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THE CURRENT FRONTIERS OF IN VITRO FERTILIZATION

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INTRODUCTION

In the early 1980s when *in vitro* fertilization (IVF) became a clinical reality it was considered therapy for diseased fallopian tubes. However, its effectiveness soon made it applicable to other causes of infertility, such as endometriosis unresponsive to other therapy, oligospermia with at least a million sperm identified in the ejaculate, and in other possible indications such as infertility of unidentified etiology, and infertility thought to be due to immunological factors.

Improvements in both clinical and laboratory technology at the turn of the millennium made IVF the treatment of choice for all forms of tubal disease (except perhaps iatrogenic sterilization), for endometriosis if infertility was the principal complaint, and for oligospermia regardless of the sperm count, and even for cases of azoospermia in which sperm could be obtained directly from the testis and intracytoplasmic sperm injection (ICSI) used for a single sperm to cause fertilization and pregnancy. It should be said up front, that it appears as if the majority of cases of oligozoospermia are due to genetic causes with the gene primarily carried on the Y chromosome. Therefore, with the use of ICSI, there is a greater transmission of genetic disorders to the next generation since the Y sperm fertilizes the egg. In spite of this, few patients reject this therapy. Occasionally, IVF therapy is used in infertility of undetermined origin and in less frequent conditions, such as the female whose mucous destroys sperm before they can ascend into the uterus.

While the above are the best possible therapeutic options, in current practice, many patients do not receive contemporary therapy. There are numerous reasons for this, but primary among them is that when IVF came into use, the health insurance industry declined coverage on the basis that it was "experimental therapy".

Although IVF is the best possible therapy for several causes of infertility, the insurers continue to deny coverage, resulting in the application of obsolescent therapy for countless patients. For example, diseased fallopian tubes which prevent pregnancy are often surgically repaired because it is covered by insurance. There is reason to believe that contemporary therapy, i.e. IVF, used when medically indicated would be less costly and less risky than the obsolescent therapy supported by the insurance carriers. While some states now have mandated insurance coverage, this is suboptimal because of the restrictions and fixed prices which are often built into the legislation. On a population basis, the United States is now far behind other countries in utilizing IVF. In a study by Collins,¹ it was shown that many other nations are far more frequent users of IVF than the US (Figure 1).

EXPECTATION OF PREGNANCY

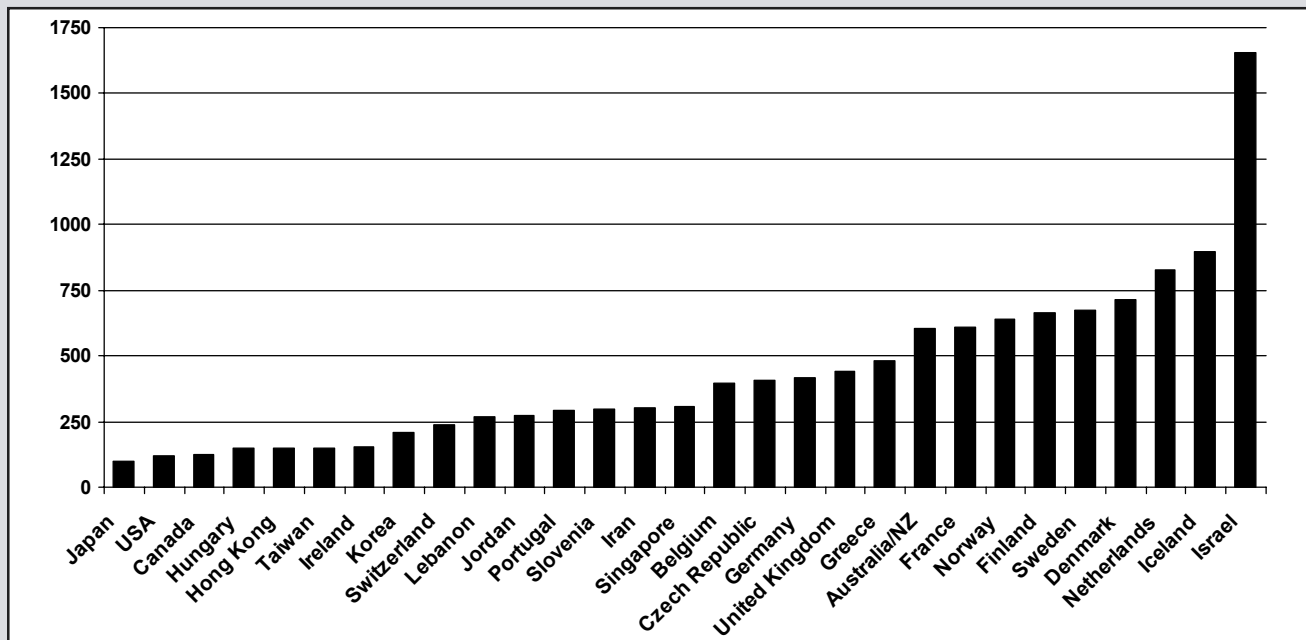
The 1998 official IVF Registry Report published in January 2002² showed that in the US there were 58,937 cycles involving IVF with a delivery rate per retrieval of 29.1% or 17,150 deliveries. There were 5,273 fresh donor oocyte cycles with a delivery rate for transfer of 41.2% (2,179 deliveries) and 11,228 frozen embryo transfer procedures with a delivery rate per transfer of 19.3% (2,167 deliveries). These percentages are as expected, as fresh donor procedures unequivocally are more successful than frozen embryo procedures. The Registry data are more than three years out-of-date and

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Figure 1

IVF/ICSI Cycles per Million Population



Adapted from Collins J. Cost-effectiveness of in vitro fertilization. *Seminars in Reprod Med* 2001;279-289.

for a variety of reasons can indeed be misleading to the unwary reader as different assisted reproductive technology (ART) programs have different performance guidelines and different methods of pooling the data.

It has long been known that fecundity, i.e. the probability of pregnancy per month of exposure, declines with the age of the female partner. This age factor cannot be overcome by the use of IVF; thus, therapeutic results reported in the ASRM/SART Registry² show a marked age related effect (Table 1). The therapeutic significance is that patients must be further educated about the eroding effect of age on the reproductive process and pregnancy should be undertaken as early as possible.

Multiple pregnancies have been a troublesome problem with IVF. Since the initiation of IVF and of ovulation induction (which also started around 1980) the multiple pregnancy rate in the US as reported by the Bureau of Vital Statistics (Figure 2) has increased each year through 2000, the last date for which data are available. Although the triplet and higher rate decreased slightly in 1999 and 2000, the increase in the rate for twins more than made up for this decrease so that the overall multiple pregnancy rate has increased each year. Examination of the 1998 ASRM/SART Registry reveals that of all deliveries 61.8% were single births, 31.7% of the deliveries were twins, 6.2% were triplets, and 0.3% were quadruplets or more. This is unacceptable and is caused by pressure from both patients and programs alike. They wish to have a high pregnancy rate which

can be accomplished with multiple transfers, but at the expense of multiple pregnancies which are undesirable. The goal should be to have a reasonable pregnancy rate with no more than 1% triplets.

Taking all these considerations into account, in 2002 a female who is a good responder, i.e. one who produces at least 5-6 mature oocytes to the required gonadic stimulation, is not over 38 years old, has both ovaries, and has a sperm producing partner, should expect to have a pregnancy 50% of the time with fresh transfer with a risk of less than 1% of having triplets and less than 4% of having twins.

