Ann. Ist. Super. Sanità, vol. 29, n. 1 (1993), pp. 27-34

Diabetes in pregnancy: experimental aspects

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Summary. - Substantial literature indicates that diabetes in pregnant rats and mice induces embryolethality, growth retardation, and a variable incidence of birth defects. All these embryopathic effects appear to be mediated by several factors, e.g. genetic disposition and the composition of the diets eaten by the animals. Studies carried out on rodent embryos cultured *in vitro* showed that numerous components of the diabetic state (keton bodies, somatomedin inhibitors, low concentrations of insulin) display a dismorphogenic potential. Besides, diabetes-induced malformations have been often related, both *in vivo* and *in vitro* studies, to morphological and physiological alterations of the yolk sac, the principal organ for the passage of nutrients from the mother to the rodent embryo. On the whole, *in vivo* and *in vitro* experiments indicate that hyperglycemia itself is not a major factor in producing diabetic embryopathies that are more likely ascribable to severe alterations of the embryonic energetic balance.

Key words: diabetes, pregnancy, congenital malformations, embryonic nutrition.

Riassunto *(Diabete in gravidanza: aspetti sperimentali)*. - Numerosi studi condotti su ratti e topi hanno evidenziato che il diabete in gravidanza influisce sullo sviluppo embriofetale provocando ritardo di sviluppo, embrioletalità e un tasso variabile di malformazioni congenite. Tutte queste embriopatie sono modulate da diversi fattori tra cui i più certi sono la predisposizione genetica e la composizione della dieta fornita agli animali. Studi condotti utilizzando colture *in vitro* di embrioni di roditori hanno evidenziato che numerosi fattori serici caratteristici della condizione diabetica (corpi chetonici, inibitori delle somatomedine, e una ridotta concentrazione di insulina) possono indurre malformazioni congenite. Inoltre le malformazioni da diabete sono spesso state associate, sia negli studi *in vivo* che in quelli *in vitro*, ad alterazioni della morfologia e funzionalità del sacco vitellino, organo primario per il trasporto di nutrienti all'embrione di roditore. Nel complesso gli studi condotti su modelli animali sia *in vivo* che *in vitro* fanno pensare che l'iperglicemia per sé non sia la causa delle embriopatie osservate che sarebbero invece imputabili a gravi scompensi dell'equilibrio energetico embrionale.

Parole chiave: diabete, gravidanza, malformazioni congenite, nutrizione embrionale.

Introduction

The risks for the conceptus due to diabetes in pregnancy, have been known for many decades.

The introduction of insulin therapy, immediately after the second world war, was a major advance. This therapy removed the almost total sterility previously associated with diabetes, and the risks associated with pregnancy for the diabetic woman. Nevertheless the risk of bearing a malformed child has remained high. This is almost certainly due to the fact that the corrective therapy starts late in the pregnancy, often in fact after the second month. At this stage the damaging effects of the diabetes have already affected the development of the embryo. The inter-relationship between maternal diabetes and malformation has been a problem which has attracted much attention from research workers, for two main reasons:

1) the identification of the factors causing the malformations;

2) the development of means to prevent the malformations.

The two aspects of the problem may have a common solution. The enormous difficulties surrounding experimentation on diabetic women have led to researchers carrying out their work on experimental animals. After the discovery of agents producing diabetes (Alloxan, and the more favored Streptozotocin) this animal based research gained impetus.

In vivo studies

The first *in vivo* studies of any significance were carried out in the early fifties using rodents, and they were aimed at establishing that the results of the animal experiments were relevant to the situation in women. Ross and Spector [1] using mice, and Bartelheimer and Kloos [2] using rats, both described an increase in embryonic malformations and embryopathies following the induction of diabetes in pregnancy.

The first methodical studies however, were carried out by the Watanabe group some years later.

By injecting Alloxan into pregnant mice between the 8.5th and the 13.5th day, they caused a high maternal death rate. In the survivors there was a high incidence of malformed fetuses and of resorptions [3]. The incidence of the malformations was linked to the timing of the treatment with the diabetogenic agent, and was much higher in the animals treated earlier in pregnancy. In a following experiment [4] the same group of researchers showed that they could prevent the malformations caused by Alloxan induced diabetes through the administration of insulin. The Alloxan was administered at the 4th day of pregnancy. In the animals afterwards treated with 0.4 i.u. of lente insulin (every 12 hours up to the 16th day of pregnancy) there was only one fetus out of 472 with palatoschisis. In the group not treated with insulin 22 fetuses out of 437 showed malformations of varying types (cranioschisis, palatoschisis, club foot).

