THE EXPRESSION OF INSULIN-LIKE GROWTH FACTORS AND THEIR RECEPTORS AT PREIMPLANTATION STAGE IN REPRODUCTIVE TISSUE OF DIABETIC MOUSE

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by

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LIST OF ABBREVIATIONS

AA	arachidonic acid
ACTH	adrenocorticotropic hormone
AGE	advanced glycation end products
Akt	protein kinase B
ALS	acid-labile subunit
ATP	adenosine triphosphate
BAD	Bcl-xL/Bcl-2-associated death protein
BSA	Bovine Serum Albumin
$CaCl_2$	calcium chloride
cDNA	complementary DNA
cds	coding region
COX-2	cyclooxygenase-2
C-peptide	connecting peptide
$cPLA_2$	cytosolic phospholipase A_2
CRK	CT10 regulator of kinase
C_T	threshold cycle
Da	Dalton
DAB	3, 3'-diaminobenzidine tetrahydrochloride
DEPC	diethylpyrocarbonate

DHA	dehydroascorbate
DNA	deoxyribonucleic acid
DPX	distyrene plasticiser xylene
ECM	extracellular matrix
EDTA	ethylenediamine-tetra acetic acid
ERα	oestrogen receptor-α
ERK	extracellular signal-related kinase
EtBr	ethidium bromide
FBP	fructose 1,6-biphosphate
FGF	fibroblast growth factor
FSH	follicle stimulating hormone
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDP	guanine diphosphate
GH	growth hormone
GLUT	glucose transporters
Grb	growth factor receptor-bound protein
GTP	guanine triphosphate
H & E	hematoxylin and eosin
H_2O_2	hydrogen peroxide
hCG	Human Chorionic Gonadotropin
HCl	hydrochloric acid
HIF-1α	hypoxia-inducible factor 1α
HPRT	hypoxanthine phosphoribosyltransferase
ICM	inner cell mass
ICR	Institute of Cancer Research

IGF-1	insulin-like growth factor-1
IGF-1R	IGF-1 receptor
IGF-2	insulin-like growth factor-2
IGF-2R	IGF-2 receptor
IGFBP	IGFs binding proteins
IGFBP-rPs	IGFBP-related proteins
IGFs	insulin-like growth factors
IgG	Immunoglobulin G
IHC	immunohistochemistry
IL-1β	interleukin-1ß
IP3	inositol triphosphate
IR	insulin receptor
IRS	insulin-receptor substrate
JNK	c-Jun N-terminal kinase
kb	kilobase
kDa	kiloDalton
LB	Luria-Bertani
LH	luteinizing hormone
M6P	mannose-6-phosphate
MAPK	mitogen-activated protein kinase
MEK	MAP/ERK kinases
MEKK	MEK kinase
$MgCl_2$	magnesium chloride
mRNA	messenger ribonucleic acid
MSA	multiplication-stimulating activity

mSOS	Son of sevenless
NaOH	sodium hydroxide
NCBI	National Center For Biotechnology Information
NIH	National Institute of Health
NOD	non-obese diabetic
NSILA	non-suppressible insulin-like activity in serum
NTC	no template control
PBS	phosphate-buffered saline
PCR	Polymerase Chain Reaction
PDFG	platelet-derived growth factor
PDK	3-phosphoinositide dependent kinases
PGE_2	prostaglandin E_2
PGF_2	prostaglandin F_2
PH	plekstrin homology
PI	phosphoinositides
PI-3K	phosphatidylinositol-3 kinase
PIP	phosphatidylinositol phosphate
PIP_2	phosphatidylinositol bisphosphate
PKB	protein kinase B
PKC	protein kinase C
PMSG	Pregnant Mare Serum Gonadotropin
PTB	phosphotyrosine binding
R^2	square regression coefficient value
RNA	ribonucleic acid
ROS	reactive oxygen species

SAPK	stress-activated protein kinase
SDS	sodium-dodecyl sulphate
SFA	sulfation factor activity
SH	Src-homology
SHC	Src homology-containing protein
SOC	sodium citrate medium
SOD	superoxide dismutase
STZ	streptozotocin
TBE	Tris-Borate-EDTA
TCA	tricarboxylic acid
TE	trophectoderm
TGF-β	transforming growth factor-β
Tm	melting temperature
TNF-α	tumour necrosis factor- α
TSH	thyrotropin
UNG	uracyl-N-glycosylase
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside

EKSPRESI "INSULIN-LIKE GROWTH FACTORS" DAN RESEPTORNYA PADA PERINGKAT PRAIMPLANTASI DALAM TISU REPRODUKTIF MENCIT DIABETES

