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Susceptibility to Pregnancy Disease in Ewes and its Relation to Gestastional Diabetes

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Sigurdsson, H: Susceptibility to pregnancy disease in ewes and its relation to gestastional diabetes. Acta vet. scand. 1988, 29, 407-414. – The ability of 8 pregnant ewes to maintain glucose homeostasis following an intravenous glucose load (glucose tolerance test) and during starvation in late pregnancy was studied. Following an intravenous load 2 out of 7 ewes tested showed an impaired insulin secretion during the first 10-15 min. An increase in plasma insulin concentration was found 50-60 min after the injection.
The results of the effect of a starvation on a number of blood constituents are demonstrated. Two of the twin-pregnant ewes developed symptoms of pregnancy disease following starvation, these were the same ewes that showed an impaired insulin secretion after the glucose load. However, no difference was demonstrated in the clinical chemical parameters whether the experimental ewes developed pregnancy disease or not.

glucose homeostasis; glucose tolerance test; sheep; metabolism; insulin.

Introduction

The balance between food and requirement is a central element in the pathogenesis of pregnancy disease. The growth of the fetuses in late pregnancy imposes additional demands on nutritional intake of the pregnant ewe.

The fetuses impose an additional drain of the glucose pool and when rate of glucose synthesis is too low, hypoglycaemia develops. Hypoglycaemia is considered to be important since *McClymont & Setchell* (1955) showed a correlation between hypoglycaemia and early clinical signs of pregnancy disease. Disorders of glucose homeostasis appear to play a role in the pathogenesis of the disease. The intravenous glucose tolerance test (IVGTT) is used for assessing the ability to maintain glucose homeostasis through secretion of insulin. The test has been used to examine the effect of fasting in late pregnancy on glucose homeostasis and to examine the glucose homeostasis in ewes with symptoms of pregnancy disease (*Reid* 1960a).

Wastney et al. (1982) hypothesized that the large variability observed between individual ewes in their susceptibility to pregnancy disease following a starvation in late pregnancy might be a result of differences in the ability to maintain glucose homeostasis. They found a higher insulin resistance in the peripheral tissues of susceptible ewes and concluded that a poor control of glucose homeostasis may be an important predisposing factor.

The purpose of the present work was to study the ability of pregnant ewes to maintain

glucose homeostasis under starvation and by an intravenous injection of glucose (IVGTT).

Materials and method

Animals

Eight pregnant Marsk-Texel crossbred ewes were used. IVGTTs were undertaken in 7 of the ewes of which 5 (no. 51, 52, 53, 54, 55) were twin-pregnant and 2 (no. 56 and 58) pregnant with 1 fetus. The tests were undertaken at about 130 days gestation, but for 1 of the ewes (no. 58) the test was performed at about 110 days gestation.

The ewes were fed indoors from 60 days of gestation with hay and concentrates. The tests was carried out after the ewes had been starved for 14-15 h.

Experimental protocol

The IVGTT procedure followed was that of *Wastney et al.* (1982), who generally used the procedure of *Reid* (1958).

A 50% solution of glucose (0.4 g/kg live wt) was injected over a 30 s period into the jugular vein. A catheter was implanted in the other jugular vein and blood samples were collected 5 and 2 min before injection and at 3, 4, 5, 7, 10, 15, 20, 25, 30, 38, 45, 52, 60, 90 and 120 min after injection. Before each collection of blood the catheter was flushed with a small amount of saline and rinsed by withdrawal and injection of blood.

The blood samples were stored in tubes containing sodium fluoride and heparin (Vacutainer) for glucose analysis and in tubes containing clot activator (Vacutainer) for insulin assay. Plasma was analyzed immediately for glucose and sera for insulin assay stored at -20° until analyzed.

About 5 days later the ewes were starved for 10 days in the same environment with free access to water. They were not subjected to an additional psychological stress such as transport. They were under an almost constant observation throughout the starvation period. The aim was to develop symptoms of pregnancy disease until the ewes became recumbent.