The dosage of insulin used in this experiment was not always sufficient to completely compensate for the hyperglycemia induced by the Alloxan, and there were some mice with glycemia values in excess of 300 mg%. Nevertheless, the incidence of malformations was very low. On the basis of this result, the authors suggested that "the teratogenic effect of the diabetes is the consequence of extensive disturbance of metabolic homeostasis, rather than the hyperglycemia of the mother itself". This was a conclusion of major importance for those times, and as we shall see is still of great relevance today.

The greater part of the experiments that followed used rats, and the results were very diverse, and occasionally false.

This was sometimes due to inadequate techniques for the assessment of malformations, and sometimes to excess zeal in attributing to the syndromes found in the experimental animals an equivalence to those described in women.

Deuchar [5], for example, made female Wistar rats diabetic either by using Alloxan (at the 9th day of gestation) or by use of Streptozotocin (at the 1st day of gestation), and reported a high frequency of congenital malformations (c.m.), both half way through gestation (malformations of the central nervous system (CNS), and of the heart), and at term (exonphalos, micrognathia, and incomplete sacral ossification).

The evaluation of these results is difficult. How can one explain how the c.m. of the CNS and the heart found at the 13th day, are not found also at full term. Furthermore, the reduced ossification of the sacrum, equated with the sacral dysgenesis in babies with diabetic mothers, is probably only retarded ossification due to the immaturity of the fetuses, and not a true malformation.

In any event, the incidence of embryonic mortality in this experiment was 28%, and the maternal glycemia was only indicated as being > 250 mg%.

Baker *et al.* [6] focused their attentions on the malformations of the sacral region, in an experiment in which female rats (Sprague Dawley strain) were rendered diabetic with Streptozotocin injections at either the 6th or the 12th day of gestation.

In the first case 25/146 fetuses had sacral malformations: 16 were of fusion failures (spina bifida occulta) and 9 were of failure of ossification. In the second case 0/54 showed sacral malformations. Treatment with semi-lente insulin from the 7th to the 13th day of pregnancy significantly reduced the incidence of sacral malformations in diabetic rats (5/104). The results of this trial show that the period of embryo sensitivity to diabetes-induced malformations is before the 12th day of gestation in the rat corresponding to the first 6-7 weeks of pregnancy in humans.

This indication of the timing of the teratogenic effect of maternal diabetes means that diabetes must be strictly controlled during the first weeks of gestation, and shows that uncontrolled diabetes after this time in gestation has no effects on the incidence of malformations.

It has to be remembered, however, that the results of experiments on rodents, and on rats in particular, are not homogeneous.

As we have already noted, some authors report a wide range of c.m., and a high frequency of malformations associated with high embryo death rates, together with delayed development. Others, like Baker *et al.* [6], focus their attention on supposedly real malformations of the sacrum. Eriksson *et al.* [7] using rats of the Sprague Dawley strain - Uppsala, reported that the fetuses of females made diabetic with Streptozotocin (Stz) show a specific syndrome characterized by caudal dysgenesis (absence of the tail and alteration of the sacrum) and micrognathia. Unfortunately, the examination of the fetuses was only skeletal. The incidence of the fetuses with the reported syndrome was around 20%, whilst the embryo death rate was around 30%. The administration of 2-6 i.u. per day of ultra-lente insulin, brought the embryo death rate back to normal values, and only 2 fetuses out of 233 showed micrognathia or caudal dysgenesis.

These results were confirmed later [8], by a study in which insulin was administered to diabetic females in discrete periods, rather than during the whole of the pregnancy. The authors were attempting to identify the periods in which the susceptibility to teratogens is most marked. However, the results are difficult to interpret as the highest embryo death rate and the most marked teratogenic effects resulted when the administration of insulin was interrupted during the pre-implantation period of the gestation (from 2nd to the 7th day after coition). This is a period in which the organogenesis of the rat embryo has not yet started.

Uriu-Hare *et al.* [9] examined the fetuses of female rats from both the Wistar and the Sprague Dawley strains, which had been made diabetic with Stz around 27 days prior to pregnancy. They did not find any significant difference between the two strains, neither in the manifestations of the diabetes (maternal and fetal glycemia, and insulinemia), nor in the effects of maternal diabetes on the conceptuses.