CRYOPRESERVATION

No program in IVF can be considered “full service” unless it offers cryopreservation which can hold frozen excess preembryos for future use. Indeed, in expressing the pregnancy rate for a particular IVF program, a misleading figure is given, unless the pregnancy potential from the frozen material is included. We have published³ a theoretical model in which a true expression of pregnancy rate resulting from stimulated cycles can be calculated. The interested reader is referred to this publication for full details. Briefly, it is quite impossible to properly evaluate the pregnancy outcome of a particular stimulation cycle unless supplementary pregnancies, if any, from cryopreservation are considered as part of the pregnancy rate of that particular stimulation cycle. This can be done by adding

Table 1

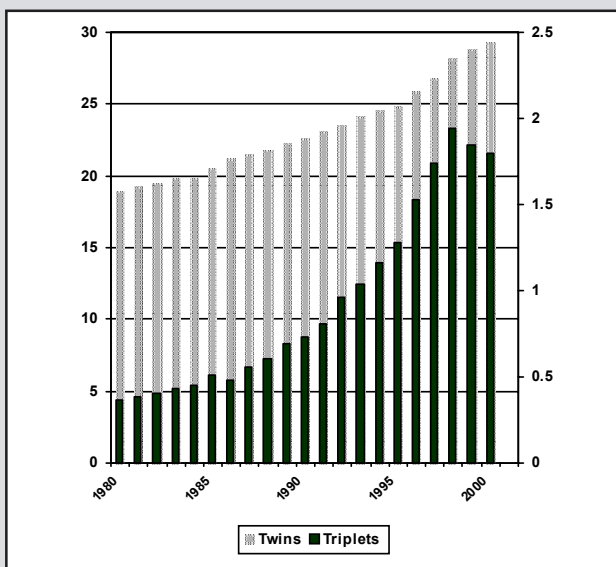
IVF procedures (with and without ICSI) by age group and cause of infertility.

1998 IVF procedures	No. of retrievals	Canceled cycles (%)	Transfers Per retrieval (%)	No. of pregnancies	No. of deliveries	Deliveries Per retrieval (%)	Multiple Births per Delivery (%)
No male factor infertility							
Women <35 years of age	16,648	10.0	93.4	6,878	5,948	35.7	43.4
Women 35-37 years of age	8,524	14.7	94.2	3,109	2,543	29.8	37.9
Women 38-40 years of age	7,063	19.5	92.7	2,006	1,498	21.2	29.0
Women >40 years of age	4,348	24.6	89.9	721	446	10.3	20.2
Male factor infertility							
Women <35 years of age	7,546	7.7	94.7	3,042	2,647	35.1	40.3
Women 35-37 years of age	3,147	11.6	94.8	1,206	1,000	31.8	35.5
Women 38-40 years of age	2,366	14.6	92.9	750	563	23.8	31.8
Women >40 years of age	1,129	19.1	91.9	231	144	12.8	13.9
1998 totals	50,771	13.9	93.6	17,943	14,789	29.1	38.2
1997 totals	44,170	14.0	93.4	15,047	12,302	27.9	39.0

SART/ASRM. ASRM/SART registry: 1998 results. Fertil Steril 2002.

Figure 2

Multiple Pregnancy Rate with IVF and Ovulation Induction



The rating of twins and triplets and more from the Bureau of Vital Statistics, U.S. Public Health Service.

all cryopregnancies to fresh pregnancies, or can be patient specific (i.e., considering cryopreservation as augmentation only among patients without a pregnancy from pre-embryos transferred fresh, or from previously transferred frozen material from the same harvest). For the patient-specific concept, cryopregnancies occurring among patients with a previous fresh or frozen pregnancy from the same harvest would be considered additive to the multiple pregnancy rate, i.e. twins, etc., but would be considered as 'delayed' multiple

pregnancies. Published results have not reflected the real purpose of cryopreservation; this is shown by the methods of presentation of cryopreservation in the publications of collecting agencies, such as the US Society for Assisted Reproductive Technology, the Great Britain Human Fertilization and Embryology Authority, the Australia-New Zealand Agency, and others. In general these publications report cryopreservation results as unrelated to a particular oocyte harvest or treat a cryopreservation as an additional transfer from the same cohort of prezygotes/pre-embryos, thus diluting the fresh pregnancy rate, as cryoresults are often not as good as fresh results.³

Generally speaking, expectation of a pregnancy from cryopreserved material is not as great as from fresh. Although the data are not exactly comparable, the ASRM/SART Registry for 1998 gave an overall pregnancy rate per transfer for fresh oocytes in IVF of 37.8% and 24.3% for cryopreserved material. With careful selection of fertilized eggs prior to cryopreservation, the pregnancy expectation from cryopreserved material approaches that of fresh material.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD has been available since about 1990.⁴ By this technique, one or two blastomeres are removed from the preembryos of the 6-10 cell stage and examined for single gene defects by the polymer chain reaction (PCR) or by fluorescent in situ hybridization (FISH) for gross chromosomal defects. Preembryos with defects are discarded and those found to be normal are transferred or frozen for future transfer.

Table 2
PGD referrals (n) according to indication

Chromosomal	647
X-linked	294
Autosomal recessive	290
Autosomal dominant	254
Mitochondrial	6
Two indications	9
Y-chromosome deletion	2
Social sexing	30
Unknown	29

ESHRE PGD Consortium Steering Committee (May 2001) Hum Reprod 17:235, 2002.

Diagnostic ability with PGD is precisely that of amniocentesis which is done at 15-18 weeks of pregnancy or chorionic villus sampling which is done at 10-14 weeks of pregnancy. PGD appeals to those who cannot morally terminate an affected fetus but who do not feel morally bound to implanting an in vitro affected preembryo. It also appeals to those who are prepared to undergo the requirements and expense of PGD and IVF simply to avoid the possibility of an elected termination, even though they may have no moral conflict in aborting an affected fetus.

The opportunity to use PGD is not offered by all centers, but its use is gradually increasing. According to data collected by the ESHRE,⁵ in 2001 there were 1,561 PGD procedures reported. The most common cause for referral was concern about chromosomal abnormalities. Specific gene disorders accounted for slightly over one-third of the cases (Table 2). Cystic fibrosis was the most common monogenic disorder.

PGD is not without an occasional error, and its efficiency in relation to fertility factors is somewhat less than IVF because of the limited number of preembryos that can be selected for transfer resulting from the screening out of affected fertilized eggs.

DONOR GAMETES

Donor *sperm* have long been used when infertility was due to sperm deficiencies. Currently, the use of donor *sperm* and *oocytes* can be considered standard practice for those who are prepared to accept nonfamilial genetic material. In some circumstances, donor gametes are used to replace gametes which are likely to or are known to harbor a mutant disease-causing gene. This is particularly valuable when the affected gene is not amenable to preimplantation genetic diagnosis.

When donor *sperm* are used either with or without IVF, the donors are vigorously screened. Requirements differ from center to center. At the Jones Institute the donors

must be 18 to 39 years of age, have a semen volume of 2 mL with a sperm count of at least 60 million, with sperm motility greater than 60%, and at least 7% of the sperm must be of normal form by strict criteria. There can be no excess of WBCs. More than 50% of the sperm must survive the cryo-survival test. The family history of the donor must be free of genetic disease. A physical examination must reveal no urethral discharge or genital warts or ulcers. Laboratory screening includes a serological test for syphilis, cytomegalovirus, hepatitis B and C, HIV-1 and HIV-2, and T-cell lymphotropic virus I and II. Serum tests must be negative for herpes, chlamydia and gonorrhea, and donors must pass a urine test for drug screening. In addition, donors must be free of cystic fibrosis and, if Jewish, tested for Hexosaminidase-A which causes Tay-Sachs disease. Black donors must be free of the sickle-cell trait. Potential Asian or Mediterranean donors with a positive hemoglobin electrophoresis for thalassemia are eliminated.

Semen quarantine is usually carried out for 6 months at which time the donor is checked for HIV and other possible potential problems before semen is released for use. All this is in accordance with the recommendations of the American Society for Reproductive Medicine (ASRM). Clinical pregnancy rates with donor *sperm*, with or without IVF, are consistent with a normal fecundity rate if there is no impediment to pregnancy on the part of the female.

