Alcohol, Vol. 6, pp. 469–473. © Pergamon Press plc, 1989. Printed in the U.S.A. 0741-8329/89 $3.00 + .00

Permanent Abnormal Response to a Glucose Load After Prenatal Ethanol Exposure in Rats

DOLORES LÓPEZ-TEJERO,* MIQUEL LLOBERA* AND EMILIO HERRERA†

**Departamento de Bioquímica i Fisiología, Facultat de Biología Universitat de Barcelona, Avda Diagonal 645, 08028 Barcelona, Spain*
†Departamento de Bioquímica, Universidad de Alcalá de Henares, Madrid, Spain

Received 26 February 1988; Accepted 29 May 1989

LÓPEZ-TEJERO, D., M. LLOBERA AND E. HERRERA. *Permanent abnormal response to a glucose load after prenatal ethanol exposure in rats.* ALCOHOL **6**(6) 469–473, 1989.—Postnatal development of the glucose and insulin balance in offspring of ethanol-treated and control rats has been studied. Newborn rats were separated from their mothers and placed with normal lactating, nonethanol-treated dams. Prenatal exposure to ethanol led to hypoglycemia on the first day of extrauterine life and a general tendency to hyperinsulinemia during the entire postnatal period studied. The glucose-tolerance test in weaned rats (30 days old) gave a greater and faster increase than controls in levels of both glucose and plasma insulin. At adult age (90 days) the response of blood glucose to an oral glucose load in offspring from ethanol-treated mothers was not different from that in offspring from controls, but the insulin response was higher. This abnormal insulin response, such a long time after the end of ethanol exposure, suggests either a permanent alteration in the pancreatic response, or a peripheral insulin resistance and/or differences in the rate of insulin degradation in these animals.

Ethanol Rat Development Glucose-tolerance-test Insulinemia

CHRONIC ethanol ingestion during pregnancy exerts deleterious effects on fetal growth and postnatal development (2). Physical abnormalities and mental retardation in offspring have been described in both clinical and experimental studies, and they are known as Fetal Alcohol Syndrome (9, 17, 23). The mechanism behind the effects of ethanol on the fetus is not clear yet, and could be caused by the direct in utero action due to the mother's nutritional deprivation and/or be secondary to the mother's metabolism disturbances (14, 15, 19, 35).

Studies in both humans and experimental animals have described many endocrine and metabolic systems that are perturbed by ethanol ingestion (18, 36, 38). Moreover, scarce attention has been paid to the effects of prenatal ethanol exposure on fetal and postnatal metabolism and endocrine balance (3, 21, 25, 33). Although the importance of the carbohydrate metabolism for a successful metabolic transition from uterine to extrauterine life (10, 11, 22) and later, in adaptation to the adult diet (4) are known, there are only a few studies on changes in the glucose/insulin interactions in the offspring of pregnant mothers under ethanol treatment.

In the present work we have studied the effects of the mother's ethanol ingestion during gestation on the postnatal glucose and insulin balance in the offspring, as well as the glucose and plasma insulin response to an oral glucose load in both weaning and grown offspring of these mothers.

METHOD

Animals and Diets

Female Wistar rats (150 ± 10 g body weight) from our colony were used, and maintained under automatically controlled temperature (23 ± 1° C) and 12 hour light-dark cycles (900 to 2100 light). The animals were divided into the following groups according to the described chronic ethanol ingestion model (31): 1) Ethanol-treated rats receiving chow diet ad lib (Panlab, Spain) and 10%, 15%, 20% and 25% ethanol (w/v) in their drinking fluid for the 1st, 2nd, 3rd and 4th weeks respectively. After this period, they were mated with nontreated males, and day 0 of gestation was established with the presence of spermatozoa in vaginal smears. Pregnant rats were maintained under 25% ethanol in drinking fluid. 2) Control rats also received ad lib water and normal diet and were handled regularly for the same amount of time as the ethanol-treated rats. The only differences between the two groups was the ethanol solution in the experimental group's water.

Pregnant rats remained on their respective diets and treatments throughout the gestational period until normal delivery. After birth, newborn rats were separated from their mothers, and after the readjustment of the litter size to 8–10 pups per litter, they were immediately placed with normal lactating rats which had delivered no more than 6 hours. No pup cannibalism was observed in these foster mothers.