The most obvious embryotoxic effects were an increase in death rate (at the limit of significance), reduction of fetal weight and increase in placental weight. The authors report a strong increase of skeletal malformations in the fetuses of diabetic rats, however, the greater part of these are minor anomalies or variations, and one can conclude that the increase in the rate of c.m. is modest, in this study.

Similar results have been reported by Giavini *et al.* [10], using Sprague Dawley strain female rats which had been rendered diabetic either before pregnancy, or on the first day of pregnancy.

The experiment was conducted on a large sample of animals, with a view to establishing the reliability of this experimental model for the study of the teratogenic effects of diabetes. It demonstrated that the diabetes induced by Stz is both severe and constant (glycemia levels > 500 mg%); it reduces fertility and increases pre-implantation losses; it reduces the fetal weight and increases placental weight; it increases the incidence of major malformations by a factor of 4 to 5. In this experiment no skeletal malformations were found. The most frequent malformations concerned the circulatory system, the absence or the reduction of the tail, and cranioschisis. Overall the c.m. did not exceed 4%. This figure, which has been characteristic of the experiments in this laboratory, finds support in the work carried out by Eriksson *et al.* [11, 12].

In fact this author, when using SD rats from Uppsala, found a high frequency of c.m. and a very specific incidence of caudal regression. When he used animals from a Hannover breeder, under the same experimental conditions, he obtained, a rate of malformations and resorptions much lower.

When Eriksson [12], crossed males and females from different substrains, he was able to demonstrate that the greater predisposition to diabetes-induced embryopathies arose from the genetic characteristics of both the mother and the embryo. The Uppsala homozygotes were decidedly more susceptible.

The results of these experiments are very important as they demonstrate the role played by genetic characteristics in the predisposition to diabetes, and they reduce the importance of hyperglycemia as an etiological factor in diabetic malformations. In fact, in this work the glycemic levels in both substrains were identical.

The evidence that hyperglycemia, by itself, is not the etiological factor in c.m. due to diabetes, has given further stimulus to research in this sector.

The observations made by several authors, that the placenta of the fetuses in diabetic mothers is both much larger than normal, and also shows serious pathological symptoms when subject to histological examination [13, 10], has led to another hypothesis. This is that the interference with the normal passage of nutrients through the placenta is the factor responsible for at least the delayed development always found in fetuses of diabetic rodents, and possibly for other embryonic pathologies also.

Eriksson and Jansson [14], found that when they measured placental blood flow using radio-active microspheres, in both normal and diabetic rats on days 20 and 22 of pregnancy, the latter was only around one half of the normal value.

On these days the placental blood flow per placental weight was drastically reduced in diabetic animals, however the placental flow per fetal weight was the same as that in the controls.

Chartrel *et al.* [15] observed that the retarded development of diabetic rat fetuses was significantly related to the reduction of the blood flow velocity in the uterine artery, the placenta and the umbilical artery, when measured at the 21st day of pregnancy. Treatment with insulin, or with the vaso-dilator nicergolina (α1-blocker) from the 9th to the 21st day of gestation restored the normal rheological values and the fetal weight. Unfortunately the authors do not give any data of the effect of this treatment on malformations.

Even if these results are not conclusive, they indicate the importance of the placental blood flow in the induction of the retarded fetal development, often seen in diabetic animals. The clinical and experimental observation that the polyuria associated with diabetes can cause high salt elimination rates [9] has led some investigators to examine the effects of the lack of elements which play an important role in embryonic development, e.g. zinc and magnesium.

Uriu-Hare *et al.* [16], fed Stz diabetic rats with low (4.5 ppm), adequate (24.5 ppm), and high (500 ppm) zinc diets, and reported a clear correlation of some embryo abnormalities (fetal weight, degree of ossification) with the zinc level in the diet. Although the highest rate of malformations was in the group of diabetic animals with a low zinc diet, it is not enough to establish a real relationship between zinc deficiency and malformations in diabetic pregnancy, as increases of zinc to quite high levels in the diet do not substantially modify the malformation picture. Eriksson [17] came to similar conclusions when he showed that additions of zinc to the drinking water of diabetic animals during gestation did not alter the frequency of gross malformations.

Giavini *et al.* [18] were also unable to demonstrate a causal linkage between lack of magnesium in the diet of diabetic rats, and c.m. Nevertheless, through these experiments some relationship between characteristics of diet, and diabetic embryopathy has been suggested.

