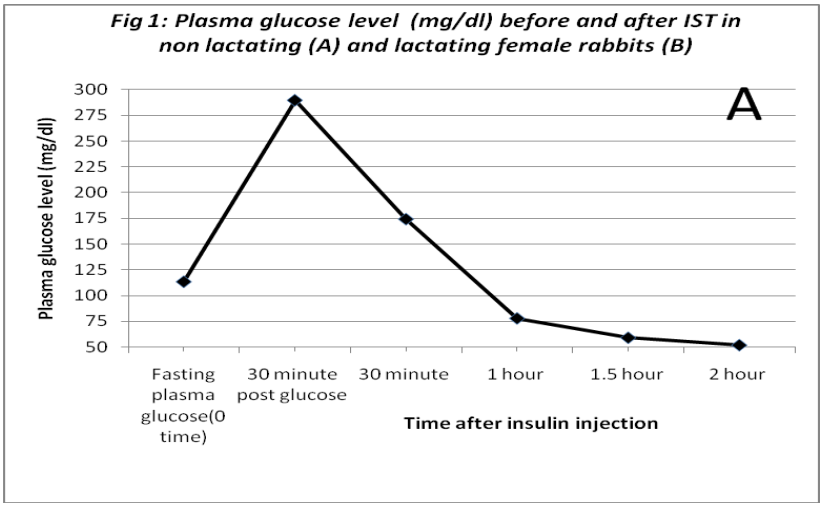
Table 2 and figure 2 showed the effect of insulin injection on plasma adiponectin level in lactating and non lactating female rabbits. Data revealed no significant differences in plasma adiponectin level before and after insulin injection in non lactating group. Concerning lactating group, there is a significant increase ($P<0.05$) in plasma adiponectin level after insulin injection in early lactation phase, While there is no significant differences in mid and late lactation phases.

Table 1: *Plasma glucose level (mg/dl) before and after Insulin Sensitivity Test (IST) in non lactating and lactating female rabbits (Mean ± SE):*

Group		*Fasting plasma glucose (0 time)*	*30 minute post glucose*	*Time after insulin injection*			
				30 minute	*1 hour*	*1.5 hour*	*2 hour*
Non lactating group		113.6^{a} ±1.16	289.8^{b} ±1.15	174.4^{c} ±4.52 (60.2%)	78^{d} ±3.56 (44.7%)	59.2^{de} ±6.17 (75.9%)	52^{e} ±5.82 (87.8%)
Lactating group	*Early*	122.6^{a} ±8.51	210.2^{b} ±9.26	155^{c} ±12.58 (73.7%)	93.8^{d} ±8.76 (60.5%)	73.6^{de} ±8.70 (78.5%)	65^{e} ±3.24 (88.3%)
	Mid	115^{a} ±5.74	196^{b} ±9.61	136.7^{a} ±16.57 (69.7%)	89^{c} ±12.04 (65.1%)	62.42^{d} ±8.29 (70.1%)	35.83^{e} ±8.46 (57.4%)
	Late	116.22^{a} ±5.88	202.4^{b} ±12.93	115.66^{a} ±4.31 (57.1%)	75.77^{c} ±3.92 (65.6%)	53.66^{cd} ±5.01 (70.8%)	42.42^{d} ±3.70 (79.1%)
LSD 0.05		22.97					

**Values having different superscripts within the same raws are significantly different at $p < 0.05$.*

** Values between brackes are the percent of change of plasma glucose level from previous values.*



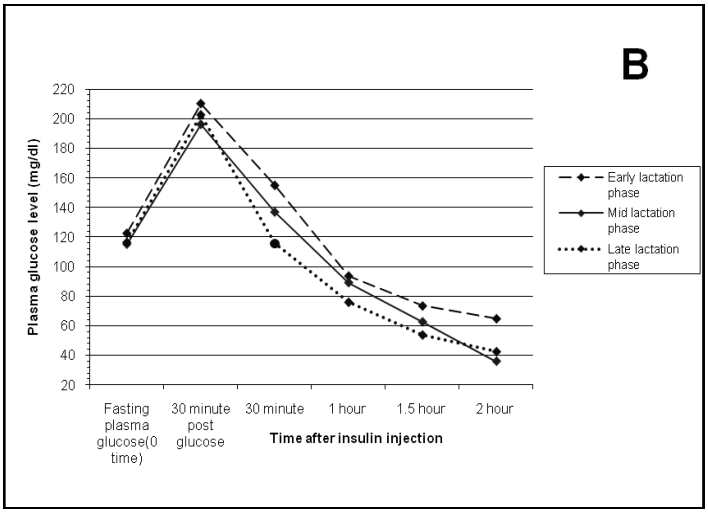
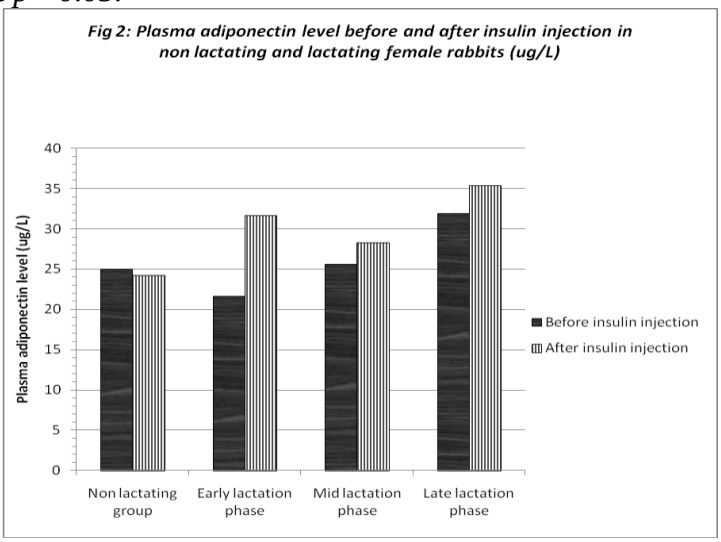