When donor *eggs* are supplied, the donor has a similar historical review for genetic problems, as well as laboratory studies. However, it is impractical to quarantine an *egg* for six months, as the *eggs* do not freeze nearly as well as the *sperm*. Therefore *egg* quarantine is essentially never done. HIV testing in the *egg* donor is done by the antigen test rather than the antibody test, as a prompt answer can be obtained, although there is some uncertainty as to the time required for the appearance of the antigen. Clinical pregnancy rates for donor *eggs* in IVF are a cut above that obtained by IVF in general - due to the younger age of the donor. The pregnancy rate with donor *eggs* is consistent with the age of the donor and unrelated to the age of the recipient. There is great uncertainty about an upper age limit for the use of donor *eggs*.

ASRM has issued a guideline indicating that donor *eggs* should not be used in a recipient at an age above a woman's normal reproductive life. This guideline probably has been left purposely vague. The guidelines must have been violated as there are accounts of recipient mothers 60 years of age and over. Each program must adopt its own standard in regard to age limit. Some variations in the standard donor egg scenario have occurred. For example, there have been

menopausal grandmothers who were prepared to receive an anonymous donor egg for their daughter - such an egg, of course, fertilized by the daughter's husband. There are no guidelines for these offbeat situations, thus each program must handle them on an individual basis. Calling for assistance might be appropriate, such as the utilization of sociologists, and/or an ethics committee, or other outside resources to establish guidelines and share responsibility for these decisions.

Suffice it to say, when donor eggs are used, and especially if the recipient's age is 40 or above, a preconception medical evaluation is in order. Such an evaluation would look for those conditions which might cause complications during pregnancy or those which might be aggravated by pregnancy, such as obesity, hypertension, and diabetes. Only those women who are totally medically fit should be considered as recipients.

An upper age limit for a prospective father is sometimes an issue *with or without* donor sperm. This seems to arise when a prospective father is 60 or above and marries a much younger wife. One must ask, "Does the program have a responsibility in this circumstance to consider the welfare of the child; specifically, is there any reason to be concerned about how a man of 60, 70 or 80 years of age can function responsibly, mentally and physically, with teenage children?" A program probably has no responsibility here, but the issue is thought provoking.

CONCLUSION AND A FINAL WORD

Prior to IVF it was common for physicians who treated infertility patients to tell them that everything had been tried, and it was now time to consider adoption or a childless future. Basic IVF technology changed much of that, as did the addition of donor gametes for those prepared to accept alien genetic material; the physician is now able to offer an option to essentially all couples. The era of IVF also has made it possible to go beyond

the mere solution of the problem of infertility. Preimplantation genetic diagnosis now makes it possible to eliminate disease-causing mutant genes. Thus, we are beginning to diminish the number of children born with handicaps. Such children previously were thought to represent an intrinsic risk of bearing children.

If the era of IVF has written a new chapter in the treatment of infertility, are there additional chapters to be written? To be sure! The aging oocytes represent a challenge. Can they be rejuvenated? I think it will be possible. IVF is inefficient, but changing this represents a problem. With eight fertilized two-cell zygotes in the dish, experience tells us that on average only two or three of these have the potential to progress to a term fetus. We are far from perfect in identifying which ones are the two or three. Can our selection potential be improved? I think it will be possible. Cryopreservation is very efficient for *sperm* but very inefficient for the *egg* due to its size. Can cryopreservation of the egg be achieved? I think it will be possible.

These are only examples. There are several other possibilities - some of which may be considered by some in the realm of science fiction, but all aimed at improving the human condition. Reproductive medicine and its developing technology have placed us in the midst of a reproductive revolution.

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Abstracts from the Literature

Genetic Screening for Maternal Uniparental Disomy of Chromosome 7 in Prenatal and Postnatal Growth Retardation of Unknown Cause

This very enlightening paper from Finland is worth reading by all pediatric subspecialists for its wealth of information. The authors first relate that uniparental disomy (UPD) associated with growth retardation has been found in at least 9 chromosomes (2,6,7,9,14,16,17,20 & 22) and concluded that UPD thus may provide explanations for some cases of growth retardation of unknown cause. Inheritance of *both*

parental genomes is essential for normal growth and development.

In their study, these authors focused on UPD of chromosome 7 and particularly on maternal or matUPD7. The study was prompted as matUPD7 has been reported in approximately 10% of patients with Russell Silver syndrome (RSS) and in a few patients with intrauterine growth retardation (IUGR) without RSS.

Basically 2 groups of patients were studied: (1) 39 patients with unequivocal RSS and, (2) 166 patients with unexplained growth retardation but who did not have RSS. The latter group was divided into 2 subgroups: (2a) those with IUGR and postnatal growth retardation (PNGR) and, (2b) those with only PNGR. For final analysis, the RSS patients were separated into 2 subgroups also: (1a) RSS with matUPD7, and (1b) those without mat-7-UPD.

Only 6 of the 205 patients studied had matUPD7 and all had RSS. Thirty-three of the 39 in the RSS group did not have UPD. Comparison of these two groups revealed that RSS infants (with or without matUPD7) were significantly shorter at birth than infants in group 2a and 2b. The birth weights and lengths of RSS patients with or without matUPD7 were equally small. However, birth weights did not differ between groups 1a, 1b, and 2a. Notable difference of parental age at birth was observed between group 1a and the other 3 groups. MatUPD7 patients had significantly higher ($p < .05$) maternal age (38 years) and paternal age (40 years) than those in the other 3 groups.

Midparental heights were near average for all groups. Maternal obstetrical complications known to possibly restrict fetal growth (e.g. toxemia, high blood pressure, and alcohol or tobacco use) were reported in 5 (15%) of 33 of group 1b, 24 (26%) of 91 in group 2a, and only in 5 (7%) of the 75 mothers of the PNGR (group 2b).

The authors point out that matUPD7 and growth hormone deficiency (GHD) can occur together as can

GHD and other causes of IUGR and PNGR, and emphasize that other metabolic disorders do not exclude matUPD7. MatUPD7 has been reported in 3 patients with cystic fibrosis, all of which were exceedingly short. Consequently the authors advise screening for matUPD7 if abnormally short stature occurs conjointly with cystic fibrosis or other recessive disorders mapped to chromosome 7. However, because matUPD7 is rare among IUGR and PNGR patients, except in RSS, screening will be primarily helpful in this group of RSS patients.

Hannula K, et al. *Pediatrics* 2002;109:441-448.

Editor's Comment: *The long-term natural history of matUPD7 is not yet clear. Fertility and possible transmission of UPD has not been evaluated. For these reasons, and others such as responsiveness to various therapies, screening in appropriate instances is important. All RSS patients should be screened and those RSS patients with and without matUPD7 should be further evaluated to determine the molecular biological differences between the two groups. The authors discuss some possibilities in their manuscript. The entire manuscript is very enlightening and is recommended both for theoretical considerations and factual data.*

Judith G. Hall, OC, MD

Quality of Life and Self-Esteem in Children Treated for Idiopathic Short Stature

This study from Leiden University in the Netherlands dealt with changes in health-related quality of life (HRQOL) and self-esteem in children with idiopathic short stature (ISS) participating in a study on the effects of growth hormone (GH) treatment. There were 36 pre-pubertal children who were randomly assigned to a treatment or to a control group. Children, their parents and their pediatricians completed a HRQOL and a self-esteem questionnaire, 3 times in 2 years. The data indicated that children with ISS did not have lower scores at the start as compared with the normal population, except for social functioning. The pediatricians noticed an improvement in HRQOL in the children in the treatment group. Those in the treatment group did grow significantly more than those in the control group. However, the parents and the children being treated reported no change in HRQOL. Indeed, in some instances they reported being worse than before. The child's satisfaction with height was more related to HRQOL than was measured height. The authors

concluded that the assumption that growth hormone treatment improves HRQOL or self-esteem in children with short stature could not be supported by this study.