Blood samples were collected daily from the jugular vein at 9.00 a.m. and whenever the ewes showed clinical signs. The samples were collected in 5 different tubes: tubes containing heparin (Nunc, sodium heparin 50 IE), siliconized tubes (Vacutainer clot activator tubes), tubes containing sodium fluorid and heparin (Vacutainer), tubes containing EDTA (Nunc, K-EDTA 30.9 μM) and 3 times during the starvation period samples were collected in heparinized tubes containing aproteinin and extra heparin for analysis of glucagon. The latter procedure was recommended by Novo (Denmark).

Heparinized plasma and heparin-fluorid plasma was separated by centrifugation immediately after the blood samples were drawn, but serum was allowed to clot for 3-4 h before centrifugation. Heparinized plasma was analyzed for calcium, magnesium, inorganic phosphate, urea, creatinin and acetoacetate, while glucose was analyzed in heparin fluorid plasma. In whole blood bicarbonate (HCO_3^-) was measured. Serum was analyzed for NEFA (non-esterified fatty acids) and insulin. Once in the starvation period (the 4th day) serum was analyzed for cortisol.

Analyses

Glucose was measured on Reaction Rate Analyzer (LKB 8600, Bromma) by the Gluc-DH method. The reagents were from Merck, Darmstadt. Calcium and magnesium were measured on Atomic absorbtion spectrophotometer, Perkin Elmer 5000. Inorganic phosphate was analyzed on Ultralab System 2074, calculating absorbtiometer. The reagents were from Merck no. 3331. Urea was measured by a rapid method from Merck

(Mercognost). Creatinin was analyzed on Reaction Rate Analyzer, LKB 8600. The reagents were from Merck no. 3384. Acetoacetate was measured on spectrophotometer (Spectronic 21) by the method of *Schilke & Johnson* (1965). Bicarbonate (HCO_3^-) was measured on ABL, ACID BASE LABORATORY (Radiometer, Copenhagen). NEFA was measured on spectrophotometer (Spectronic 21) by the method of *Ko & Royer* (1967). Insulin was analyzed by a radioimmunoassay using a double antibody system. The analysis was carried out by Statens Husdyrbrugsforsøg, Copenhagen. Glucagon was analyzed by Novo (Denmark) by radioimmunoassay using antibody K 5563. Cortisol was measured by Medical Laboratory, Copenhagen using a charcoal separation radioimmunoassay and I-125 labelled cortisol as tracer.

Model and calculations

Glucose tolerance as the half-life (T1/2) of the injected glucose was calculated from a linear regression of log of change of glucose from basal over the 15-90 min period. From the T1/2 the fractional turnover rate, K was calculated (*Kaneko* 1980).

From the model of *Sherwin et al.* (1974) for insulin kinetics in man the extravascular concentration was calculated by solving the Sherwin model in the same way as done by *Wastney et al.* (1982).

Insulin resistance index (R) was calculated from the product of T1/2 and an extravascular insulin concentration when plasma glucose was 5.5 mmol/l above basal value (*Wastney et al.* 1982).

Results

IVGTT

Four ewes (no. 51, 52, 53, 56) showed up to tenfold increase in serum insulin within 15 min after the glucose injection, but 2 out of 7 ewes tested (no. 54 and 55) showed an impaired insulin secretion during the first 10-15 min. The low insulin production rate in these 2 ewes was accompanied by a slow disappea-