Table 2: *Effect of insulin injection on plasma adiponectin level in non lactating and lactating female rabbits (mean±SE):*

Group		*Before insulin injection*	*After insulin injection*
Non lactating group		25.01 ± 1.80	24.27 ± 1.57
Lactating group	*Early*	21.63[a] ± 2.81	31.64[b] ± 3.88
	Mid	25.60 ± 2.35	28.26 ± 2.78
	Late	31.90 ± 2.36	35.36 ± 3.05

**Values having different superscripts within the same raws are significantly different at p <0.05.*



DISCUSSION

Adiponectin is an adipocyte-derived hormone with multiple biological functions. It represents the most abundant protein secreted by adipose tissue. It has been reported that adiponectin may have putative anti-atherogenic properties in vitro and it has been shown to play important roles in the regulation of energy homeostasis and insulin sensitivity ***(Yamauchi et al, 2003).***

Concerning Insulin Sensitivity Test (IST), plasma glucose level increased significantly ($P<0.05$) 30 minutes after glucose administration (peak hyperglycemia) at which insulin was injected (table 1 and figure 1). The plasma glucose levels were gradually decreased 30 minutes after insulin injection in both lactating and non lactating groups. The slow reduction in glucose levels after insulin injection in mid and late lactation phases as demonstrated in this study (table 1) suggests low insulin sensitivity at these phases to promote glucose supply to the mammary tissue. These results are consistent with the results obtained by ***Ohtani et al, (2012)*** and explained as a decrease in glucose uptake in skeletal muscle and adipose tissue ***(Komatsu et al, 2007)***. In fact, these data are in concordance with previous studies of ***Foss et al (1995) and Reis et al (1997)*** which indicate that prolactin hormone (PRL) is capable of antagonizing insulin action loading to insulin resistance.

The results presented in table 1 in this study revealed a decrease in insulin sensitivity demonstrated by higher levels of glucose 1 hour after glucose administration at early and mid lactation phase. These results support the previous results indicating tendency of insulin decrease in early lactation which is a part of series of coordinated changes that occur around the time of parturition in support of lactation. This reduces glucose uptake by insulin - responsive tissues (adipose tissue and muscle) and facilitate greater uptake by the mammary gland ***(Bauman and Elliot, 1983)***. The data explained that increase in glucose supply to the mammary tissue during lactation is believed to result from an increase in insulin resistance as well as insulin – independent glucose uptake in tissue except mammary gland ***(McDowell et al, 1987)***.

It has been demonstrated a decrease in adiponectin sensitivity in adipose tissue after calving, which might be involved in the reduced insulin sensitivity of adipose tissue during early lactation in Holstein dairy cows ***(Lemor et al, 2009)***. ***Satoh et al, (2005)*** reported that physiologically and patoh physiologically elevated levels of adiponectin in rats enhance insulin sensitivity in skeletal muscle via an AMPK-dependent pathway without affecting hepatic and adipose insulin sensitivity. ***Kubota et al, (2007)*** reported that serum adiponectin

concentrations increased with fasting and returned with refeeding.