Theunissen NCM, et al. *J Pediatr* 2002;140:507-515.

First Editor's Comments: *It is widely assumed that short stature may be a handicap and that this condition may result in psychosocial problems, such as ridicule, and mascotism. Indeed, short people might be victims of discrimination and prejudice, often referred to as "heightism". For that reason, many have opted to receive GH with the intent to accelerate growth and improve the final adult height, and in that way improve their psychosocial status. The response to GH treatment in these children appears to be modest, resulting in a possible gain in final height of 5–9 cm, after many years of treatment. However, few studies have approached the concept of HRQOL as an outcome measure of this treatment. In this study, children with a height of more*

than two standard deviations below the mean for age and sex, who were not GH deficient, were found to have appropriate HRQOL and self-esteem, and did not show improvements after GH treatment. The parent's opinion about their social competence after treatment was also not changed. Of interest was the lack of agreement between the informants, who were the patients and parents, with the pediatrician's perception of the effects on quality of life after GH. The relationship between stature, growth, HRQOL and self-esteem might be determined by the expectations of the participants rather than by the improvements in growth. These children, as well as their parents, might have had unrealistic expectations and, therefore, not be satisfied with the treatment, despite improved standard deviation scores for height. Therefore, when we undertake treatment of a non-growth hormone deficient short child, we should consider aspects other than height. GH treatment should not be initiated just because the child is short. An interesting editorial accompanied this article and was written by Basil J. Zitelli in the same issue of the journal, and the reader is encouraged to review that as well. (*Journal of Pediatrics* 2002;140:493-495).

Fima Lifshitz, MD

Second Editor's Comment: Dr. Zitelli in his commentary points out with emphasis that offering

children and parents therapy for short stature raises expectations of success. Motivation to be included in GH trials frequently involved the hope of gaining height, yet if expectations were not met through therapy, poor self-esteem and parental anxiety and disappointment were acutely felt by the child. With the variability and unpredictability of results for any particular child, GH therapy becomes an intervention that may be more detrimental than the original complaint of short stature.

Investigators have added another layer of therapy to enhance growth. To delay epiphyseal fusion, gonadotropin releasing hormone agonists have been added to GH treatment regimens. This may potentially compound the iatrogenically introduced fear in the normal short child of being abnormal or affected with a disease that requires 2 medications to treat.

The last issue (GGH 2002 Vol 18:3) has an abstract and commentary regarding the use of LHRHa in advanced puberty. The conclusion of the authors was "these data suggest that advanced puberty (as differentiated from sexual precocity defined as sexual development in girls before the age of 8 years and boys below 9 years) decreases the growth potential by about 5 cm and that GnRHa therapy does not prevent this".

Robert M. Blizzard, MD

A Gene as a Major Cause of Sotos Syndrome has been Identified

Sotos syndrome is a relatively common neurologic disorder characterized by prenatal and postnatal overgrowth, advanced bone maturation, large skull with acromegalic features, and significant developmental delay. Most cases are sporadic, but autosomal dominant inheritance has been suggested in some instances and autosomal inheritance in a few rare instances. Reports of balanced translocations have pointed to several chromosomal sites as the location of a gene responsible for the syndrome. One of these has led to the identification of mutations of a nuclear hormone receptor cofactor as a major cause of this syndrome.

Kurotaki et al analyzed DNA from a patient with a de novo translocation 46,XX,t(5;8)(q35;q24.1) that had been reported previously by Imaizumi et al. From analysis of a series of overlapping clones, a contig, that covered the break point, they identified a partial sequence that corresponded to a gene originally cloned in mice, *NSD1*. They then isolated and characterized the human *NSD1* showing that it encoded a protein of 2,696 amino acids that is expressed in many tissues including fetal brain, skeletal muscle and kidney, and that the 5q35 breakpoint is located within *NSD1*.

The group next analyzed DNA from 38 patients with the clinical diagnosis of Sotos syndrome. De novo point mutations that would predict truncated gene products with loss of function were identified in four individuals. Fluorescent in situ hybridization (FISH) analysis revealed a common 2.2 Mb deletion in 18 and a smaller deletion in one of 30 patients in whom a suitable chromosomal spread was available. These deletions included the entire *NSD1* gene. In total, a loss of function mutation or a deletion of *NSD1* was found in 77% of patients implicating haploinsufficiency of *NSD1* as a cause of Sotos syndrome.

NSD1 is thought to act as a co-activator or co-repressor of nuclear hormone receptors, such as the androgen receptor, depending on the promoter context of the target gene and the cellular context. In other words, in one cell type *NSD1* may interact with a combination of regulatory factors unique to the cell type to activate a target gene, whereas it may interact with another set of factors to inhibit expression of target genes in another cell type. The mutations thus alter expression of target genes in relevant tissues.

Clinically, the authors state that the identification of a deletion or mutation of this mutated gene on

chromosome 5 will sometimes help in the diagnosis of Sotos syndrome. Investigatively, the knowledge reported in this article will eventually shed light on some of the underlying mechanisms producing human mental retardation and physical growth.

Imaizumi K, et al. *Am J Med Genet* 2002;107:58-60.
Kurotaki N, et al. *Nat Gen* 2002;30:365-366.

First Editor's Comments: *Sotos syndrome has been considered to be a relatively heterogeneous entity. The identification of the responsible gene(s) will undoubtedly lead to a better definition of the syndrome and a better understanding of the features observed. Sotos syndrome can now be added to the growing list of disorders with microdeletions in which fluorescent probes are available to identify affected individuals.*

In the last few years, identification of individuals with translocations has been instrumental in identifying the genes responsible for many genetic disorders. Sotos syndrome has been considered to be sporadic, even though there were a few reports of parent/child involvement. This discovery clearly confirms that an abnormality in only one allele leads to the syndrome.

As in other microdeletions, the size of the deletion may indicate how severely an individual is affected.

Judith G. Hall, OC, MD

Second Editor's Comment: *The results reported in this paper argue strongly that Sotos syndrome is caused by a partial loss of NSD1 function. The range of nuclear receptors whose action is affected by NSD1 is not known, nor are the target genes whose level of expression are influenced by NSD1. Given the overgrowth features of Sotos syndrome, one would conclude that the relevant genes are involved in controlling growth and maturation, probably at a very basic level. Moreover, one would expect that the mutations lead to loss of co-activation of growth inhibiting genes, loss of repression of growth promoting genes, or some combination of the two. Questions still remain regarding which cell types are involved. NSD1 is known to be expressed in the fetal brain, which presumably explains the CNS manifestations, but the cells responsible for the skeletal features are still not known.*

William A. Horton, MD

β -Cell-Specific Deletion of the IGF-I Receptor Leads to Hyperinsulinemia and Glucose Intolerance but does not Alter β -Cell Mass

Global deficiency of IGF-I receptors result in hypoplasia of pancreatic islet β -cells. In order to examine the role of the IGF-I receptor in an individual tissue, the investigators from the Joslin Clinic and elsewhere developed a mouse model in which there is "knock-out" of the IGF-I receptor on only the pancreatic islet β -cells. All other tissues continue to express the IGF-I receptor normally, and circulating IGF-I concentrations are comparable to values in controls, indicating no generalized absence of IGF-I presence or action. The investigators did so by breeding animals with conditional *Igf1r* targeting by a neomycin selection cassette for exon 3 flanked by *loxP* sites that was subsequently excised with mice expressing *cre* linked to the rat insulin promoter.