Sacrifice and Biochemical Methods

Pups of both sexes from the two experimental groups before suckling (day 0) or on days 1, 4, 15 and 30 of life, were sacrificed by decapitation between 9 and 10. To minimize differences in the birth time, pups studies at day 0 were selected from those mothers having delivered between 7 and 8 and both the newborn and their

TABLE 1

BODY WEIGHT AND CIRCULATING GLUCOSE AND INSULIN LEVELS AT DELIVERY IN ETHANOL-TREATED AND CONTROL RAT MOTHERS

	Body Weight (g)	Blood Glucose (mg/100 ml)	Plasma Insulin (μU/ml)	Glucose/ Insulin Ratio
Control	291 ± 4	79 ± 1	30 ± 7	0.92 ± 0.15
Ethanol	238 ± 7†	59 ± 5*	25 ± 8	0.81 ± 0.15

Values are mean ± SEM of 5–7 rats. Statistical comparisons versus controls were done by Student's *t*-test: *$p<0.01$, †$p<0.001$.

mothers were decapitated 2 hours after the onset of delivery. The blood of each litter was collected into dry heparinized beakers, and aliquots were deproteinized (29). The supernatant was used for blood glucose assay (16). Plasma samples were used for insulin quantification with a rat-specific radioimmunoassay kit (13) generously provided by Novo Industry A/S (Denmark). Pieces of liver from three pups per litter were immediately frozen and pooled for glycogen extraction and quantification (12).

Glucose-Tolerance Test

In vivo experiments were carried out on 30-day-old male and female rats from both experimental groups. In order to study the animals with an empty stomach but without the prolonged food deprivation period which would decrease sensitivity in the response to glucose load (8), and therefore reduce potential differences between the groups, it was decided to carry out the glucose-tolerance test in all animals after 6 hours' fasting. They were given 2 g glucose/kg body weight intragastrically using a 30% glucose solution. Blood samples were then collected into heparinized tubes, by decapitation of different animals at 0, 5, 10, 15, 30, 45, 60, 90 or 180 minutes after the glucose load.

After weaning (on day 30), other rats from the two experimental groups were maintained without handling and on normal adult diet. When they were 90 days old, the same glucose-tolerance test was performed. In this case, blood from the tail tip was collected into heparinized beakers at 0, 5, 15, 30, 45, 60, 90 and 180 minutes.

Deproteinized blood aliquotes and plasma samples were frozen until glucose (16) and insulin (13) measurements could be made.

RESULTS

Maternal Parameters

After a phase of adaption to progressively increasing amounts of ethanol, rats received a stable dose of ethanol throughout pregnancy (25% in drinking water). This protocol was previously used by us to establish the manner in which this treatment affects maternal food and caloric intake (31), and it was found that whereas ethanol ingestion accounted for more than 30% of total calories ingested, total energy intake during pregnancy in these rats did not differ from that of controls (25.3 ± 1.3 vs. 24.6 ± 0.4 kcal/100 g b.wt./day respectively). As shown in Table 1 at delivery time, both body weight and blood glucose concentration in the ethanol-treated mothers was significantly lower than in controls, whereas plasma insulin and the glucose/insulin ratio did not differ between the two groups.

Postnatal Parameters: Body Weight, Blood Glucose, Plasma Insulin and Liver

As shown in Table 2, the body weight for newborn offspring of ethanol-treated mothers is significantly lower than in controls at 0, 1, and 4 days after birth, and this difference disappeared from day 15 on. Blood glucose levels and liver glycogen did not differ in offspring from ethanol-treated mothers and controls throughout the studied development period, except on the first day of life, when the newborn from ethanol-treated mothers presented evident hypoglycemia (Table 2). The mean plasma insulin levels were slightly higher and the glucose/insulin ratio slightly lower in the pups from ethanol-treated mothers than in the controls' pups, although the difference between the groups was not significant at any of the age-times studied (Table 2).

Glucose-Tolerance Test

To clarify whether ethanol-treated mother's offspring have any

TABLE 2

BLOOD GLUCOSE, PLASMA INSULIN, AND LIVER GLYCOGEN IN 0, 1, 4, 15 AND 30-DAY-OLD RATS BORN FROM ETHANOL-TREATED OR CONTROL MOTHERS