In fact, when the standard diet of the rats was replaced with a synthetic diet with the same ratios between the main elements (60% glycides, 20% protein, 4.5% fats, 4.5% fiber), the results on the conception product were dramatic. The resorptions reached a level of over 50%, the malformations 24%, and the fetal weight was drastically reduced. A partial improvement was seen when diabetic animals were given an unbalanced diet of 60% protein, and only 20% carbohydrate [19].

These results are an indication of the fact that a serious metabolic alteration like diabetes will be affected in its pathological effects by the characteristics of the food administered. These moderate the effects of the diabetes, in ways that are not entirely clear, and can affect the development of the products of conception. This supports the hypothesis of a "fuel mediated teratogenesis" firstly announced by Freinkel [20] in diabetic pregnancy. A further relevant result can be added to these findings: when arachidonic acid (200-400 mg/day) was injected subcutaneously into pregnant diabetic rats during the organogenesis period, the frequency of neural tube fusion defects was reduced from 11% to 3.8%, the frequency of cleft palate from 11% to 4%, and the frequency of micrognathia from 7% to 0.4%. The treatment did not alter maternal glycemia, maternal weight gain, or fetal weight [21].

The high concentration of tissue glucose to be found in diabetic subjects can lead to relevant quantities of sorbitol being formed, through the action of aldose reductases.

This is the cause of numerous complications of diabetes, like cataract and the slowing of nerve transmissions.

Eriksson *et al.* [22] wished to assess the effect of the transplacental accumulation of sorbitol and whether this could have a role in the genesis of malformations in diabetes. They verified the accumulation of sorbitol in diabetic rat embryos and that the accumulation could be eliminated through the administration of aldose reductase inhibitors (Statil, Imperial Chemical Industries). However this did not lead to a reduction of the malformations, nor of the embryo-fetal pathology.

At this point it is opportune to note that in our researches on diabetes in pregnancy, more than 90% of all the fetuses in the experiments had congenital cataracts.

When we studied the evolution of this severe alteration in the post-natal period we found that the lens lesions are permanent [23]. We followed the morphogenesis, and confirmed that the genesis of this alteration of the crystalline begins around the 16th day of gestation in the rat fetus.

Vacuoles form in the lens fibers and with the passage of time these swell and become hydropic, and finally degenerate and give rise to large cysts full of amorphous material, in the centre of the lens [23]. The pathogenesis is very similar to that observed in the adult lens, and suggests that there is an osmotic disturbance behind the alteration, possibly due to an accumulation of sorbitol. Strange to say, we have only found one report in the literature, an old work by Koskenoia [24], which reports two cases of congenital cataract in children of a diabetic mother. We fear that the lack of data concerning this type of pathology in humans is due to a lack of research in this field.

In vitro studies

Taken together, the results of the *in vivo* experiments have supplied a considerable quantity of data and suggestions for the identification of the mechanisms and the factors responsible for teratogenesis in diabetes, however there is a long way to go before the crucial mechanism is identified.

In recent years the development of methods for the cultivation of rodent embryos *in vitro* has allowed their cultivation during the most relevant phases of their morphogenesis. It has also supplied a valid research tool for the study of the causes of diabetic malformations which can be studied without the interfering complications of the events happening in the maternal body. The use of *in vitro* cultures also allows the examination of the several factors which are suspected to have a role in the induction of diabetic malformations, one at a time.

The first to demonstrate the dysmorphogenic properties of high concentrations of glucose were Cockcroft and Coppola [25]. They cultivated rat embryos *in vitro* in a culture medium (homologous serum) containing 1,200 to 1,500 mg% of glucose. The results were delayed development, and severe malformations. These results were confirmed by Freinkel *et al.* [26], and Reece *et al.* [27] also using cultured rat embryos (from 9.5 to 11.5 days of development postcoitum), in serum to which variable concentrations of glucose had been added (between 600 and 900 mg%). Obviously the experimental conditions are very unlike reality. Deuchar [28], in fact, did not obtain any particular dysmorphogenic effect when she cultivated rat embryos under the same conditions, in rat serum that had been made diabetic using Streptozotocin. She actually demonstrated that both controls and experimental embryos developed better in diabetic serum. Similar results have been obtained by Styrud and Eriksson [29], and in unpublished work in our own laboratory.