β -cell-specific IGF-I receptor "knock-out" mice (KO) survived normally *in utero* and after birth. β -cell mass, insulin, and glucagon content were normal in control and KO animals at 6 months. *In vitro*, islets from KO mice failed to release insulin in response to glucose in a normal manner and basal insulin secretion was not suppressed by IGF-I added to the incubation medium. *In vivo*, fasting glucose levels were similar, but basal insulin and C-peptide concentrations were higher in KO than in control mice. There was impaired glucose tolerance following intraperitoneal glucose. The

immediate first phase of insulin secretion was absent, and the second phase was blunted in KO animals while the insulin secretory response to L-arginine was comparable in KO and control mice. KO mice had reduced islet cell expression of the genes encoding important glucose-sensing proteins, including the GLUT-2 glucose transporter, and glucokinase which is the enzyme necessary for glucose phosphorylation. Thus, the β -cell IGF-I receptor is not necessary for β -cell growth, but it is needed for the selective β -cell insulin secretory response to glucose.

Kulkarni RN, et al. *Nature Genet* 2002;31:111-115.

Editor's Comment: *Present technology has opened the portal to the investigation of the function of cell-specific proteins. One wonders if patients with impaired glucose tolerance, paradoxically increased basal insulin values, and subnormal insulin glucose-specific insulin secretion, present a loss-of-function defect in β -cell IGF-I receptors. This article and the one on page 62 (β -cell Expression...) are related and have potential importance in the future treatment of diabetes mellitus.*

Allen Root, MD

Leptin Acts as a Growth Factor on the Chondrocytes of Skeletal Growth Centers

In order to examine the mechanism(s) by which obesity might lead to enhanced linear growth and advanced skeletal maturation relative to chronologic age, these investigators studied the effects of leptin, a 16-kDa protein product of adipocytes with anorexigenic properties, upon cartilage cell growth and function *in vitro*. They employed mandibular condyles from 6-day-old mice in organ culture for their model of endochondral ossification. Leptin-specific receptors were identified in chondrocytes in the cartilage growth plate; the molecular weight (148 kDa) of these receptors suggested that they were likely to be the intact, biologically active isoform of this class I cytokine receptor. Addition of leptin (0.5 and 1.0 $\mu\text{g}/\text{mL}$) to the organ culture stimulated chondrocyte division in a dose dependent manner, thereby increasing the width of the proliferative zone and the size of the mandibular condyle. Enhanced functional chondrocyte maturation was demonstrated by increased production of chondroitin sulfate and collagen type II after incubation with leptin. The authors also found that leptin increased expression of the IGF-I receptor in chondrocyte precursors and that immunoneutralization of IGF-I prevented the growth and functional effects of leptin, thus suggesting that leptin's actions are mediated by the IGF-I/IGF-I receptor unit. The authors concluded that leptin has direct effects upon cartilage growth and differentiated function.

Maor G, et al. *J Bone Miner Res*;17:1034-1043.

Editor's Comment: *It has been previously reported that leptin stimulates osteoblast differentiation and maturation. However, leptin levels do not correlate with bone mineral density, an index of bone strength that is more closely related to lean body mass than to body fat content or total body weight. Indeed, experimentally central administration of leptin actually reduces bone mass by an as yet unrecognized mechanism. Of concern and consideration in evaluating this study is the need to employ very high concentrations of leptin to demonstrate biological effects, levels far greater than those achieved in vivo even in the most obese subject. Furthermore, there was a biphasic effect of leptin in this system in that, when incubated with 1.5 $\mu\text{g}/\text{mL}$, most of the reported effects were attenuated. Nevertheless, the data are of interest in furthering our understanding of how obesity might mediate its effects on linear growth and cartilage maturation - particularly in the interesting patients who grow despite complete GH deficiency as after neurosurgical removal of a craniopharyngioma or those with septo-optic dysplasia.*

Root AW, Diamond FB Jr. *Pediatric Endocrinology* 2nd ed, Saunders, Philadelphia, 2002, p 65-95.

Allen W. Root, MD

Effect of Supplemental Zinc on the Growth and Serum Zinc Concentrations of Prepubertal Children: A Meta-Analysis of Randomized Controlled Trials

This study performed meta-analyses of all randomized controlled intervention trials that completed the assessment of the effects of zinc supplementation on the serum zinc concentrations and physical growth of pre-pubertal children. A total of 33 acceptable studies with appropriate data were identified by MEDLINE searches and other methods. Weighted mean effect sizes were calculated for changes in height, weight, weight-for-height, and serum zinc concentrations. The authors used random-effects models, extrapolated by meta-regression techniques.

Zinc supplementation produced highly significant, positive responses in height (+0.35 SDS) and weight (+0.39 SDS) increments. Zinc supplementation caused a large increase in the children's serum zinc concentrations (+0.82). Growth responses were greater in children with low initial weight-for-age z scores, and in those aged more than 6 months with low initial height-for-age z scores.

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The authors concluded that interventions to improve the zinc nutriture of children should be considered in populations at risk of zinc deficiency, especially and particularly in those where there are elevated rates of children who are underweight or experience stunting.

Brown KH, et al. *Am J Clin Nutr* 75:1062-1071.

Editor's Comments: *The benefits of zinc supplementation for children's growth have been debated for many years. This meta-analysis conducted by Brown et al showed that zinc supplements probably are of benefit for children in developing countries. It is not surprising that in such populations there are nutrient deficits which can be corrected by specific nutrient supplementation. Underlining the potential nutritional deficiency status of the population studied and reported, there was a higher significant aggregate zinc effect on children's growth in those who exhibited deficits of body weight for height. It might also be inferred that children who do not exhibit growth retardation or body weight-for-height deficits might not be nutrient-deficient, and may, therefore, not benefit from zinc supplementation. It should also be kept in mind that zinc deficiency is difficult to document, and that zinc supplementation, either alone or in combination with other nutrients, is*

not easily accomplished nor tolerated by children. Zinc supplements are also expensive where they might be needed the most, namely in developing countries. The foods richest in zinc are from animal sources which are also often not accessible in these countries. Children in the United States and other developed countries who ingest a wide variety of meat products are highly unlikely to be zinc deficient.

I agree with the authors who state in the last paragraph of this article "Because of the important functional consequences of zinc deficiency for children's growth and other health outcomes, interventions to improve zinc nutriture should be considered in those populations at particularly high risk of zinc deficiency. Additional research will be needed to determine whether the mean serum zinc concentration of a population is a useful predictor of response to zinc supplementation. On the other hand, the population mean serum zinc concentration does increase after supplementation, so this measure can be used to indicate whether public health interventions to promote increased zinc intakes are successful." For those interested in this topic, reviewing the original manuscript and its excellent and extensive graphic expression of data will be appreciated.

Fima Lifshitz, MD

Placental-Specific IGF-II is a Major Modulator of Placental and Fetal Growth

A substantial proportion of imprinted genes, i.e., genes expressed from only one parental chromosome, are involved in placental development and fetal growth in mammals. In the mouse for example, *Igf2* is expressed paternally in the placenta and fetus, while its receptor is expressed maternally. Imprinted genes can act directly on the fetus by influencing cellular proliferation and apoptosis; they can also affect fetal growth by influencing placental structure and physiology and the supply of maternal nutrients. Debate over the evolutionary significance of imprinting in mammals has led to the so-called genetic conflict hypothesis or theory of imprinting. It predicts that paternally expressed genes act on the placenta to promote extraction of resources from the mother to enhance fetal growth while maternally expressed genes act to restrain fetal growth to conserve maternal resources for long-term reproductive fitness of the mother. Testing this hypothesis has been difficult because the relevant genes are expressed in both placenta and fetus and their tissue-specific inactivation has not been achieved.

Recently, it has been shown that the mouse *Igf2* has four promoters, one of which, designated P0, directs paternal expression of *Igf2* in the labyrinthine trophoblasts of the placenta. Deleting this promoter

through gene targeting enabled Constância and colleagues to study the impact of paternally-directed placental IGF-II on fetal growth. The P0 knockout for *Igf2* was confirmed by in situ hybridization that revealed a marked reduction of *Igf2* expression specifically in the labyrinthine trophoblasts. Expression of *Igf2* from its other promoters was normal in mutant placentas and fetal tissues as were levels of IGF-II in the fetal circulation.