ABSTRAK

Kami menghipotesis bahawa embrio praimplantasi yang berkembang dalam persekitaran diabetes mengalami kekurangan faktor pertumbuhan tertentu. Tujuan utama kajian ini adalah untuk menentukan kesan diabetes ke atas perkembangan embrio praimplantasi mencit secara *in vitro* dan menganalisis ekspresi mRNA dan protein IGF-1, IGF-2, IGF-1R dan IGF-2R dalam tisu fallopio dan uterus mencit kontrol dan diabetes. Mencit ICR betina yang matang secara seksual berusia 6 hingga 8 minggu dijadikan diabetes dengan suntikan streptozotosin (200 mg/kg, intra peritoneum). Embrio normal dalam peringkat 2-sel diperolehi daripada mencit kontrol dan diabetes yang diseperovulasi pada 48 jam pasca rawatan korionik gonadotropin manusia (hCG). Embrio dikultur secara berasingan dan diperiksa di bawah mikroskop songsang selama 3 hari berturut-turut. Tisu fallopio dan uterus diperolehi daripada mencit kontrol dan diabetes yang disuperovulasi pada 48, 72 dan 96 jam pasca rawatan hCG. Kuantifikasi ekspresi mRNA menggunakan tindak balas polimerase masa nyata menggunakan piawai internal homologus yang dibangunkan secara spesifik untuk setiap gen. Ekspresi protein menggunakan pewarnaan imunohistokimia dijalankan ke atas tisu dan skor semikuantitatif dibuat berdasarkan sistem 5-skala piawai. Bilangan embrio 2-sel yang diperolehi daripada mencit diabetes

adalah sangat berkurangan apabila dibanding dengan mencit kontrol. Walau bagaimanapun, tiada perbezaan yang signifikan dalam peratus perkembangan embrio 2-sel mencit kontrol dan diabetes. Ekspresi mRNA IGF-1 tisu fallopio dan uterus mencit diabetes rendah secara signifikan masing-masing pada 72 dan 96 jam pasca rawatan hCG. Ekspresi mRNA IGF-1R kekal tinggi dalam tisu fallopio tetapi rendah secara signifikan dalam tisu uterus mencit diabetes pada 96 jam pasca rawatan hCG. Ekspresi mRNA IGF-2 tisu fallopio mencit diabetes tinggi secara signifikan pada 48 dan 96 jam pasca rawatan hCG tetapi rendah secara signifikan dalam tisu uterus mencit diabetes pada 96 jam pasca rawatan hCG. Ekspresi mRNA IGF-2R tisu fallopio dan uterus mencit diabetes tinggi secara signifikan masing-masing pada 48 dan 96 jam, dan 48 jam pasca rawatan hCG. Untuk ekspresi protein, skor imunohistokimia IGF-1 dan IGF-1R berkurang secara signifikan dalam tisu fallopio pada 96 jam pasca rawatan hCG. Skor IGF-2 dan IGF-2R sebaliknya bertambah secara signifikan dalam tisu fallopio mencit diabetes masing-masing pada 48 dan 72 jam, dan pada 72 jam pasca rawatan hCG. Walau bagaimanpun, tiada perbezaan skor yang signifikan bagi IGFs dan reseptornya dalam tisu uterus mencit kontrol dan diabetes. Sebagai kesimpulan, peratus perkembangan embrio 2-sel kepada blastosista adalah serupa di dalam mencit kontrol dan diabetes tetapi tidak dapat disahkan sama ada kualiti embrio tersebut sama atau tidak. Ekspresi mRNA dan protein IGFs dan reseptor masing-masing mengalami perubahan yang signifikan akibat kesan diabetes ibu, mencadangkan wujudnya peranan mereka dalam patogenesis embriopati diabetes.

THE EXPRESSION OF INSULIN-LIKE GROWTH FACTORS AND THEIR RECEPTORS AT PREIMPLANTATION STAGE IN REPRODUCTIVE TISSUES OF DIABETIC MOUSE