This experimental model does not seem therefore to be suitable for the identification of the factors in play in diabetic pregnancy. However the *in vitro* model has been

widely used to verify the role played by other factors involved in diabetic metabolic disorder. Bearing in mind that ketone bodies are a serological characteristic of diabetic individuals, Horton and Sadler [30], decided to test the effect of B-Hydroxybutyrate (B-HOB) on the development of the mouse embryo, *in vitro*. They demonstrated that this ketone body slowed the development and the closure of the neural tube, according to the dose used (8, 16 and 32 mM/l), and provokes the formation of cytoplasmic vacuoles. These latter, upon ultra structural examination, were shown to be mitochondria which had become excessively blown up, with the loss of both the matrix and the cristae.

The dysmorphogenetic effects of high concentrations of B-HOB were later confirmed by Horton *et al.* [31] in mice and by Freinkel *et al.* [32] in rat embryos. These latter authors observed that concentrations of 4 mM of B-HOB do not have any effects, and that doses of 8 mM only give rise to minor effects.

However, the culture of rat embryos in serum containing B-HOB at 8 mM and glucose at 600 mg% (minimum efficacious doses) showed that these concentrations were intensely embryopathic. This suggests that there may be a synergic effect of the two compounds [32].

Other factors that may play a role in the induction of diabetic embryopathy are the somatomedin inhibitors (SI), that are found in the serum of diabetic rats, and which reduce the activity of the somatomedin when this is analyzed in the cartilage bioassay [33].

The *in vitro* cultivation of mice embryos in the presence of a serum fraction with somatomedin inhibitory activity isolated from Stz-diabetic rats, resulted in cranio-facial and CNS malformations in embryos grown from the stage of 3-5 somites, and telencephalic and facial malformations in embryos cultivated from the 18-19 somite stage [34]. The presence of SI in the culture media leads to a reduction in the incorporation of H^3-thymidine in the embryo and a reduction of its content in RNA and protein.

At the level of the yolk sac (the external membrane of the rodent embryo which plays a fundamental role in the transport of nutrients) the presence of SI leads to the formation of ample vacuoles in the endodermic cells, suggesting an alteration of their functionality, and therefore of the nutrition of the embryo itself [35, 36]. According to Hunter *et al.* [37] the SI alter two processes of yolk sac hystiotrophic function:

1) decrease pynocitotic activity;

2) alter protein processing, ultimately resulting in a lower availability of substrates for the embryo.

The change in the functionality of the yolk sac is a recurring result in the experimental studies of diabetic embryopathy.

Zusman and Ornoy [38] and Zusman *et al.* [39] described the changes in the endoderm of the yolk sac of *in vivo* developing 9 to 13 day old embryos from diabetic rats (Stz, or genetically determined diabetes). The pathological cellular changes were first observed on day 9 and were more severe by day 11 of pregnancy. The endodermal cells appeared columnar, with a significant reduction of the number and size of the microvilli. Cells had a high number of lipid droplets and cytoplasmic inclusions. Similar results were described by Pinter *et al.* [40]. The yolk sac of rat embryos cultured in serum containing 950 mg% of glucose were characterized by a marked reduction of vitelline vessel formation, a decrease in rough endoplasmic reticulum, and an increase in lysosome-like structures in the visceral endodermal cells.

The arachidonic acid added to the culture serum (20 µg/ml) prevented embryopathies and ultrastructural abnormalities of the visceral endodermal yolk sac cells [41].

These lesions at the level of the yolk sac, have prompted the suggestion that some diabetes related embryopathies observed in animal models may be ascribed to primary damage of the yolk sac cells, and the resulting impairment of nutrient processing and transfer causing secondary changes in the embryo. This putative sequence of events has already been demonstrated with other teratogens such as trypan blue [42], or yolk sac antisera [43]. In this connection it must be stressed that pinocytosis and intralysosomal digestion of proteins by the visceral yolk sac is the principal source of amino acids for the early post implantation rodent embryo [44].

Of course, under diabetic conditions, the high blood glucose level is merely a manifestation of the low insulin level.

Travers *et al.* [45] examined the effect of hypo-insulinemia on rat embryos cultured *in vitro* in normoglycemic sera.

Sera poor in insulin (0.055 to 0.18 ng/ml) did not support normal embryonic growth and development, however these were restored when the medium was supplemented with porcine insulin.

The insulin need of the early embryo has been demonstrated by another elegant experimental approach. Guinea pig insulin is inactive on rat systems. Travers *et al.* [46] cultured post implantation rat embryos in guinea pig serum. The results were dramatic: a 92% malformation rate, and general signs of embryopathies. Supplementation of guinea pig serum with porcine insulin significantly improved rat embryo growth and development, and reduced the malformation rate to 46%.