Lack of the P0 *Igf2* transcripts with paternal transmission primarily resulted in placental growth restriction, which was detected early in gestation at embryonic day 12 (E12) of the 19-day mouse gestation. The impaired growth of the mutant placentas remained relatively constant throughout the remainder of the pregnancy (weight of mutant placentas 76%, 82%, 68%, 68% of normal at E12, E14, E16, E18, respectively) suggesting that the paternally-directed, labyrinthine trophoblast-specific *Igf2* transcripts are required to sustain normal growth of the placenta.

In contrast to the early decrease in placenta size, the indirectly affected fetuses became growth restricted only toward the end of gestation. Their weight was 96% of normal at E16, but dropped to about 70% at birth. The ratio of fetal to placental weight increased as

gestation proceeded and was significantly higher for mutant compared to normal pregnancies reflecting the small placenta size.

To address the discrepancy between placental and fetal growth, the authors compared normal and mutant placentas structurally and functionally. Other than size, no obvious differences in tissue organization or cell morphology were detected. They next compared maternal-fetal transport of different radiolabelled compounds, one transferred by passive diffusion and the other by active transport. Their results showed that passive diffusion declines proportionate to the relative reduction in placental size. Active or system A transport, however, increases during mid gestation, apparently compensating for the loss of passive transfer until near the end of gestation when this compensation is insufficient to meet the needs of the fetus and fetal growth drops off. Importantly, the system A transporter has been shown to be a determinant of fetal growth.

In summary, deletion of a placental-specific imprinted transcript results in fetal growth restriction, primarily through a decrease in total nutrient transfer across the placenta. This example of a morphologically normal but small placenta affecting fetal growth supports the genetic conflict theory of imprinting, in which a placental-specific gene expressed from the paternal allele regulates the supply of nutritional resources to the fetus. On the other hand, fetal demand for nutrients is genetically regulated by the level of growth factors such as IGF-I and IGF-II. Increasing fetal size therefore requires a higher level of demand (for example, higher fetal IGF-II) as well as a higher level of supply (by increasing, for example, placental surface area). Reduced fetal size can be the outcome of reduced supply (as in the P0 mutant described here) or of reduced demand (for example *Igf1* knockout, which reduces fetal but not placental size). The mouse *Igf2* gene is remarkable in combining the

control of both the supply and the genetic demand for maternal nutrients in a single gene.

Constância M, et al. *Nature* 2002;417:945-948.

First Editor's Comment: *This work supports the genetic conflict theory of imprinting showing that placental-specific genes expressed from the paternal allele contribute substantially to the supply of nutrients a fetus receives from its mother. It also shows that the placenta can partially compensate at least for the loss of this paternal effect. It will be interesting to learn more about the nature of the compensation, which represents a potential mechanism to exploit in treating intrauterine growth retardation. It is important to acknowledge, that the relationship between mother and fetus differs substantially between mice and humans, especially with regard to size and duration.*

William A. Horton, MD

Second Editor's Comment: *As a pediatric endocrinologist who has had a special interest in IUGR for many years, I found the reading of the original article most informative. Not mentioned in the abstract or First Editorial comment was the following brief statement, "At birth, P0 mutant pups were 69% of normal birth weight. This was followed by postnatal catch-up growth which was complete by three months of age." While, as Dr. Horton stated above that mice and humans (may) differ substantially, there is a corollary between the catch up growth in these IUGR mice and the catch up growth that is seen in most IUGR human neonates (primarily those without associated dysmorphology) in the first two years of life. Subsequent studies dealing with the genetic conflict theory in humans should be very informative and intriguing.*

Robert M. Blizzard, MD

Insulin-like Growth Factor I and Leptin in Umbilical Cord Plasma and Infant Birth Size at Term

Umbilical cord blood samples were collected from 12,804 consecutive deliveries, and cord plasma samples were collected from 585 singleton infants born in Norway at term after uncomplicated pregnancies. These were analyzed for plasma leptin, IGF-I, IGFBP-1 and IGFBP-3. Data were analyzed following log transformation of IGFBP-1 and leptin values. Linear regression analysis was used to determine the contribution of maternal and infant factors to umbilical levels of these hormones. The mean age of the mothers of these infants was 28 years. Seven percent had smoked at the beginning of the pregnancy, and 36 percent were primiparous. Male

infants had a higher birth weight and length than girls, but girls had a higher ponderal index. Leptin and IGF-I levels were higher in the cord blood of female infants than in males. None of the maternal factors which were analyzed, including pre-pregnancy weights, smoking, or number of previous pregnancies were significantly associated with levels of cord leptin. IGF-I, IGFBP-3, and leptin increased proportionately with increasing birth weight. Levels of IGF-I and leptin were the strongest predictors of both birth weight and birth length, and were independent of length of gestation, maternal age, parity, pre-pregnancy weight, smoking and offspring sex.

The authors conclude that their data suggest that the sexual dimorphism in the regulation of leptin and IGF concentrations, which previously was demonstrated in later childhood, may already be established at birth. They also suggest a possible role for leptin and/or the IGF-I system in relation to birth size and to the risk of diseases such as non-insulin dependent diabetes and cardiovascular disease which have been shown to be frequent in low birth weight infants.

Vatten LJ, et al. *Pediatrics* 109:1131-1135.

Editor's Comment: *These findings have important implications for understanding the relationship between low birth weight and adult morbidity - especially*

cardiovascular disease, hypertension, and type 2 diabetes. It would appear that leptin, IGF-I, and IGFBP-1, which have been shown to be important factors in growth in utero, may be important in understanding the risk of developing these adult diseases. It would be very important to follow a cohort of children from birth through adulthood with serial measurements of IGF-I, IGFBP-3, and leptin in order to better understand how these factors change over time and how they might contribute to the development of serious adult disorders. Studies such as those by Vatten et al in Norway support the importance of conducting such difficult epidemiological studies.

William L. Clarke, MD

A Longitudinal Study of the Effects of a Gluten-Free Diet on Glycemic Control and Weight Gain in Subjects With Type 1 Diabetes and Celiac Disease

Amin et al from Oxford reported their findings of longitudinal growth characteristics and glycemic control in children with type 1 diabetes along with celiac disease (CD). Annually, from 1994 and 1998, 230 children with type 1 diabetes were screened starting in the first year after the onset for the presence of IgA and anti-endomysial antibodies (EMA). A total of 10 children were EMA positive and another one was AGA positive, which was 4.8% of the clinic population. Only one patient demonstrated symptoms typical of CD, including failure to thrive and steatorrhea; four complained of some mild abdominal discomfort. Jejunal biopsy showed classical histopathology of CD in all eleven patients. These subjects were matched for age, sex, and diabetes duration with two control diabetic children who were negative for EMA. Height, weight, and HbA_{1c} were measured at the time of diagnosis of CD and every 3 months. Antibody levels were tested every 3 months until negative, and then yearly. The ANOVA model was used to determine the influence of CD on both HbA_{1c} and BMI SDS. The data are presented as mean \pm SEM.