Concerning the effect of insulin on plasma adiponectin concentration (table 2 and figure 2), there was no significant change in plasma adiponectin level before and after insulin injection in non lactating group, While in lactating group, there was a significant increase ($P<0.05$) in plasma adiponectin level after insulin injection in early lactation phase and no significant differences in mid and late lactation phases . Several studies have investigated the effect of insulin on adiponectin production and secretion, where in vitro studies found a stimulating effect on adiponectin gene expression (***Halleux et al, 2001; Viengchareun et al, 2002 and Seo et al, 2004)*** or secretion ***(Bogan and Lodish, 2004; Pereira and Draznin, 2005). Regje et al (2008)*** reported that hyperglycemia prevent the suppressive effect of hyperinsulinemia on plasma adiponectin levels. The study concluded that, insulin suppresses plasma adiponectin levels. This suggests that, in contrast to hyperglycemia, hyperinsulinemia could be involved in the down regulation of plasma adiponectin. ***Weyer et al (2001)*** showed that fasting hyperinsulinemia is associated with low plasma adiponectin concentration. As well as low plasma adiponectin concentration at baseline proceeds a decrease in insulin sensitivity ***(Stefan et al, 2002).***

In conclusion the present study revealed that plasma adiponectin level affected by insulin injection.

REFERENCES

Annison E.F. and Linzell J.L. (1964): The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their overall metabolism and metabolism and milk formation. J.Physiol.; 175: 372–385.

Baird G.B.; Lomax M.A. and Symonds H.W. *et al.*, (1980): Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows in vivo in relation to lactation and nutrient supply. Biochem. J.; 186:47-57.

Bauman D.E. and Elliot J.M. (1983): Control of nutrient partitioning in lactating ruminants. Biochemistry of Lactation; 437-468.

Bogan J.S. and Lodish H.F. (2004): Two compartments for insulin stimulated exocytosis in 3T3-L1 adipocytes defined by endogenous ACRP30 and GLUT4. J Cell Biol; 146: 609–620.

Catalano P.M.; Hoegh M. and Minium J. *et al.*, (2006): Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. Diabetologia; 49: 1677–1685.

Chaiyabutr N.; Preuksagorn S. and Komolvanich S. *et al.*, (1999): Glucose metabolism in crossbred Holstein cattle feeding on two types of roughage at different stages of lactation. Comparative

Biochemistry and Physiology Part A; 125: 121–130.

El-Darawany and Siam A. (1995): Number of coitus, LH and reproductive performance relationships in doe rabbit. Der tropen Laudwirt, Beitrage Journal of agiculture; 96: Issue 0041-3180, p.227.

Foss M.C.; Paula F.J. and Paccola G.M. *et al.*, (1995): Peripheral glucose metabolism in human hyper-prolactinemia. Clinical Endocrinology; 43: 721–726.

Georgiev I.P.; Kanelov I.N. and Dimitrova S.S. *et al.*, (2006): An experimental model for evaluation of glucose tolerance in rabbit. Bulgarian J Veter Med.; 9:27-35.

Halleux C.M.; Takahashi M. and Delporte M.L. et al., (2001): Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. Biochem Biophys Res Commun.; 288: 1102–1107.

Komatsu T.; Itoh F. and Sakumoto R. *et al.*, (2007): Changes in the gene expression of adiponectin and glucose transporter 12 (GLUT 12) in lactating and non-lactating cows. Animal Science Journal; 78:98-102.

Kubota N.; Terauchi Y. and Yamauchi T. *et al.*, (2002): Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem.; 277(29): 25863-6.

Lemor A.; Hosseini A. and Sauerwein H. *et al.*, (2009): Transition period-related changes in the abundance of the mRNAs of adiponectin and its receptors, of visfatin, and of fatty acid binding receptors in adipose tissue of high-yielding dairy cows. Domestic Animal Endocrinology; 3: 7 37–44.

McDowell G.H.; Gooden J.M. and Leenanuruksa D. *et al.*, (1987): Effects of exogenous growth hormone on milk production and nutrient uptake by muscle and mammary tissues of dairy cows in mid-lactation. Australian J. of Biol. Sci.; 40:295-306.

Ohtani Y.; Takahashi T. and Sato K. *et al.*, (2012): Changes in circulating adiponectin and metabolic hormone concentrations during periparturient and lactation periods in Holstein dairy cows. Animal Science Journal doi.; 10: 1740-0929.

Pereira R.I. and Draznin B. (2005): Inhibition of the phosphatidylinositol 3kinase signaling pathway leads to decreased insulin-stimulated adiponectin secretion from 3T3-L1 adipocytes. Metabolism; 54: 1636–1643.

Raddatz, Julia (2008): Measurement of adiponectin in lactating dairy cows and adiponectin, insulin, NEFA, and Glucagon concentrations during an IVGTT and an IVITT in lactating vs. Non-lactating Holstein Cows. Master thesis. Animal science, faculty of North Carolina State University.

Regje M. E.; Saskia N. C. and Michiel E. S. *et al.*, (2008):

Hyperglycemia prevents the suppressive effect of hyperinsulinemia on plasma adiponectin levels in healthy humans. Am J Physiol Endocrinol Metab.; 295(3):613-7.