	Postnatal Age (days)	Body Weight (g)	Blood Glucose (mg/100 ml)	Plasma Insulin (μU/ml)	Glucose/ Insulin Ratio	Liver Glycogen (g/100 g)
Control	0	5.6 ± 0.1	58.8 ± 5.8	49.8 ± 5.8	1.2 ± 0.1	4.4 ± 0.7
	1	6.4 ± 0.2	69.8 ± 3.1	45.8 ± 9.0	1.5 ± 0.2	1.1 ± 0.1
	4	10.5 ± 0.3	98.5 ± 1.8	29.3 ± 3.0	3.4 ± 0.4	1.5 ± 0.7
	15	27.2 ± 0.1	104.3 ± 5.1	20.7 ± 5.8	5.0 ± 0.7	2.5 ± 0.4
	30	91.7 ± 2.2	120.1 ± 3.5	25.8 ± 2.0	4.7 ± 0.4	6.3 ± 0.6
Ethanol	0	5.1 ± 0.1†	67.1 ± 7.1	54.7 ± 5.6	1.2 ± 0.2	4.8 ± 0.7
	1	5.8 ± 0.1*	55.7 ± 5.5*	55.7 ± 7.2	1.0 ± 0.2	1.3 ± 0.2
	4	9.2 ± 0.5*	94.3 ± 1.7	33.2 ± 5.7	2.7 ± 0.5	2.6 ± 0.3
	15	27.2 ± 1.3	108.1 ± 4.2	21.7 ± 3.0	4.8 ± 0.5	2.0 ± 0.1
	30	91.8 ± 2.7	123.5 ± 2.3	32.2 ± 3.5	3.8 ± 0.4	5.0 ± 0.2

Values are mean ± SEM of 8–10 litters. Statistical comparisons versus controls were done by Student's *t*-test: *$p<0.05$, †$p<0.001$.

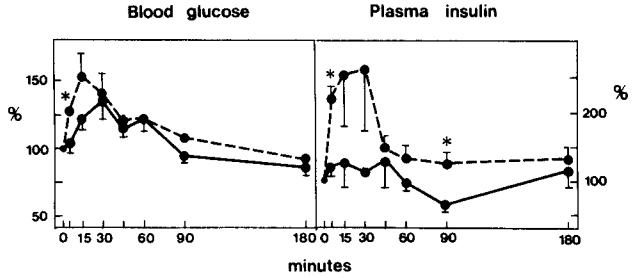

FIG. 1. Glucose-tolerance test in 30-day-old rats born from ethanol-treated (●---●) or control (●—●) mothers. Values are expressed as percentage of zero time and are mean ± SEM of 5–7 litters. Statistical comparisons versus controls were done by student's *t*-test: $*p<0.05$. Zero time values were for blood glucose (mg/100 ml): 103.9 ± 5.6 in the ethanol-treated group vs. 109.9 ± 4.7 in controls (nonsignificant); and for plasma insulin (μU/ml): 23.2 ± 3.5 vs. 32.8 ± 5.0 respectively (nonsignificant).

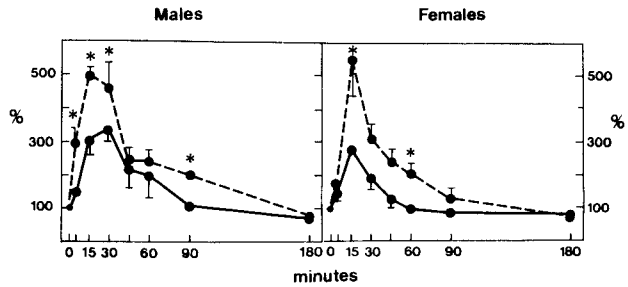

FIG. 2. Plasma insulin levels after an oral glucose load, in 90-day-old male and female rats, born from ethanol-treated (●---●) or control (●—●) mothers. Values are expressed as percentage of the zero time value and are mean ± SEM of 5–7 rats/group. Statistical comparisons versus controls are made by Student's *t*-test: $*p<0.05$. Zero time values in μU/ml for males were 48.3 ± 7.8 in the ethanol group vs. 74.8 ± 11.5 in controls (nonsignificant) and, for females, 27.5 ± 7.8 and 67.5 ± 15.5 respectively ($p<0.05$).

other glucose homeostatic alteration, glucose-tolerance tests were carried out in other experimental animals at the end of the weaning period. The results of the glucose-tolerance test in 30-day-old pups are shown in Fig. 1. When expressed as percentual glucose value at zero time, the blood glucose increase at 5 minutes was significantly greater in pups born from ethanol-treated mothers than in controls' pups. Whereas glucose values in the ethanol group peaked at 15 minutes, in the control group this occurred only at 30 minutes. Values at greater times did not differ between the two groups. Plasma insulin levels also increase higher and faster in the pups born from ethanol-treated mothers than in the pups from controls.