ABSTRACT

We hypothesized that the alteration in the expression of IGFs and their receptors may create an abnormal intrauterine environment thus affect embryos development. Therefore, the aims of the present study were to determine the effects of diabetes on *in vitro* development of mouse preimplantation embryos and to determine the mRNA and protein expression of IGF-1, IGF-2, IGF-1R and IGF-2R in the fallopian tube and uterine tissue of control and diabetic mice. Sexually mature female ICR mice of 6-8 weeks old were made diabetic by streptozotocin (200 mg/kg, intraperitoneal). The normal two-cell embryos were obtained from superovulated control and diabetic mice at 48 post-hCG treatment. Embryos were separately cultured and examined under an inverted microscope for 3 consecutive days. Fallopian tubes and uterine tissues were obtained from the superovulated control and diabetic mice at 48, 72 and 96 hours post-hCG treatment. The mRNA expression was measured using Real-time PCR using specifically developed homologous internal standards for each gene. Protein expression was measured by immunohistochemical staining and a semiquantitative scoring was performed using a standardized 5-scale system. The number of normal two-cell embryos obtained from diabetic mice was much reduced when compared to control mice. However, there was no significant

difference in the percentage of two-cell embryo development in control and diabetic mice. The mRNA expression of IGF-1 in the fallopian tube and uterus of diabetic mice was significantly low at 72 hours and 96 hours post-hCG treatment, respectively. The mRNA expression of IGF-1R remained high in the fallopian tube but was significantly low in the uterus of diabetic mice at 96 hours post-hCG treatment. The mRNA expression IGF-2 in the fallopian tube was significantly high at 48 and 96 hours post-hCG treatment but was significantly low in the uterus of diabetic mice at 96 hours post-hCG treatment. The mRNA expression of IGF-2R in the fallopian tube and uterus of diabetic mice was significantly high at 48 and 96 hours, and at 48 hours post-hCG treatments, respectively. For protein expression, the immunohistochemical scoring for both IGF-1 and IGF-1R was significantly decreased in the fallopian tube of diabetic mice at 96 hours post-hCG treatment. In contrast, the score for IGF-2 and IGF-2R was significantly increased in the fallopian tube of diabetic mice at 48 and 72 hours; and at 72 hours post-hCG treatment, respectively. However, there was no significant difference in the score of IGFs and their receptors in the uterus of control and diabetic mice. In conclusion, the percentage of the two-cell stage embryos which developed to blastocysts was similar in control and diabetic groups but whether the quality of these embryos were the same could not be confirmed. Both the mRNA and protein expression of IGFs and their receptors were significantly altered by maternal diabetes, which suggest their role in the pathogenesis of diabetic embryopathy.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 PHYSIOLOGY OF PREIMPLANTATION EMBRYO DEVELOPMENT

1.1.1 Proliferation and differentiation of preimplantation embryo

Fertilization of the mouse ovum by sperm occurs in the ampullary region of the fallopian tube. Subsequent development occurs as the embryo moves down the fallopian tube and into the uterus over a period of about four days in mice, as compared to five and seven days in rats and humans, respectively (Figure 1.1). Approximately 24 hours after fertilization, the embryo undergoes relatively synchronous cell division resulting in the formation of two cells or blastomers. There are no junctions established between the individual cells, which are held together by ionic attractions on the opposing plasma membranes (Chavez, 1984). The cells are constrained within a physical shell, the zona pellucida, which is a matrix of four glycoproteins (Wassarman and Mortillo, 1991). During this period, each blastomere is totipotent, retaining the capacity to form a complete fetus. Further mitotic divisions occur asynchronously at progressively shorter intervals so that from the eight-cell stage onwards, one cell cycle is approximately six hours. At the eight-cell stage, generation of two distinct lineages commences with the process of compaction when individual blastomers polarize, become epithelial-like and flatten on each other,