Reis F.M.; Reis A.M. and Coimbra C.C. (1997): Effects of hyperprolactinemia on glucose tolerance and insulin release in male and female rats. Journal of Endocrinology; 153: 423–428.

Sato A.M.; Okamoto M. and Endo M. *et al.*, (2006): Hypoadiponectinemia in lean lactating women: prolactin inhibits adiponectin secretion from human adipocytes. Endocrine Journal; 53: 555–562.

Satoh H.; Audrey Nguyen M.T. and Maria Trujillo *et al.*, (2005): Adenovirus-mediated adiponectin expression augments skeletal muscle insulin sensitivity in male wistar rats. Diabetes; 54:1304–1313.

Seo J.B.; Moon H.M. and Noh M.J. *et al.*, (2004): Adipocyte determinationand differentiation-dependent factor 1/sterol regulatory element-binding protein 1c regulates mouse adiponectin expression. J Biol Chem.; 279: 22108–22117.

Stefan N.; Vozarova B. and Funahashi T. *et al.*, (2002): Plasma adiponectin levels are not associated with fat oxidation in humans. Obes Res.; 10: 1016–1020.

Taylor V.J.; Beever D.E. and Wathes D.C. (2003): Physiological adaptations to milk production that affect fertility in high yielding dairy cows. Br. Soc. Anim. Sci.; 37-71.

Tschritter O.; Fritsche A. and Thamer C. *et al.*, (2003): Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes; 52:239–243.

Veerkamp R.F. (1998): Selection for economic efficiency of dairy cattle using information on live weight and feed intake: A review. J. Dairy Sci.; 81:1109-1119.

Viengchareun S.; Zennaro M.C. and Pascual-Le T.L. *et al.*, (2002): Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. FEBS Lett.; 532: 345–350.

Weyer C.; Funahashi T. and Tanaka S. *et al.*, (2001): Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab.; 86:1930–1935.

Yamauchi T.; Kamon J. and Waki H. *et al.*, (2003): Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem; 278:2461–8.

Yin W.; Yuan Z. and Tsutsumi K. *et al.*, (2003): A lipoprotein lipase-promoting agent, NO-1886, improves glucose and lipid metabolism in high fat, high sucrose-fed New Zealand white rabbits. Int J Exp Diabesity Res.; 4: 27-34.

الملخص العربى

أجريت هذه الدراسة لمعرفة تأثيرهرمون الأديبونيكتين فى فترة الرضاعة فى الأرانب. حيث أجريت على ٢٤ أرنب نيوزيلندي أبيض، ٢٠ من الإناث و ٤ من الذكور البالغة، متوسط وزنهم ٣ كجم تقريبا. قسمت الإناث إلى مجموعتين : المجموعة الأولى تضم ٥ إناث غير مرضعة (المجموعة الضابطة)، والمجموعة الثانية تضم ١٥ أنثى مرضعة تشمل كل مراحل الرضاعة: المرحلة المبكرة من الرضاعة (من اليوم الأول حتى اليوم العاشر)، المرحلة الوسطى من الرضاعة (من اليوم الحادى عشر حتى اليوم العشرين)، المرحلة المتأخرة من الرضاعة (من اليوم الحادى والعشرين حتى اليوم الثلاثين).تم إجراء اختبار حساسية الأنسولين، الذى من خلاله تم حقن الأنسولين أثناء ذروة إرتفاع السكر فى الدم ،ثم تم قياس مستوى الجلوكوز فى الدم وذلك لمعرفة مدى استجابة الجلوكوز للأنسولين. تم تجميع عينات الدم فى المراحل المختلفة من الرضاعة من كل حيوان على حدة فى مجموعة الأرانب المرضعة وكذلك الغير مرضعة قبل وبعد اختبار الحساسية للأنسولين. وتم فصل بلازما الدم لقياس الأديبونيكتين.

وأسفرت نتائج الدراسة عن مايلى: بخصوص اختبار حساسية الأنسولين، عند حقن الأنسولين عند ذروة ارتفاع مستوى الجلوكوز فى البلازما،بدأ مستوى الجلوكوز ينخفض معنويا بعد ٣٠ دقيقة وحتى ساعتين من حقن الأنسولين وذلك فى مجموعتى الإناث المرضعة (الضابطة) والغير مرضعة. بشأن تأثير حقن الأنسولين على مستوى الأديبونيكتين، فقد أظهرت النتائج عدم وجود اختلاف معنوى فى مستوى الأديبونيكتين قبل وبعد حقن الأنسولين فى المجموعة الغير مرضعة، ومجموعة الإناث المرضعة فى المرحلة الوسطى والمرحلة المتأخرة من الرضاعة. فى حين كانت هناك زيادة معنوية فى مستوى الأديبونيكتين بعد حقن الأنسولين فى المرحلة المبكرة من الرضاعة.