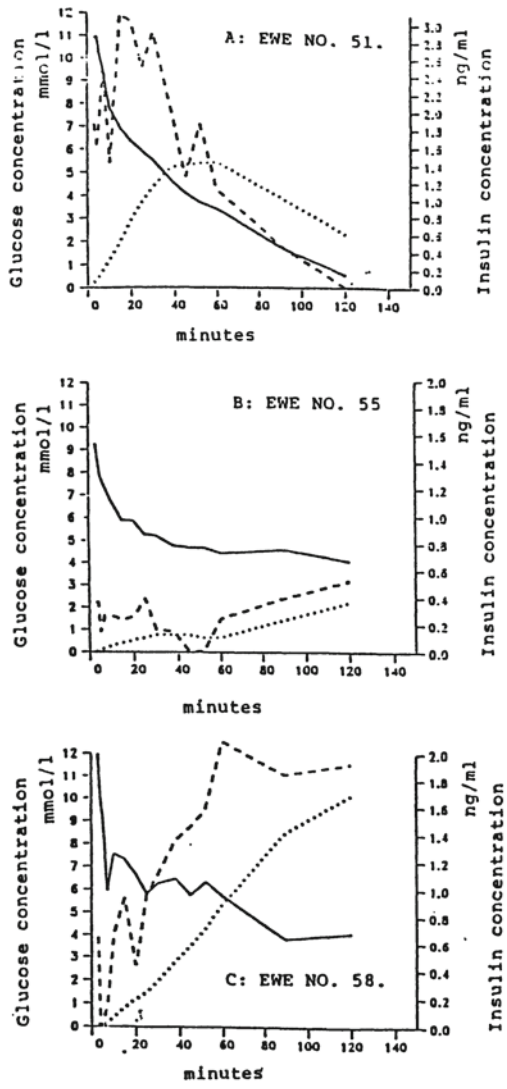

Figure 1. Changes in concentration of glucose (____), serum insulin (----) and extravascular insulin (....) after IVGTT of 3 pregnant ewes, 2 twin-pregnant and 1 pregnant with one fetus (A. ewe no. 51, B. ewe no. 55, C. ewe no. 58).

rance rate of glucose. The responses of 3 ewes (no. 51, 55, 58) to IGVTT are shown in Fig. 1 A, B, C. The figures show the changes in plasma glucose, plasma insulin and extravascular insulin concentration. One ewe (no. 58) showed an irregular insulin secretion the first 10-15 min, but thereafter a high insulin production (Fig. 1c). Impaired glucose disappearance resulted in a high extravascular insulin concentration.

A second peak in plasma insulin concentration was found 50-60 min after the injection (from $\bar{x} = 1.03$ ng/ml, SD = 0.52 to $\bar{x} = 1.44$ ng/ml, SD = 0.47). Table 1 shows the half time (T1/2), the fractional turnover rate (K) and the insulin resistance index (R).

Table 1. Half time (T1/2), fractional turnover rate (K) and insulin resistance index (R) in 7 pregnant ewes in late pregnancy.

Ewe no.	T(1/2) min.	K-values %-min	Insulin resistence index*
51	38.6	1.80	43.6
52	52.9	1.31	40.7
53	63.1	1.10	34.1
54	91.4	0.76	30.2
55	201.6	0.34	22.2
56	85.7	0.81	156.1
58	82.1	0.84	75.5

*mg min/ml

Clinical signs

The diagnosis of pregnancy disease depended upon both clinical symptoms and biochemical changes. Blood samples were collected when the ewes showed symptoms (Table 2).

In general the animals became dull and 3 ewes showed clinical symptoms. On the 4th day one of the ewes (no. 53) showed muscular tremors and rapid breathing. The serum calcium level was low (Table 2). Intravenous administration of calcium gluconate was carried out and the ewe responded well. The ewe did not show further symptoms for the rest of the starvation period. Two out of 8 pregnant ewes showed both clinical and biochemical changes of pregnancy disease. One of the ewes (no. 54) showed clinical signs which were consistent with the sub-acute form of pregnancy disease described by *Reid* (1960b), e.g. twitching of the ear, atypical postures (»star gazing«) and reluctance to move. The other (no. 55) became recumbent on the 7th day and appeared moribund which is consistent with the acute syndrome described by *Reid* (1960b). Glycerol and anabolic steroid were administered and the ewe recovered. Three of the ewes lambed the last days of the starvation period and the others just afterwards except ewe no. 58

Table 2. Results of analyses on some clinical chemical blood constituents in blood samples drawn in relation to nervous symptoms under a starvation period.