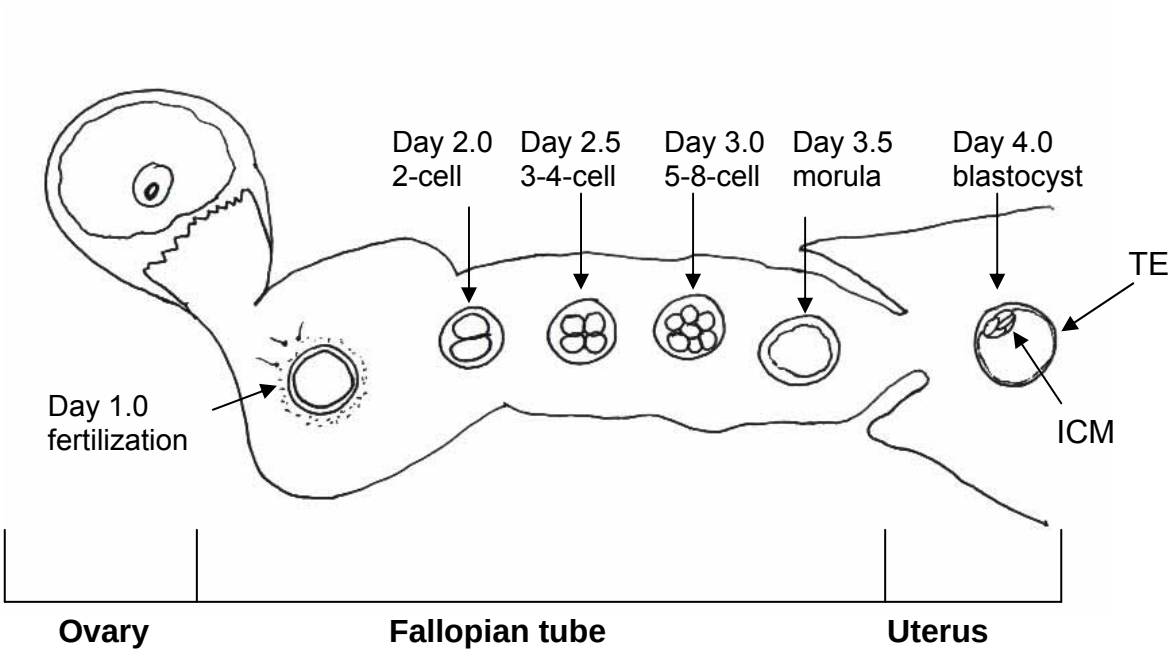

Figure 1.1 Development of the preimplantation embryo in mice from embryonic Day 1 through Day 4

(Adapted from Hogan *et al*., 1986)

The fertilization takes place in the ampullary region of the fallopian tube and the developing embryo traverse down to the site of implantation in the uterine cavity.

maximising cell contact and forming tight and gap junctions (Ducibella *et al*., 1977; Magnuson and Epstein, 1981; Chavez, 1984; Fleming *et al.,* 1992). This compacted morula stage coincides with the arrival of the embryo at the utero-tubal junction. In the compacted morula, fluid is transported across the newly formed epithelium to form a blastocoel and at this point the embryo is referred to as a blastocyst. At this stage, two distinct cell populations can be recognized (Johnson, 1981). The eccentrically placed inner cell mass (ICM) eventually forms the embryo proper and some extraembryonic tissues. The trophectoderm (TE), which is a single epithelial layer of flattened cells surrounding the blastocoel and ICM, establishes the foci of adhesion with the uterine epithelium and gives rise to the fetal component of the placenta (Hogan *et al*., 1986).

1.1.2 Metabolic activity of preimplantation embryo

Mouse oocyte and zygote have an absolute requirement for pyruvate (Biggers *et al*., 1967); i.e. glucose cannot support early embryo development until the eight-cell stage (Biggers, 1971). From the two-cell to the blastocyst stage, the embryos experience an increase in the tricarboxylic acid (TCA) cycle metabolites and a dramatic increase in fructose 1, 6-biphosphate (FBP). The dramatic switch from a dependence on the TCA to a metabolism based on glycolysis occurs at the time of compaction. The only source of adenosine triphosphate (ATP) for the preimplantation embryo would be conversion of glucose to pyruvate and lactate via glycolysis.

The blastocyst stage marks a new peak in cellular proliferation and growth. These changes create new biosynthetic demands on the embryos.

Maintenance of a high rate of glycolysis is important for providing a “dynamic buffer” of metabolic intermediates for the biosynthesis of macromolecules (Newsholme and Newsholme, 1989) and increasing amount of glucose are converted to lactate at this stage in humans and rodents (Leese and Barton, 1984). Interspecies variations in the rate of glycolysis have been reported, higher in human blastocysts (Leese *et al*., 1993) and lower in mouse blastocysts (Leese, 1991) compared to rat embryos.

Blastocysts are actively engaged in the uptake and metabolism of maternally derived nutrients such as glucose (Leese, 1991). The major site of uptake regulation is likely to be the system of facilitative glucose transporters (GLUT) situated at the basolateral surface of the TE in mouse blastocysts (Aghayan *et al*., 1992).

1.1.3 Influence of maternal factors on preimplantation embryo development

Although the activation of the embryonic genome provides the conceptus with a number of vital developmental signals (Kidder, 1992; Schultz and Heyner, 1992), its progression through the preimplantation period is also influenced by maternal factors present in the oviductal and uterine environment.

Biggers (1981) proposed a theoretical model summarizing the physiological processes that influence the microenvironment of the preimplantation embryo. The composition of the microenvironment is determined by several transport mechanisms: between the embryo and the

secretions in which it is bathed, between the fallopian tube and uterus and the bathing secretions and further mixing is produced as the secretions flow up and down the reproductive tract as shown in Figure 1.2.