To determine whether observed changes in glucose-tolerance tests persist during a longer time after birth, the test was also performed in rats from both experimental groups but at 90 days after birth. In these experiments, male and female animals were studied separately and the values of the glucose-tolerance test are shown in Table 3 (blood glucose levels) and in Fig. 2 (plasma insulin levels) expressed both as percentage of the zero time values. Blood glucose values at different times after glucose load did not differ between adult males and females born from either ethanol-treated or control mothers (Table 3). Higher plasma insulin levels were detected in both adult males and females born from ethanol-treated mothers than in control offspring after the glucose load (Fig. 2), and this change is comparable to that found in the weaning rats (Fig. 1).

TABLE 3

BLOOD GLUCOSE LEVELS AFTER AN ORAL GLUCOSE LOAD IN 90 DAY OLD MALE AND FEMALE RATS BORN FROM ETHANOL-TREATED OR CONTROL MOTHERS

	Males		Females	
Minutes After Glucose Load	Control	Ethanol	Control	Ethanol
0	100	100	100	100
5	130 ± 11	116 ± 6	134 ± 10	114 ± 5
15	148 ± 8	155 ± 2	154 ± 10	153 ± 4
30	156 ± 4	169 ± 9	160 ± 9	150 ± 6
45	155 ± 10	154 ± 5	159 ± 5	148 ± 2
60	171 ± 8	156 ± 5	162 ± 6	149 ± 7
90	150 ± 4	146 ± 7	152 ± 6	140 ± 7
180	122 ± 8	130 ± 12	131 ± 12	112 ± 4

Values are expressed as percentage of the zero time value and are mean ± SEM of 5–7 rats/group. No significant differences ($p<0.05$) were found by Student's *t*-test between ethanol and control values. Zero time values in mg/100 ml were for males, 94.3 ± 2.7 in the ethanol group and 89.2 ± 2.9 in controls (nonsignificant) and for females 96.7 ± 4.2 and 79.4 ± 5.8 ($p<0.05$) respectively.

DISCUSSION

The present study shows that offspring from rats receiving ethanol during pregnancy have an enhanced response to oral glucose load as far as their plasma insulin is concerned. Nursing with untreated foster mothers from birth allowed the experimental pups to recuperate both a normal nutritional condition during the suckling period and their reduced birth weight. However, at 90 days they still had an enhanced response to oral glucose load indicating that this defect is a consequence of their exposure to ethanol during intrauterine life. Although a slightly augmented increase in blood glucose was found at the first minutes after glucose load in the 30-day-old rats born from ethanol-treated mothers, this was not the case when the test was performed in 90-day-old rats. This indicates the presence of an enhanced sensitivity of the pancreatic β-cell to glucose stimulus, although some degree of insulin resistence also seems to be present due to glycemia stability.

Present findings on unmodified plasma glucose and liver glycogen concentration in newborn rats from ethanol-treated mothers agree with some previous reports (27,34) but not with others (15, 26, 30, 32, 37). It is accepted that pups born from alcoholic pregnant rats show important variations in glucose-circulating levels in the course of the first hours of life (27), and this, together with differences in dose and experimental design, could explain the discrepancies. There is, however, general agreement on the tendency of sustained-enhanced plasma insulin levels in the offspring of ethanol-treated mothers (26, 27, 32, 34, 37). Our present findings clearly show that the pancreatic hypersensibility to glucose existing in these offspring may be responsible for the persistent hyperinsulinemia. We suspect that the mechanism underlying the response may be influenced by intrauterine malnutrition secondary to ethanol intake during pregnancy. This malnutrition occurs even when the mothers taking ethanol receive the same caloric intake as the controls (35). Another factor

affecting fetal malnutrition is the impaired metabolite placental transfer from the mother (14). Gestational malnutrition with intermittent hypoglycemia may cause glucose insulinotropic hypersensitivity in the newborn rat (28). It is therefore hypothesized that intrauterine malnutrition caused by the mother drinking during pregnancy could affect normal maturation of the insulin secretory mechanism accounted during the first 2–3 days of life in the rat (5) and so cause permanent pancreatic hypersensitivity. Maintenance of normoglycemia in the presence of enhanced plasma insulin levels after glucose loading also indicates the presence of some level of insulin resistence in the pups from ethanol-treated mothers, but further studies are necessary to clarify this point.

