

Role of Insulin and/or Fasting in a Protocol for Inducing Pregnancy Toxemia in Twin-Bearing Zaraibi Goats

Abd Elghany Hefnawy¹, Saad Shousha^{2,*}, Seham Youssef³

¹ Department of Internal Medicine, ² Department of Physiology, ³ Department of pharmacology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Egypt.

ABSTRACT

In this study we determined the effect of insulin injection and /or short fasting on the induction of pregnancy toxemia in Zaraibi goats. Eighteen twin-bearing Zaraibi goats (around 130 days of gestation) were divided into control group (Group C, n = 6) fed ad libitum, feed deprived only group (Group A, n = 6) and feed deprived with insulin injection group (Group B, n = 6). Clinical signs of pregnancy toxemia appeared after 24 and 72 h in group B and A, respectively. Refeeding and 50 ml of 500 gm dextrose /l were injected after appearance of the clinical signs every 12 h for two successive doses. Plasma concentrations of glucose and β -hydroxybutyrate were significantly ($p < 0.01$) decreased by 24 and 36 h respectively in group-A, and these variables were significantly decreased by 12 and 24 h in group- B. Concentrations of glucose and β -hydroxybutyrate were returned to the control levels ($p > 0.1$) at 48 h of refeeding and injection of dextrose in both experimentally induced groups. There was significant ($P < 0.05$) decrease in the length of gestation in both experimental groups (A & B) than that of control one. The lengths of gestation were 140.3 ± 1.1 , 139.2 ± 0.8 and 149.1 ± 1.4 days in group A, B and C, respectively. Simple protocol of short fasting combined with insulin injection can induce pregnancy toxemia and this protocol might be used for valuable studies for pregnancy toxemia in twin-bearing Zaraibi goats.

KEY WORDS: Insulin; Short fasting; Zaraibi goats; Pregnancy Toxemia.

INTRODUCTION

Pregnancy toxemia is a metabolic disorder with a high mortality rate and occurrence in twin-bearing ewes in late gestation [1]. Maternal hypoglycemia is a characteristic symptom of the disease and has been attributed to an increase in glucose uptake by the twin-bearing uterus [1]. Also undernutrition or stress resulting in decrease energy intake that seems to be critical for the development of ovine ketosis [2].

Insulin is well recognized for its hypoglycemic effects. For a long time and ruminal tissues have been considered less sensitive to insulin than non-ruminant tissues [3]. Insulin inhibits hepatic glucose output due to stimulation of glycogen synthesis as well as inhibits gluconeogenesis which seems more responsive than glycogenolysis to the inhibitory effects of insulin in ruminants [4]. Insulin exhibits selectivity among extra hepatic tissue and facilitates uptake of glucose by muscles [5, 6] and adipose tissues [7, 8]. Insulin does not alter the removal of glucose by pregnant uterus [6]. When energy intake is low, such as during fasting, low insulin promotes utilization of glucose for fetal growth by reducing the uptake by extra uterine tissues [3].

An induction protocol has been developed to produce a metabolic ketosis in lactating dairy cows that appears to be very similar to naturally occurring spontaneous ketosis either by food restriction or administration of 1, 3-butanediol [9-12]. There are many reports on experimental ketosis induced by undernourishment or starvation of multiple pregnant ewes [13]. Typical laboratory findings in experimentally starved pregnant ewes are moderate hyperketonemia, hypoinsulinemia and loss of gluco-stasis [14, 15].

It is found that limiting feed intake at four weeks postpartum without a supplemental ketone bodies precursor will not sustain hyperketonemia and causes clinical ketosis to develop in cow [16, 17], while other published papers indicated that a relatively simple protocol of prolonged energy deficit combined with an influx of ketone body precursors can induce experimental lactation ketosis in overfed cows [18, 19]. The aim of this study was to investigate the role of short fasting with and without insulin injection in a protocol for inducing pregnancy toxemia in twin-bearing Zaraibi goats.