Maternally-derived nutrients/factors can either be transudates from the maternal circulation such as glucose (Leese *et al*., 1979, Wales and Edirisinghe, 1989; Gonzalez *et al*., 1994) and insulin (Heyner *et al*., 1989; Smith *et al*., 1993), or secretions by various uterine cells into the lumen such as growth factors (Pollard, 1990; Song *et al.*, 2000) and cytokines (Pampfer *et al*., 1991; Robertson *et al*., 2001) during the preimplantation period.

1.2 DIABETIC PREGNANCY

The association between maternal diabetes and the increased risk of congenital malformations has a long history and was first reported by LeCorche (1885). It is generally accepted that congenital malformations are the leading cause of death in the offspring of diabetic women (Kitzmiller *et al*., 1978). The incidence of congenital malformations is approximately 6 to 9% in diabetic pregnancies, which is three- to four-fold higher than in the general population, and accounts for 33 to 66% of perinatal deaths (Reece and Hobbins, 1986). The congenital malformations most commonly associated with maternal diabetes are listed in Table 1.1 (Reece and Hobbins, 1986). However, none of the reported congenital malformations is pathognomonic for the diagnosis of diabetic embryopathy.

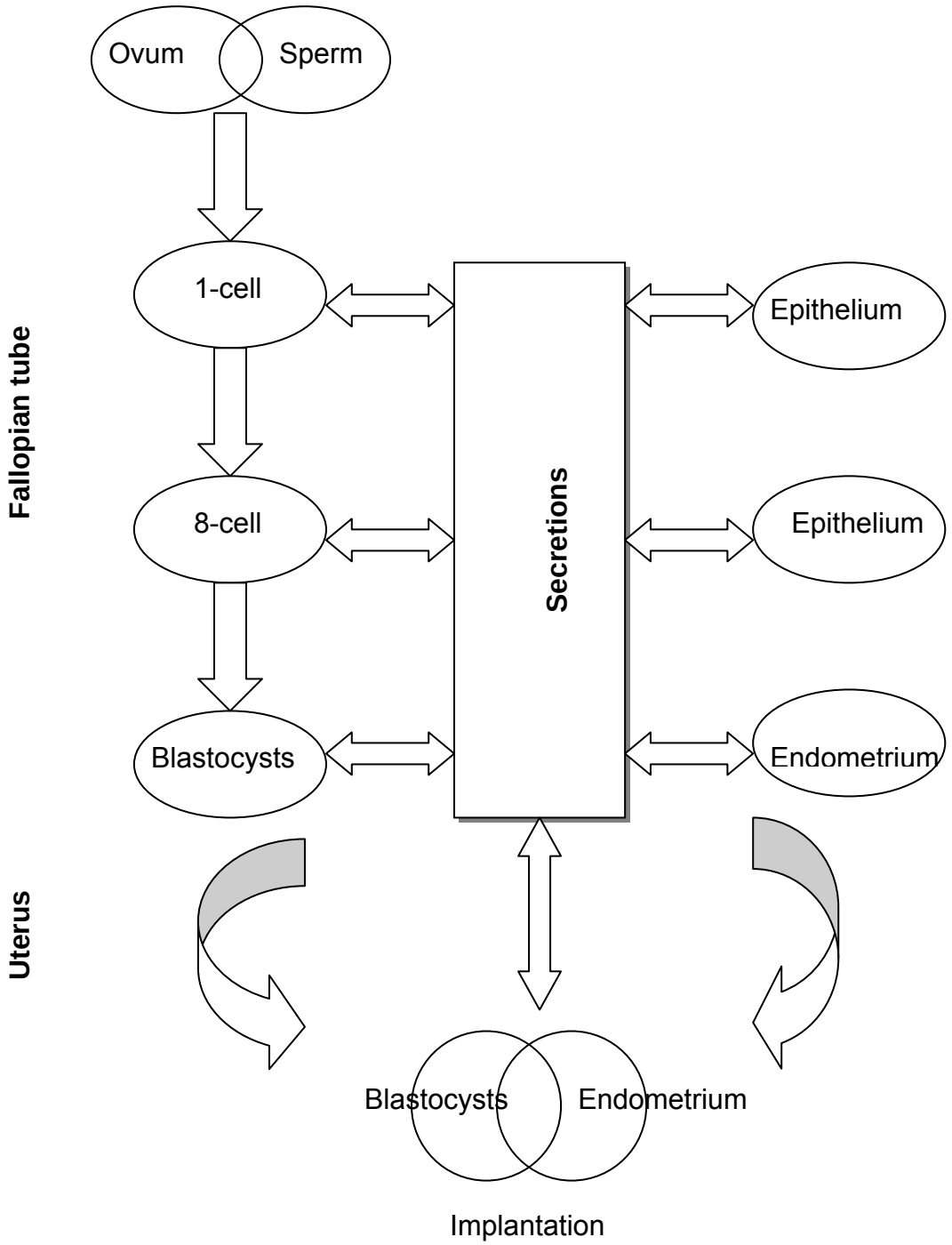

Figure 1.2 A theoretical model summarising the physiological processes that influence the microenvironment of the preimplantation embryo (Adapted from Biggers, 1981).