Blood samples no.	Ewe no.	Calcium mmol/l	Magnesium mmol/l	Acetoacetate mmol/l	Glucose mmol/l
1	55	2.15	0.74	1.81	1.17
2	54	1.94	0.88	0.88	1.24
3	54	1.91	0.88	0.76	1.50
4	53	1.66	0.72	1.04	1.10
5	55	1.92	0.81	2.23	1.40
6	55	2.06	0.78	1.47	2.00

Table 3. Concentration of some blood constituents in 8 pregnant ewes before and under a starvation period.

	Ewe no. 51			Ewe no. 52			Ewe no. 53			Ewe no. 54		
Parameters	1	2	3	1	2	3	1	2	3	1	2	3
Calcium mmol/l	2.46	2.18	2.15	2.35	2.03	2.01	1.99	1.74	1.90	2.14	1.94	2.15
Magnesium mmol/l	0.92	0.78	0.68	0.93	0.81	0.75	0.86	0.75	0.76	1.02	0.88	0.75
Inorg.phos mmol/l	1.19	1.10	1.45	1.29	1.37	1.52	2.04	1.60	2.18	1.34	1.76	1.70
Acetoacet. mmol/l	0.00	0.47	1.22	0.00	1.08	1.39	0.00	1.04	1.14	0.00	0.82	1.06
NEFA mEqui mmol/l	0.57	1.53	2.47	0.91	2.26	2.49	0.89	1.86	1.53	1.03	2.00	1.90
Glucose mmol/l	3.07	1.65	1.68	2.56	1.17	1.24	2.83	1.32	1.55	2.08	1.24	1.43
Insulin ng mmol/l	0.56	0.52	0.23	0.37	0.63	0.07	0.25	0.19	0.17	0.13	0.13	0.08
Creatinin mmol/l	-	82.51	106.70	-	106.08	100.10	-	97.24	86.50	-	97.24	94.00
HCO_3 mmol/l	-	23.20	22.10A	-	25.90	20.50A	-	24.10	19.60A	-	26.20	23.60A
Urea mmol/l	4	4	4	5	8	8	4	5	8	4	8	12
Days of Starvation		10			10			8			8	

	Ewe no. 55			Ewe no. 56			Ewe no. 57			Ewe no. 58		
Parameters	1	2	3	1	2	3	1	2	3	1	2	3
Calcium mmol/l	2.25	2.15	2.06	2.39	2.21	2.28	2.44	2.15	2.21	2.39	2.31	2.40
Magnesium mmol/l	0.87	0.74	0.82	0.97	0.89	0.71	0.85	0.79	0.70	0.83	0.82	0.68
Inorg.phos mmol/l	1.51	1.57	1.86	1.00	0.99	1.49	1.42	1.36	2.11	1.33	1.29	1.62
Acetoacet. mmol/l	0.46	1.81	2.08	0.00	0.44	1.38	0.00	0.65	0.95	0.00	0.31	0.61
NEFA mEqui mmol/l	0.37	1.84	1.88	0.56	1.63	2.74	-	1.98	2.71	0.46	1.52	2.81
Glucose mmol/l	2.56	1.17	1.23	2.65	1.62	1.41	3.91	1.60	2.03	3.35	1.93	2.04
Insulin ng mmol/l	0.40	0.14	0.14	0.28	0.15	0.21	0.16	0.26	0.33	0.34	0.23	0.39
Creatinin mmol/l	-	97.24	83.40	-	113.30	113.70	-	119.30	122.90	-	107.50	112.20
HCO_3 mmol/l	-	21.10	19.90A	-	22.60	21.40A	-	-	-	-	23.70	21.70A
Urea mmol/l	2	6	7	4	6	6	3	4	5	5	4	4
Days of Starvation		7			10			10			10	

1: Results before starvation; 2: Results from day 3 in the starvation period; 3: Results from day 7 in the starvation period; A. Results from day 8 in the starvation period.

which lambed 20 days later. All the lambs were born alive, but 3 of them showed impaired movements.