MATERIALS AND METHODS

Experimental design: Eighteen twin –bearing Zaraibi goats 3-4years old (they were diagnosed as bearing twins by ultrasonography), 20-27 Kg body weight and 139–149 days of gestation were used for induction of

*Corresponding Author: Saad Shousha Ph.D., Department of Physiology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Egypt. E-mail: physiology2009@yahoo.com

pregnancy toxemia by the effect of short fasting (fasting for 72 hours) with access to water only and short fasting (fasting for 72 hours) with S/C injection of one dose of insulin. Does were well-fed up to 120 days of gestation. 250 g corn/ day plus 0.3 kg concentrate /day at 2 months gestation, increased gradually up to 1 kg during the last 6 weeks of gestation. Fresh water was freely available. Animals were divided into three groups. Group (A) for inducing of pregnancy toxemia by short fasting only, group (B) for inducing of pregnancy toxemia by short fasting with single dose of zinc insulin (Mixtard 40 I U) by the dose of 5 I U / kg body weight S/C and allowing access to drinking water in both group until appearance of clinical signs of pregnancy toxemia (i.e. muscle tremors, staggering in gait, partial loss of vision and acetone odor in the breath). The third group (Group C) as control one. NIH guidelines for the care and use of animals have been followed.

At the time at which pregnancy toxemia was induced and the clinical signs were observed, feed was reintroduced in normal amounts, using the same diet as described with intravenous injection of two successive doses of glucose with interval 12 hours (50 ml of 500 g dextrose / L) for both pregnancy toxemic induced groups then the animals were observed until parturition.

Preparation and analysis of the samples: Serum samples were collected at zero time and every 12 hours until the clinical sings of pregnancy toxemia were appeared and then at 12, 24 and 48 hours after glucose injection and refeeding and stored at +4°C (≤ 48 h) until assay of β -hydroxybutyrate [20] and glucose concentrations were determined using glucose oxidase (Sigma Trinder kit number 315; Sigma Chemical Co.).

Statistical Analysis: For presentation of results, the means and their standard errors (SEM) were calculated. Analysis of variance (ANOVA) was performed using the Statistical Analysis System software [21]. Results were considered statistically significant when ($P < 0.05$).

RESULTS

Clinical examination of the animals revealed that the clinical signs of pregnancy toxemia appeared after 24 hours in the group which induced by fasting and insulin while after 72 hours in the group which induced by fasting only in the form of dullness, ruminal stasis, sternal recumbency with acetone odor of the mouth and urine and general weakness with pale mucous membrane.

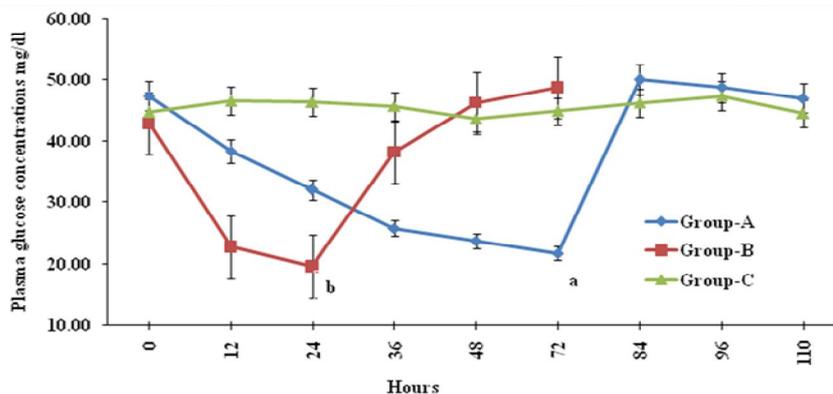


Figure 1: plasma glucose concentrations (mg/dl) in pregnancy toxemic and control groups. letters a and b indicate the time of appearance of clinical signs and administration of glucose in group A and B, respectively.

The concentration of β -hydroxybutyrate was significantly increased ($P < 0.01$) at 36 and 24 hour of induction of pregnancy toxemia in group A and B respectively than that of control group as shown in Figure 2. While glucose concentration was significantly ($P < 0.01$) decreased at 12 and 24 hours of induction of pregnancy toxemia in group A and B respectively than that of control one as shown in Figure 1. There was significant decrease in plasma glucose level in both pregnancy toxemia induced groups but plasma glucose levels were significantly decreased in group B than that of group A, until 24 hour where the signs of pregnancy toxemia were appeared in group B while at 72 hour where the clinical signs of pregnancy toxemia were appeared in group A. plasma glucose levels were not significantly different with that of group B at the time in which the clinical signs of pregnancy were appeared in this group.

β -hydroxybutyrate concentrations were significantly higher in group A and B after 36 and 24 hour respectively than that of control one, but the concentration of β -hydroxybutyrate was significantly higher in

group A than that of group B at the time in which the clinical signs of pregnancy toxemia were appeared i.e. at 72 and 24 hour in group A and B, respectively as in figure 2.