1.3 DEFINITION OF "DIABETIC EMBRYOPATHY"

The term "embryopathia diabetica" was coined by Mayer (1952) and was later replaced by the term "diabetic embryopathy" (Passarge and Lenz, 1966). The concept of diabetic embryopathy initially encompassed the long-recognized newborn features such as macrosomia and organomegaly but was later broadened to include congenital malformations (Mayer and Camara, 1964). Presently, the same concept was separated into two different entities, diabetic embryopathy and diabetic fetopathy. Diabetic embryopathy occurs during embryogenesis, mainly from the end of blastogenesis until the period of organogenesis (between the 3rd and 7th week of gestation) and is associated with congenital malformations (Kousseff, 1999). In contrast, diabetic fetopathy occurs during fetal development, after the 10th week of gestation, and is not associated with malformations (Kousseff, 1999). Occasionally, diabetic embryopathy is associated with diabetic fetopathy. However, these two entities, both induced by maternal diabetes mellitus, have different windows of vulnerability and perhaps, different pathogenesis.

1.4 AETIOLOGICAL FACTORS ASSOCIATED WITH DIABETIC EMBRYOPATHY

It has been suggested that the absence of a specific malformation pattern for diabetic embryopathy signals the presence of several aetiological factors and mechanisms in diabetic pregnancy (Khoury *et al*., 1989). Likewise, the number of different teratogenic agents identified indicates that diabetic embryopathy is of complex aetiology (Sadler *et al*., 1989; Zusman *et al*., 1989; Buchanan *et al*., 1994).

1.4.1 Maternal Hyperglycemia

Hyperglycemia-induced teratogenic effects have been demonstrated in animal studies both *in vivo* and *in vitro* (Cockroft and Coppola, 1977; Baker *et al*., 1981; Horton and Sadler, 1983; Kalter and Warkany, 1983a & 1983b; Freinkel *et al*., 1986; Reece and Hobbins, 1986). The percentage of congenital malformations correlated with blood glucose levels (Reece *et al*., 1985) and glycosylated hemoglobin levels (Rose *et al*., 1988).

The period and time of exposure to hyperglycemia as well as the level of hyperglycemia are all important for dysmorphogenesis to occur. The critical period of exposure to hyperglycemia is during organogenesis, which is considered to be between days 9.5 to 11.5 in rats and 8.0 to 9.6 in mice (Freinkel, 1988), corresponding to the first 5 or 6 weeks of human pregnancy. A minimum exposure time of two or more hours is needed to induce these malformations. A 20% malformation rate was induced at glucose levels that were approximately two-fold above normal concentrations; an almost 50% malformation rate was seen at glucose levels three-fold above normal concentrations; and approximately 100% rate at six times above normal concentrations (Reece *et al*., 1985).

Maternal hyperglycemia adversely affects not only the postimplantation embryos but also preimplantation progression from one-cell to the blastocyst stage in a streptozotocin (STZ)-induced or a non-obese diabetic (NOD) mouse model (Diamond *et al*., 1989; Moley *et al*., 1991 & 1994). In the NOD model at 96 hours after superovulation and mating, only 20% of the recovered embryos

reached blastocyst stage in the diabetic compared to 90% among the non-diabetic. This developmental delay is reversible by treating the mothers with insulin before superovulation and mating and during the first 96 hours of gestation. This early preimplantation delay may be manifested later in gestation as fetal loss, early growth delay or congenital malformation. There is now convincing evidence that severe developmental anomalies leading to fetal resorption or malformation can occur as a consequence of subtle damage inflicted to the embryos before or at the time of implantation (Rutledge, 1997).

The mechanism of hyperglycemia-induced congenital anomalies remains unclear. Hyperglycemia-induced reduction in GLUT has been proposed to be one of the possible mechanisms. A paradoxical reaction to hyperglycemia has been demonstrated in preimplantation embryos (Moley, 1999). In embryos of diabetic mice, a pronounced intracellular hypoglycemia was found despite maternal hyperglycemia (Moley *et al*., 1998b). The decreased intracellular glucose concentration was associated with decreased GLUT namely, GLUT-1, GLUT-2 and GLUT-3 isoforms; both at the protein and mRNA levels (Moley *et al*., 1998b).