These results support the hypothesis that early rat embryos need exogenous insulin, and that low levels of exogenous (maternal) insulin may contribute to the increased risk of developmental abnormalities. It is not

yet clear where this insulin source is located *in vivo*. It seems probable that it is of maternal origin, and that during the early stages of organogenesis there is no placental barrier to insulin.

Certainly insulin receptors are present in the embryo during the early stages of embryogenesis [47].

Conclusions

The enormous quantity of data obtained from the *in vivo* and the *in vitro* studies seems to present an impossible analytical task. However if there is no single clear road to follow there is at least a logical path.

We need to ask ourselves if the animal studies have made a contribution to the knowledge on diabetes-induced embryopathies, and if the animals used are suitable for this type of study. Even though the results obtained to date are of a scattered nature, the animal model used, *in vivo*, seems to be adequate, provided that the evaluations are done carefully and with skill. This affirmation is based on comparisons of well thought out experiments *in vivo* with the results of clinical practice:

1) the frequency of c.m. in the offspring of diabetic rats not prone to particular malformations, is around 3 to 4%, i.e. 6 to 8 times the normal rate. The same is true of diabetic women, in whom the malformation rate is around 6 times that of the normal population;

2) the most frequent malformations observed were: cardiovascular, schisis of the CNS or abdomen, and caudal regressions. The same typology has been described in babies of diabetic women;

3) the increased frequency of resorptions (dead embryos) in diabetic rats, can mimic the high rate of abortions described in diabetic women;

4) the retarded development always observed in rat fetuses from diabetic mothers, is also typical of human babies. A reduced lung maturation has also been described in fetuses of diabetic rats [48, 49];

5) an evident difference between the rat and the human, is the weight of the conceptus at the end of pregnancy. Rat pups have low weight, and human babies a very high weight.

These differences can be ascribed to two reasons: a) the different relative length of the fetal period; b) the severe lesions observed in rat placentas.

Concerning contributions to knowledge on the mechanisms, and the factors in play in the genesis of diabetic embryopathy, we can draw the following conclusions:

1) the teratogenic effects of diabetes are not due to the maternal hyperglycemia itself. This conclusion can be drawn from: a) experiments in which diabetic animals with the same blood glucose levels had very different rates of c.m. as a function of their diet; b) the incidence of malformations was very low in diabetic mice treated with doses of insulin which were inadequate to reduce their high glycemic values; c) in the majority of experiments *in vitro* carried out using the serum of diabetic animals as culture medium, the embryos did not show signs of suffering;

2) genetic predisposition, also in terms of metabolism and metabolic disorders, could play a major role in inducing malformations;

3) *in vitro* studies showed that a number of specific components of the diabetic status (ketone bodies, hyperglycemia, SI, low insulin levels) can be implicated in diabetic embryopathies, suggesting a multifactorial basis for the origin of these pathological conditions [50];

4) maternal and embryonic nutrition play major roles in causing embryopathies.

The quality and quantity of maternal diet, modulate the frequency of malformations and embryo deaths [18, 19]. The diabetic status *in vivo*, or the hyperglycemic conditions *in vitro*, alter the morphology and the physiology of the visceral yolk sac. The significance of this event for the rodent embryo is quite clear as the yolk sac is the organ providing embryo nutrition prior to the establishment of a functional chorioallantoic placenta [51]. It is more difficult to extrapolate this result to humans, as little is known about the function of the yolk sac in this species. Recently a re-evaluation of the yolk sac role in human embryos has been made using as a basis the sonographic studies of Crooij *et al.* [52]. They produced a correlation between the embryonic crown-rump length and the yolk sac diameter. However, further information is needed about the physiology of this phylogenetically important organ before making specific claims about its role in human diabetic embryopathies.

There is also experimental evidence of nutritional alteration at the embryonic level: hyperglycemic conditions alter the mitochondrial morphology of neural cells, with a consequent alteration of cellular energy production [53].

Thus the basic idea of a "fuel mediated teratogenesis" first advanced by Freinkel [20], still seems to be valid: the diet of a diabetic mother can affect the consequences of her metabolic disorder, and a worsening can have effects on the embryo nutrition through an alteration in the passage of nutrients and/or through the passage of substances affecting embryo energy production.

This seems to be a viable approach to understanding the mechanisms in play in diabetic embryopathies.

Submitted on invitation.
Accepted on 25 September 1992.

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