Mean BMI SDS in the CD group was significantly lower (-1.2 ± 0.1 vs. -0.1 ± 0.1 , $P=0.005$), as was mean weight SDS (-0.7 ± 0.3 vs. 0.5 ± 0.3 , $P=0.002$) than in those without CD. However, there was no difference between the two groups mean height or C-peptide level. Mean age of diagnosis of CD was 11.2 years (2.2-17.3). The mean duration of diabetes at diagnosis was 3.8 years (0.9-7.2). Mean HbA_{1c} was significantly lower at diagnosis in the children with CD ($8.9\% \pm 0.3\%$ vs. $9.8\% \pm 0.3\%$, $P=0.002$), but there was no difference in the mean daily insulin dose in the two groups. The difference in mean BMI SDS between the subjects and the controls was eliminated by 12 months of gluten-free diet (1.1 ± 0.13 vs. 1.0 ± 0.1 , $P=0.11$). HbA_{1c} levels were lower

than in the controls during the period of gluten-free diet (8.3 ± 0.2 vs. 10.0 ± 0.2 , $P=0.002$). Insulin requirements increased in both groups, but no difference in those requirements developed between the two groups. Using a general factorial linear model, CD was associated with lower BMI SDS and lower HbA_{1c} across time, independent of other factors such as insulin dose and regime. Also, while on a gluten-free diet, the children with CD had lower HbA_{1c} which was independent of BMI SDS or the insulin dose or regimen. The EMA antibodies tended to disappear while the patients were on the gluten-free diets.

The authors reviewed recent reports regarding the association in children between type 1 diabetes and CD. Prevalence rates range between 1.7 to 10%. However the data on whether intervention with gluten-free diet would be of benefit remain controversial. This is, in part, because there are few longitudinal follow-up data and few age and sex matched controlled studies. The authors note that their findings could have been influenced by the small sample size or the increased input by dieticians which was received by case subjects. They stress, that because the long-term complications of CD include gastrointestinal malignancy, lymphoma, infertility, and osteoporosis, the screening of children with type 1 diabetes at a young age may be cost effective and warranted.

Amin R, et al. *Diabetes Care* 25:1117-1122.

Editor's Comment: *These findings are very intriguing. Many pediatric endocrine clinics are now screening children with type 1 diabetes for EMA or tissue transglutaminase IGA to identify CD. There is controversy as to whether or not children who are*

asymptomatic with their CD will benefit from a gluten-free diet, and whether or not there is any effect of a gluten-free diet on the management of their diabetes. Amin and co-workers have demonstrated that indeed children with CD and type 1 diabetes are anthropometrically different from those children without CD, and that treatment reverses this finding. In addition, there appears to be a treatment benefit on overall glucose control. The authors noted that their data could

have been influenced by the frequent visits to the dietician by case subjects. It will be important to determine whether gluten-free diet is of benefit in all children with diabetes, and or whether similar nutritional input to all type 1 diabetic children could improve HbA_{1c} to the extent observed in this study.

William L. Clarke, MD

Risk for Abnormal Outcomes is Increased with Assisted Reproductive Technology

The advent of assisted reproductive technologies (ART) has increased the complexity of care in newborn nurseries. An increased number of premature infants and multiple births are among a variety of risks that occur with the increased frequency of ART. These risks should be shared with all perspective parents (patients).

An article by Schieve et al studied 42,463 infants who were born between 1996 and 1997, and who had been conceived utilizing ART. These infants were compared to the three million plus infants born in the United States during that period. Among singleton births conceived by ART, and born at 37 weeks or after, the risk for low birth weight was 2.6 times that in the general population. The use of ART was also associated with an increased rate of multiple births which also increases the rate of IUGR births and many other complications.

Hansen et al reported on 301 infants conceived by intracytoplasmic sperm injection and 837 infants conceived with in vitro fertilization (IVF). These were compared to naturally conceived infants from the same region. The infants conceived with ART had an increase of birth defects which was greater than double the occurrence among the naturally conceived. The abnormalities involved a broad spectrum of congenital anomalies. The etiology for the increased risk was unclear. However, advanced maternal age, the usual underlying causes of infertility, medications used to induce ovulation and maintain pregnancy, factors associated with procedures such as freezing and thawing of embryos, and delayed fertilization of the oocyte individually or collectively, contributed to this increased risk.

Strömberg et al studied the neurologic sequelae of children born after IVF. Through a population based retrospective cohort assessment, they compared the neurologic outcome of 5,680 children born after IVF against the neurological outcome of 11,360 matched controls. For each of the 2,060 twins born after IVF, a second set of twin controls was used. Children born after IVF demonstrated an odds ratio of 1.7 of needing habilitation services. Among singletons born after IVF,

the risk was 1.4. The most common neurologic disorder was cerebral palsy, with a relative risk of 3.7 for all children born after IVF and 2.8 for singletons. Data concerning twins born after IVF was essentially the same as control twins in respect to neurologic sequelae. Twins with low birth rate and prematurity were more likely to require habilitation services. Maternal age did not seem to be a factor in this study.

Multiple births have an increased risk factor for neurologic sequelae and, consequently, Ozturk et al. strongly recommend that no more than two embryos be placed in the uterus while performing IVF.

Hansen, et al. *N Engl J Med* 2002;346:725-730.

Ozturk, et al. *Lancet* 2002;359:232.

Schieve, et al. *N Engl J Med* 2002;346:731-737.

Strömberg, et al. *Lancet* 2002;359:461-465.

First Editor's Comment: Information regarding the increased risk of problems associated with ART must be shared with the families who are considering using them. Healthcare providers must also be aware of these risks. The increased expenditures associated with ART are not just the cost of the procedure, but also involve the long-term health care costs. Healthcare costs have become more expensive because of these complications, and these are not usually considered when assessing the expenditures of ART.

Judith G. Hall, OC, MD

Second Editor's Comment: A dictum of physics is only rarely violated. Specifically every positive force has a negative force and vice versa. Chances are what we take daily. There are no positive assurances about anything except death. Therefore, we should expect that every technology will not be perfect – either in construction of the technology itself, or carrying out of a procedure with the technology and in the results thereof. Thus, we should not be disturbed by some imperfections of the system, although we should continue to try to make it perfect.

Human error as well as errors of nature also complicate life, including life related to IVF. The Associated Press on July 10th released in newspapers around the world a report entitled "Test Tube Baby Mix-Up Causes Alarm: Birth of Black Babies to White Couple Raises Questions About Reliability of the Program". This

occurrence was in England. Such occurrences of error undoubtedly are very rare, but inevitably occur.

Life goes on, but not always without error. The positivities of what IVF has, does, and will accomplish, far outweigh the negativity of the errors of nature and man.

Robert M. Blizzard, MD

Hypovitaminosis D Prevalence and Determinants Among African American and White Women of Reproductive Age: Third National Health and Nutrition Examination Survey, 1988-1994

This study addressed the issue of the prevalence and the determinants of hypovitaminosis D among 1,546 African American and 1,426 white women of reproductive age (15-49). These women were not pregnant and participated in the Third National Health and Nutrition Examination Survey (1988 – 1994). Hypovitaminosis D was defined as serum 25-hydroxyvitamin D concentrations of < 37.5 nmol/L. The prevalence of hypovitaminosis D was 42.4% among African American women as compared to only 4.2% among white women. The presence of hypovitaminosis D was independently associated with low consumption of milk or cereal, less than ideal use of vitamin D supplements, cold seasons, urban residence, low body mass index, and use of oral contraceptives. Even among the 243 African Americans who consumed an adequate intake of vitamin D from supplements (>200 IU/d), 28.2% had hypovitaminosis D. The authors concluded that the high prevalence of hypovitaminosis D among African American women warrants further examination of the vitamin D recommendations for these women. The determinants of hypovitaminosis D among women should be considered when these women are advised regarding dietary intake and supplement use.

Nesby-O'Dell S, et al. *Am J Clin Nutr* 2002;76:187-192.

Editor's Comments: *The report by this group of investigators provided compelling data with irrefutable evidence that vitamin D deficiency constitutes a major unrecognized epidemic in many young black adult women and in 5% of white women of childbearing age. This survey might have shown a much higher prevalence of hypovitaminosis D if it had been performed in the winter. We may also assume that vitamin D deficiency*

might be equally prevalent among males of the same age and race, although this was not studied. This article clearly documents it is still currently possible to frequently find vitamin D deficiency in the United States, which plagued our ancestors during the 19th century. There are vulnerable populations, such as those who are not exposed to the benefits of sunlight irradiation, and in those who are dark skinned. The latter may not be able to synthesize sufficient vitamin D from the skin to prevent vitamin D deficiency, and may be in need of higher levels of vitamin D intake as compared to their white counterparts. Therefore, the recommendation to examine the dietary recommendations for young black women and men should be quickly undertaken. Since the black population has a high incidence of lactase deficiency and, therefore, not able to tolerate milk, oral vitamin D supplements may be needed.