Reduced availability of glucose associated with decreased GLUT in diabetic embryos, results in significantly lower FBP and higher pyruvate, indicating decreased glycolysis and increased pyruvate uptake by the embryos, respectively. The glycolytic changes lead to dysfunction of the outer mitochondrial membrane and subsequently trigger the apoptotic cascade (Chi *et al*., 2002).

The decrease in GLUT especially GLUT-1 and -3 isoforms also explains the elevated extracellular dehydroascorbate (DHA) and reduced intracellular ascorbic acid (Rumsey *et al*., 1997) related to hyperglycemia as described previously (Ely, 1981). Ely (1981) proposed that reduced intracellular ascorbic acid resulted in decreased hexose monophosphate shunt activity (DeChatelet *et al*., 1972), which might suppress deoxyribonucleic acid (DNA) synthesis as shown in Figure 1.3. Reduced DNA synthesis may slow cell division, leading to impaired cell proliferation or anomalies. It has also been suggested that elevated extracellular DHA may inhibit mitosis or cell proliferation (Edgar, 1970).

This decrease in intracellular glucose concentration leads to a lower cell number in the ICM, either by increased apoptotic rate (Pampfer *et al*., 1997b; Moley *et al*., 1998a), or by diminished proliferation of these cells (Pampfer *et al*., 1990). Decrease in glucose transport and metabolism is not only related to progressive decrease in embryo viability but served as important regulatory points in the early apoptotic cascade (Johnson *et al*., 1996; Li *et al*., 1998; Shim *et al*., 1998; Bialik *et al*., 1999, Lin *et al*., 2000). Maternal hyperglycemia, moreover, can cause direct disruption of the highly regulated gene program that controls the expression pattern of crucial developmental determinants during early embryogenesis, including apoptosis (Phelan *et al*., 1997; Cai *et al*., 1998; Moley *et al*., 1998a; Pampfer *et al.,* 2001).

Three cell death paradigms that are linked to decreased GLUT include, (i) induction of ATP depletion and stimulation of the mitochondrial death cascade or (ii) induction of oxidative stress and triggering of Bax-associated