Clinical chemical and endocrine parameters

Table 3 shows the concentrations for blood parameters, before the starvation period and on the 3rd and the 7th day of the period. No difference was demonstrated in clinical chemical parameters whether the experimental ewes developed symptoms or not. The concentrations of glucose and calcium dropped while there was a rise in NEFA and *acetoacetate* concentrations. The degree of *hypoglycaemia* failed to indicate whether or not the ewes would be affected by pregnancy disease. The concentrations of urea was within the reference range based on approximately 80 healthy pregnant ewes in 4 herds of the same breed in the last month of pregnancy ($\bar{x}$=8.86 mmol/l, SD = 2.96). There was a fall in the insulin concentration during starvation to very low levels and on the third day a lower concentration was found in the blood of the ewes that showed symptoms of pregnancy disease than in the other twin-pregnant that did not, although not significant ($\bar{x}$=0.135 ng/ml, SD = 0.007 and $\bar{x}$=0.43 ng/ml, SD = 0.23). Table 4 shows the concentration of glucagon before and for the 4th, 8th and 10th day of the starvation period. The table also shows the concentration of cortisol in the blood samples drawn on the 4th day of the starvation pe-

Table 4. Glucagon and cortisol in the blood of starved pregnant ewes.

Ewe no.	Glucagon (pmol/l) The day before starvation	Day 4	Day 8	Day 10	Cortisol (nmol/l) Day 4
51	<10	20	15	<10	34
52	<10	28	35	29	40
53	<10	22	46	117	38
54	<10	<10	<10	<10	68
55	<10	39	21	103	89
56	<10	15	34	17	29
57	<10	26	31	16	<26
58	<10	24	27	27	35

riod. There is a rise in glucagon concentration in the blood of all the ewes but one. The concentration of cortisol was significantly higher in the blood of the ewes that showed symptoms of pregnancy disease than in the blood of the other twin-pregnant that did not ($p < 0.001$).

Glucose tolerance and symptoms of pregnancy disease

Two of the twin-pregnant ewes (no. 54 and 55) developed symptoms of pregnancy disease following starvation. These were the same ewes that showed an impaired insulin secretion after the glucose load. Fig. 1c shows the response of ewe no. 58 to IVGTT. The ewe did not show any signs of disease during starvation. The ewe was pregnant with 1 fetus and was 20 days behind the others in gestation.

Discussion

The responses of the ewes to IVGTT, which showed up to tenfold increase in serum insulin within 15 min are in agreement with responses observed by *Boda* (1964) and by *Horino et al.* (1968). A second peak in plasma insulin concentration was found 50-60 min after the injection. Similar observation is made in human beings, especially newborn infants, where the insulin production rate is slow (*Pedersen & Fahrenkrug* 1976). This second peak is attributed to the »de novo« synthesis of insulin by the beta-cells. Investigation on insulin secretion have shown that beta-cells have 2 different insulin pools: one reacting on an acute stimulus by secretion of prefabricated insulin and another that secretes insulin under normal conditions and synthesizes insulin under protracted stimulation (*Pedersen & Fahrenkrug* 1976).