Earlier studies in the rat and other species show enhanced insulin response to glucose load in the offspring of ethanol-treated mothers around parturition. Chronic ethanol exposure in the rat during pregnancy produces a high insulin response to glucose load in newborns up to three days after birth (34). In the sheep, acute ethanol exposure in the mother also enhances the insulin response to glucose load in the fetus (7). An enhanced insulin pancreatic response and/or a peripheral insulin resistance in children affected with Fetal Alcohol Syndrome has also been shown (6). Our data from the glucose tolerance test in 90-day-old rats born from ethanol-treated mothers evidences the permanence of this alteration and suggests that this abnormality is responsible for some of the metabolic alterations shown to be present in the offspring of alcoholic mothers.

It is, however, important to note that besides interspecies and intraspecies variations caused by different sensitivities to ethanol, the reproducibility of these findings must take to consideration the delivery (vaginal or cesarian), ethanol dose and time of treatment as well as administration mode, since all of these factors are known to affect any response to ethanol intake during pregnancy (1,24). There is also an additional factor which may influence the observed findings. We have previously shown that, as occurs in humans, offspring from ethanol-treated mother rats suffer high neonatal mortality (20) and this factor may introduce a very important degree of natural postnatal selection. These indirect effects, together with those caused directly by ethanol during intrauterine life, may be responsible for both the reduced newborn weight and the permanent alteration in the insulin balance. Further studies are needed to establish the precise mechanism by which maternal ethanol intake during pregnancy causes these alterations and how they participate in the other pathological manifestations present in the Fetal Alcohol Syndrome.

ACKNOWLEDGEMENTS

The authors thank Carol F. Warren of the ICE of Alcalá de Henares University for her editorial help. The work was supported by a grant from the "Comisión Asesora de Investigación Científica y Técnica," Ministerio de Educación y Ciencia, Spain.

REFERENCES

1. Abel, E. L. Procedural considerations in evaluating prenatal effects of alcohol in animals. Neurobehav. Toxicol. Teratol. 2:167–174; 1980.
2. Abel, E. L. Consumption of alcohol during pregnancy: a review of effect on growth and development of offspring. Human Biol. 54: 421–453; 1982.
3. Anderson, R. A. Endocrine balance as a factor in the etiology of the fetal alcohol syndrome. Neurobehav. Toxicol. Teratol. 3:89–104; 1982.
4. Angel, J. F.; Back, D. W. Weaning and metabolic regulation in the rat. Can. J. Physiol. Pharmacol. 63:538–545; 1985.
5. Asplund, K.; Westman, S.; Hellerström, C. Glucose stimulation of insulin secretion from the isolated pancreas of fetal and newborn rats. Diabetologia 5:260–262; 1969.
6. Castells, S.; Mark, E.; Abaci, F.; Schwartz, E. Growth retardation in fetal alcohol syndrome. Dev. Pharmacol. Ther. 3:232–241; 1981.
7. Castro, M. I.; Koritnik, D. R.; Rose, J. C. Fetal plasma insulin and thyroid hormone levels during *in utero* ethanol exposure in a maternal-fetal sheep model. Endocrinology 118:1735–1742; 1981.
8. Codina, J.; Herrera, E. Effect of fasting on the circulating glucose and insulin levels after glucose, arginine, pyruvate and palmitate administration in the rat. Mol. Physiol. 2:193–201; 1982.
9. Colangelo, W.; Jones. D. G. The fetal alcohol syndrome: A review and assessment of the syndrome and its neurological sequelae. Prog. Neurobiol. 19:271–314; 1982.
10. Gain, K.; Watts, C. Glucose homeostasis in the developing rat. II. Blood glucose, plasma insulin and hepatic glycogen in the newborn rat. Biol. Neonate 30:35–39; 1976.
11. Girard, J.; Ferré, P. Metabolic and hormonal changes around birth. In: Jones, C. T., ed. Biochemical development of the fetus and the neonate. Amsterdam: Elsevier Biomed. Press; 1982:517–551.
12. Good, C. A.; Kramer, H.; Somogyi, M. The determination of glycogen. J. Biol. Chem. 100:485–491; 1933.
13. Heding, L. G. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. Diabetologia 8:260–266; 1972.
14. Henderson, G. I.; Patwardhan, R. V.; MacLeroy, S.; Schenker, S. Inhibition of placental aminoacid uptake in rats following acute and chronic ethanol exposure. Alcohol.: Clin. Exp. Res. 6:495–505; 1982.
15. Herrera, E.; Llobera, M. Ethanol toxicity: lipid and carbohydrate metabolism and ethanol in pregnancy and the fetal alcohol syndrome. In: Brown, S. S.; Davies, D. S., eds. Organ-directed toxicity. Chemical indices and mechanisms. Oxford: Pergamon Press; 1981: 11–23.
16. Hugget, A. G.; Nixon, D. A. Use of glucose oxidase, peroxidase, and Odianisidine in determination of blood and urinary glucose. Lancet ii:368–370; 1957.
17. Jones, K. L.; Smith, D. W.; Ulleland, C. N.; Streissguth, A. P. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet 7815:1267–1271; 1973.
18. Lieber, C. S. Metabolism and metabolic effects of alcohol. Med. Clin. North Am. 68:3–31; 1984.
19. Lin, G. W. Fetal malnutrition: a possible cause of the fetal alcohol syndrome. Prog. Biochem. Pharmacol. 18:115–121; 1981.
20. López-Tejero, D.; Arilla, E.; Colás, B.; Llobera, M.; Herrera, E. Low intestinal lactase activity in offspring from ethanol-treated mothers. Biol. Neonate 55:204–213; 1988.
21. Ludeña, M. C.; Mena, M. A.; Salinas, M.; Herrera, E. Effects of alcohol ingestion in the pregnant rat on daily food intake, offspring growth and metabolic parameters. Gen. Pharmacol. 14:327–332; 1983.
22. Margolis, R. N. Regulation of hepatic glycogen metabolism in pre- and postnatal rats. Endocrinology 113:893–902; 1983.
23. Reyes, E.; Rivera, J. M.; Saland, L. C.; Murray, H. M. Effects of maternal administration of alcohol on fetal brain development. Neurobehav. Toxicol. Teratol. 5:263–267; 1983.
24. Samson, H. H. Maternal ethanol consumption and fetal development in the rat: A comparison of ethanol exposure techniques. Alcohol.: Clin. Exp. Res. 5:67–74; 1981.
25. Schweistal, M. R.; Gingerich, R. L. The effects of ethanol diet on insulin release in maternal, fetal and neonate rats. Nutr. Rep. Int. 32:863–871; 1985.
26. Singh, S. P.; Snyder, A. K.; Singh, S. K. Effects of ethanol ingestion on maternal and fetal glucose homeostasis. J. Lab. Clin. Med. 104:176–184; 1984.
27. Singh, S. P.; Snyder, A. K.; Pullen, G. L. Fetal alcohol syndrome: Glucose and liver metabolism in term rat fetus and neonate. Alcohol.: Clin. Exp. Res. 10:54–58; 1986.
28. Sodoyez-Goffaux, F.; Sodoyez, J. C. Effects of intermittent hypoglycemia in pregnant rats on the functional development of the pancreatic β-cells of their offspring. Diabetologia 12:73–76; 1976.
29. Somogyi, M. Determination of blood sugar. J. Biol. Chem. 160: 69–73; 1945.