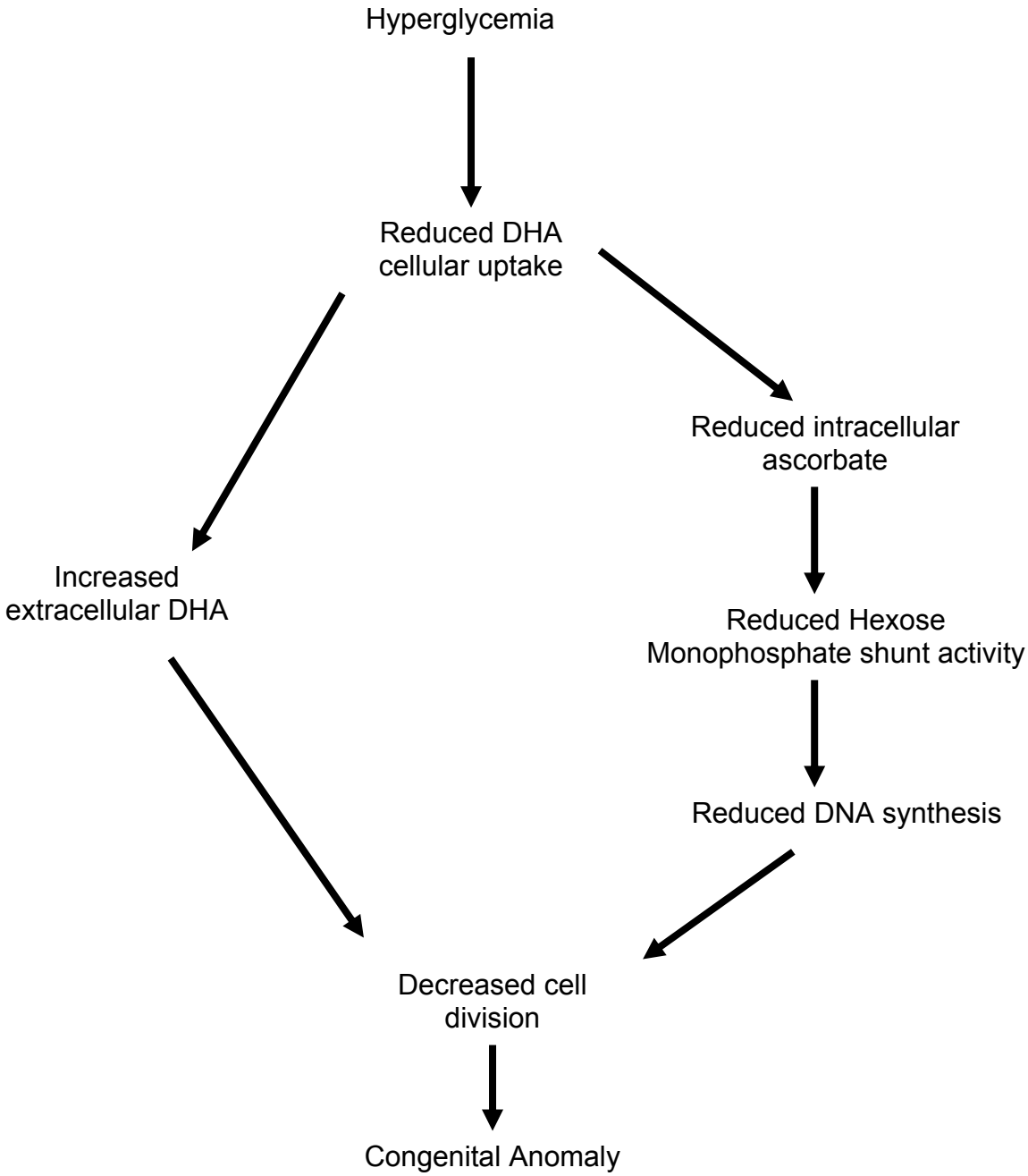

Figure 1.3 Mechanism of hyperglycemia-induced alterations in cell division and congenital anomalies

(Adapted from Ely, 1981)

events including the c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) signalling pathways or (iii) regulation of expression of the gene encoding hypoxia-inducible factor 1α (HIF-1α) and the stabilization of p53 by HIF-1α binding, leading to an increase in p53-associated apoptosis and, in turn, increased expression of Bax, and thus exaggerated apoptosis within blastocysts during glucose deprivation (Chi *et al.,* 2000; Moley and Mueckler, 2000; Keim *et al.,* 2001).

The outcome of apoptosis during preimplantation stage will depend on the percentage of cell death, if more than 60% of ICM undergo cell death, the pregnancy may result in fetal loss and resorption. However, the death of fewer cells (e.g. 40-45%) can result in either fetal resorption or malformation if this cell death involves key progenitor cells in development (Tam, 1988; Moley, 2001).

Another possible mechanism of hyperglycemia-induced congenital anomalies that has been put forward is related to dysregulation in the uterine cytokine secretion (Pampfer, 2001). Diabetes-induced modifications in the oviductal and uterine concentrations of nutrients (such as increased glucose levels), hormones (such as decreased insulin levels), growth factors, and cytokines (increased local synthesis of inhibitory factors or decreased local synthesis of stimulatory factors) are likely to elicit alterations in embryo development before implantation and organogenesis.

Studies in STZ- and alloxan-treated diabetic mice exhibit an increased amount of tumour necrosis factor-α (TNF-α) messenger ribonucleic acid

(mRNA) and protein in the uterus and placenta of diabetic mice (Pampfer *et al*., 1995; Flein *et al*., 2001) as well as a marked reduction in pregnancy rate and a high incidence of litters with severely malformed fetuses (Torchinsky *et al*., 1997, Machado *et al*., 2001). In addition, overexpression and excessive secretion of TNF-α by uterine cells in diabetic pregnancy may induce a decrease in cell number of the ICM as reported in an earlier study (Pampfer *et al*., 1997b; Wuu *et al.,* 1999).

TNF-α acts in a cell type- and stimulus-dependent manner, to generate apoptotic-signalling pathways (Baud and Karin, 2001; Gupta, 2001). The apoptotic action of TNF-α could occur mainly through its binding to the type 1 receptor TNF-α, followed by the activation of caspase 8 (Slee *et al*., 1999; Mohr *et al*., 2002; Torchinsky *et al*., 2003), which is considered to be among the main mediators of apoptosis (Baud and Karin, 2001; Gupta, 2001). The apoptotic action of TNF-α could be mediated through interleukin-1ß (IL-1ß) secreted by macrophages (Pampfer *et al*., 1999) localized at the subepithelial region of the uterine stroma (Takacs *et al*., 1988).

The mechanism of hyperglycemia-induced IL-1ß secretion has been proposed to be via the formation and interaction of advanced glycation end products, AGE (Vlassara *et al*., 1988). Macrophages have a receptor that recognizes the AGE moiety and mediates the uptake and degradation of AGE proteins. This removal process is associated with the production and secretion of TNF-α and IL-1. The localized release and action of these cytokines may play