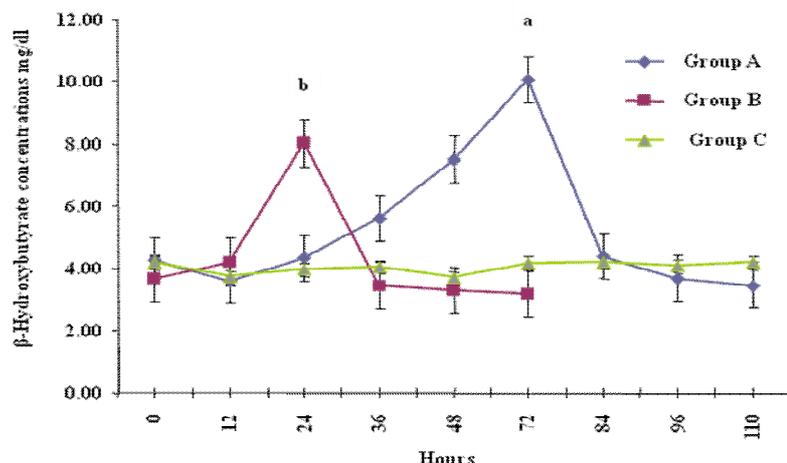


Figure 2: plasma β -hydroxybutyrate concentrations (mg/dl) in pregnancy toxemic and control groups. letters a and b indicate the time of appearance of clinical signs and administration of glucose in group A and B, respectively.

After glucose administration plasma glucose levels were returned to normal levels with 48 hour in both groups (A & B) but at 12 hour after treatment the glucose level in group A was significantly higher than that of group B at the same time post-treatment, while at 48 hour post-treatment there was no significant difference in plasma glucose level in group A, B and C as shown in figure 1. Also concentrations of β -hydroxybutyrate were not significantly different between group A, B and C at 48 hours post-treatment. There was significant ($P < 0.05$) decrease in the length of gestation in both experimental groups (A & B) than that of control one, the lengths of gestation were 140.3 ± 1.1 ; 139.2 ± 0.8 day and 149.1 ± 1.4 days in group A, B and C respectively while still birth kids were 4 (33.3%), 7 (58.3%) and 2 (16.6 %) in group A, B and C respectively from 12 kids of each group.

DISCUSSION

Insulin is the most important regulator of glucose disposal and production. It inhibits gluconeogenesis and the output of glucose from the liver, apparently increases uptake and the incorporation of amino acids into muscle protein and promotes lipogenesis in adipose tissue. As a result of these actions of insulin, glucose production is reduced, its utilization by peripheral tissue is promoted (indeed administration of exogenous insulin to ruminants induces hypoglycemia) [22, 23]. From this point of view, insulin injection was used for rapid induction of pregnancy toxemia in Zaraibi goats and that leads to rapid regression of plasma glucose concentrations by using insulin injection with fasting than that of fasting only.

Alimentary ketogenesis begins to diminish within 24 hour after fasting in ruminant but compensatory increases in hepatic ketogenesis maintains total splanchnic release until the 48 hour and 72 hour and increasing of the circulating ketone bodies concentrations during fasting in ruminants and this may be due to acceleration of hepatic ketogenesis and lipolysis [2, 24]. Also, it is reported that hepatic ketogenesis at least during starvation induced ketosis in sheep [25]. Similar observations have been found in fasted dairy cow [26]. Significant increase in β -hydroxybutyrate in group A than that of control at 36 h while group B was significantly increased at 24 hour of fasting indicates that insulin injection with fasting may accelerate hepatic ketogenesis and hepatic uptake of free fatty acids was increased [26, 27]. In addition, high rates of ketogenesis from endogenous fatty acids occurred in the liver in fasted pregnant sheep [28]. However, alimentary ketogenesis ceased because of lack of exogenous substrate of glucose during fasting.

Clinical recovery of the pregnancy toxemic does within 48 hours of refeeding and intravenous administration of glucose agreed with the result obtained earlier [29]. Also significant reduction of gestation period in pregnancy toxemic does agreed with the observations recorded before [30]. The production of dead kids from the pregnancy toxemic does more than that of the control group is more pronounced in field cases in ewes [31, 32] that often have a higher maternal mortality rate.

In this study depending on the evaluated parameters, we proved that a simple protocol of short fasting combined with insulin injection can induce experimental pregnancy toxemia in twin-bearing Zaraibi goats and this protocol can be used for valuable studies for pregnancy toxemia research in Zariaby goats.

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