30. Tanaka, H.; Suzuki, N.; Arima, M. Hypoglycemia in the fetal alcohol syndrome. Brain Dev. 4:97–103; 1982.
31. Testar, X.; López-Tejero, D.; Llobera, M.; Herrera, E. Ethanol administration in the drinking fluid to pregnant rats as a model for the fetal alcohol syndrome. Pharmacol. Biochem. Behav. 24:625–630; 1986.
32. Testar, X.; Llobera, M.; Herrera, E. Comparative metabolic effects of chronic ethanol intake and undernutrition in pregnant rats and their fetuses. Alcohol.: Clin. Exp. Res. 121:197–200; 1988.
33. Thadani, P. V. Fetal alcohol syndrome: Neurochemical and endocrinological abnormalities. Prog. Biochem. Pharmacol. 18:83–98; 1981.
34. Villarroya, F.; Mampel, T. Glucose tolerance and insulin response in offspring of ethanol-treated pregnant rats. Gen. Pharmacol. 16: 415–417; 1985.
35. Weinberg, J. Effects of ethanol and maternal nutritional status on fetal development. Alcohol.: Clin. Exp. Res. 9:49–55; 1985.
36. Winston, G. W.; Reitz, R. C. Effects of chronic ethanol ingestion on glucose homeostasis in males and females. Life Sci. 26:201–209; 1980.
37. Witek-Janusek, L. Maternal ethanol ingestion: effect on maternal and neonatal glucose balance. Am. J. Physiol. 251:178–184; 1986.
38. Ylikahri, R. H.; Huttunen, M. O.; Härkönen, M. Hormonal changes during alcohol intoxication and withdrawal. Pharmacol. Biochem. Behav. 13:131–137; 1980.