Two out of 8 pregnant ewes showed clinical symptoms of pregnancy disease described by *Reid* (1960b). No difference was demonstrated in clinical chemical parameters whether the experimental sheep developed symptoms or not. The degree of hypoglycaemia failed to indicate whether or not the ewes would be affected by pregnancy disease, but the 2 twin-pregnant ewes that developed symptoms were the same ones that showed an impaired insulin secretion after the glucose load. The impaired glucose tolerance appears to be similar to type I diabetes mellitus or insulin-dependant diabetes mellitus in humans. It appears that there is no prefabricated insulin in the beta-cells which is released due to the acute stimulation by the glucose load. This could be a result of some kind of blocked secretion of insulin by other hormonal abnormalities or that the constant load of the fetal drain af glucose has resulted in reduced insulin production, especially the prefabricated part.

In the present investigation all the lambs were born alive. *Wastney et al.* (1982) classified the ewes as susceptible or non-susceptible to

pregnancy disease after a 10 days starvation period. In the nonsusceptible group some of the ewes were found to be carrying dead fetuses. These ewes respond with a slow insulin secretion and a slow disappearance rate of glucose when the IVGTT was carried out. In the present work exactly the same type of response was found in ewes that developed symptoms of pregnancy disease (Fig. 1b). The lambs were born alive. *Wastney et al.* (1982) found relationship between high insulin resistance indices and susceptibility to pregnancy disease. One ewe (Fig. 1c) showed the same response to IVGTT. The ewe did not show any signs of disease during starvation. The ewe was pregnant with 1 fetus and was 20 days behind the others in gestation. The ewe was not fat but very scared when dealt with, so adrenalin could have caused the irregular insulin release when the IVGTT was carried out. These results are in disagreement with the results obtained by *Wastney et al.* (1982) which may be explained by a possible existence of different types of gestational diabetes.

During the starvation period 1 of the ewes developed hypocalcaemia and that emphasis the importance of measuring the calcium concentration in the blood in this period. There is also an accumulating evidence that a combination of hypocalcaemia and pregnancy disease occurs in the field (*Simesen* 1971, *Bath* 1983, *Kjölleberg et al.* 1984, *Sigurdsson* 1986). A normal secretion of insulin depends on an adequate calcium concentration in the blood (*Ganong* 1977).

Reid (1960c) postulated that a state of insulin deficiency in pregnancy disease is an attractive explanation of the *pathogenesis* and that the possibility remains that the inhibitory effects of cortisol on glucose utilization may be enhanced under conditions of severe insulin insufficiency. In that case the severity of ketosis could depend on the balance between cortisol and insulin rather than on the absolute amount of each hormone secreted. The degree of inhibition of glucose utilization and an appearance of clinical signs could depend on this balance. In the present investigation the concentration of cortisol was significantly higher in the blood of the ewes that showed symptoms of pregnancy disease than in the blood of the other twin-pregnant that did not.

It is concluded that the results are in agreement with the postulate of *Reid* (1960c) and the ewes' susceptibility to pregnancy disease might be a result of difference in the ewes ability to maintain glucose homeostasis under late pregnancy.

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Sammendrag

Modtagelighed for drægtighedssyge hos får og dens relation til gestastional diabetes.

Højdrægtige fårs evne til at opretholde glukose homeostasen under en belastning blev undersøgt, både ved en intravenøs injektion af glukose (glukose tolerance test) og ved at sulte fårene. Hos to får fandtes ingen stigning i insulinkoncentrationen i løbet af de første 10-15 min. efter glukoseinjektionen. Der registreredes en sekundær stigning i plasma insulinkoncentrationen 50-60 min. efter injektionen af glukose. Effekten af sult på en række klinisk kemiske parametre er belyst. Resultatet af sulteforsøget blev, at to tvillingdrægtige får udviklede symptomer på drægtighedssyge. Hos præcis de samme får fandtes ingen stigning i insulinkoncentrationen ved glukose tolerance testen. Herudover fandtes ingen forskel på blodparametre registreret hos de to tvillingdrægtige får som udviklede symptomer på drægtighedssyge, og de øvrige får som ikke viste symptomer på sygdommen. De opnåede resultater diskuteres, især i forhold til tidligere teorier om patogenesen for drægtighedssyge.

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