In this study there were no measurements of parathyroid hormone levels or the active metabolic vitamin D (25-D hydroxy vitamin D), both of which are very sensitive indicators of calcium homeostasis and vitamin D deficiency. The high prevalence of hypovitaminosis D among "healthy young female adults" is important as vitamin D deficiency is associated with osteomalacia, bone pain, muscle aches, muscle weakness, and fibromyalgia. It also causes secondary hyperparathyroidism, which can precipitate and exacerbate osteoporosis by increasing mobilization of mineral and matrix from the skeleton. Therefore, there is reason for each of us to pay attention to an easily remedied medical problem that affects many of our patients whether they are adults or children.

Fima Lifshitz, MD

β-Cell Expression of IGF-I Leads to Recovery from Type 1 Diabetes

A method by which to reverse the process that leads to destruction of pancreatic islet cells and type 1 diabetes mellitus is the "Holy Grail" that all diabetologists seek.

In the present report from Barcelona, the investigators of the School of Veterinary Medicine and Gene Therapy Center succeeded in doing just that in an animal model

in which the key is selective overexpression of IGF-I in β -cells.

Transgenic mice were developed in which mouse IGF-I was linked to the rat insulin promoter and thus targeted to the β -cell, where IGF-I expression was many fold greater than in control animals. In these mice, at 6 months of age there was a 1.5 fold increase in β -cell mass but normal pancreatic insulin content. Circulating concentrations of IGF-I were comparable in control and transgenic animals. The latter did not develop hypoglycemia, hyperinsulinemia, or neoplasms and had normal life span and reproduction.

At two months of age, administration of streptozotocin (STZ) led to the development of insulinitis, hyperglycemia, hypoinsulinemia, and death at four months of age in the control groups from two strains of mice (C57BL and CD-1) utilized. In the C57BL mice which overexpressed IGF-I only in the β -cell, STZ led to transient modest hyperglycemia, impaired insulin secretion, mild but reversible insulinitis, and subsequent normal life span. In the CD-1 transgenic mice, hyperglycemia and hypoinsulinemia following STZ were extreme, but again transient with long term survival (Figure). After recovery from hyperglycemia, the growth was normal in the β -cell-targeted IGF-I transgenic animals.

Histological examination in C57BL mice revealed a mild decrease in islet β -cells and budding of insulin containing cells from pancreatic ductal epithelium. Thus, IGF-I appeared to at least partially protect β -cells from destruction while also increasing generation of new β -cell precursors. Since the β -cell IGF-I receptor is found on the β -cell membrane, the high levels of IGF-I synthesized by the β -cell specific IGF-I transgenic mice must be acting in a paracrine or autocrine manner to protect β -cells insulted by STZ.

Histological examination in the CD-1 mice revealed much less severe insulinitis in the transgenic STZ treated mice than in the control STZ treated animals. There was slow recovery from insulinitis, but with β -cell proliferation and neogenesis, blood sugar and insulin serum levels were restored to normal.

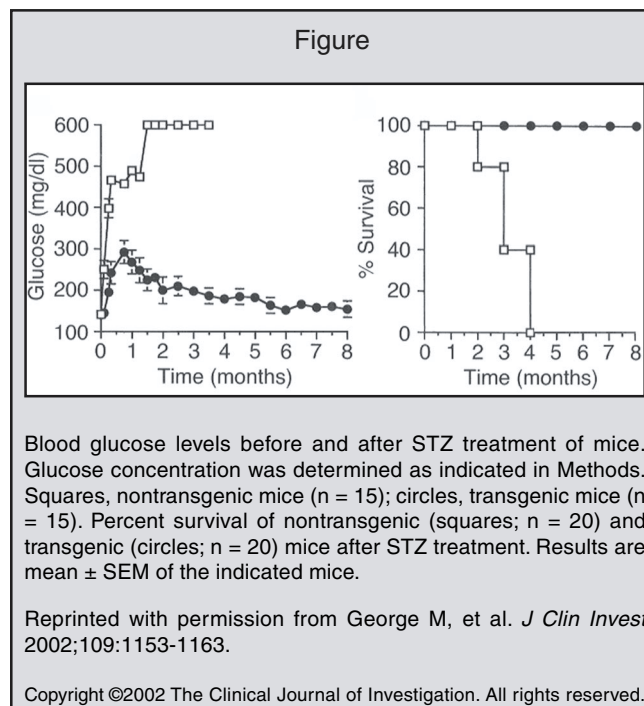
The authors concluded that co-expression of IGF-I and insulin in β -cells protected these cells from permanent destruction by STZ by increasing resistance to the inflammatory insult itself, augmenting β -cell division, and encouraging differentiation of new β -cells. They suggest that IGF-I may be a candidate gene for

transfer to pancreatic β -cells in the gene therapy of patients developing type 1 diabetes mellitus.

George M, et al. *J Clin Invest* 2002;109:1153-1163.

Editor's Comment: *This exciting paper raises the possibility that IGF-I might be capable of halting the progression of β -cell loss in patients developing type 1 diabetes mellitus if a method can be found to target this growth factor to the insulted β -cell in the intact patient. Perhaps equally feasible, and possibly even more beneficial, might be the insertion of IGF-I into the β -cells of patients at risk for development of type 1 diabetes mellitus to "protect" or to help them recover from the anticipated insults in the future that will lead to insulinitis. The latter objective may be more useful because the present experiments, which were successful, were conducted in animals that had high IGF-I pancreatic islet contact before the STZ insult. Such an approach would, hopefully, simulate the successful experiment recorded in this article.*

Allen Root, MD



Growth and Maturation in Marfan Syndrome

The Marfanoid habitus is well known to pediatric clinicians; it is characterized by tall, asthenic habitus. In Marfan Syndrome (MFS), there is multi-organ involvement including eye, heart and muscular/skeletal abnormalities. Erkula et al, largely from Johns Hopkins

data, have retrospectively compiled growth pattern data on 180 clinically diagnosed MFS patients. They have generated growth charts and growth velocity charts for infant, children and adolescent males and females. Not unexpectedly, males and females with MFS are larger

at birth, grow at a greater velocity, and end up taller than average. Interestingly, skeletal maturation is also advanced and puberty is earlier when compared to the general population.

These data are extremely important and very helpful for those caring for children with MFS to determine whether a child is outside the expected range for MFS. This and further accumulated data will be very important in respect to the management of the spinal deformities common in MFS, as well as considering either surgical or hormonal therapies to decrease ultimate height.

The study was done using retrospective measurements, primarily from familial cases where the diagnosis had been made on a clinical basis. The authors express some concern about precision of height and weight measurements since they were collected by non-auxologists and because longitudinal data early in life were very limited. Nevertheless, the data are extremely useful in defining the overall natural history of growth in MFS. The authors point out that the excessive linear growth seen in MFS begins prenatally. The growth velocity is consistently higher than that observed in the general population, although body mass does not exceed that in the general population. This combination leads to the slender habitus in MFS.

An important consideration in MFS is the development of idiopathic scoliosis. On average, it develops earlier in children with MFS than in children in the general population. Since it is a common occurrence in MFS, it needs to be screened early and treated aggressively.

The study also documented that skeletal maturation occurs earlier in MFS than in the average population. This is an important consideration when thinking about various therapeutic modalities such as the timing for

surgical epiphysiodesis or hormonal therapy to produce cessation of growth and for considering utilizing braces to treat scoliosis.

Erkula G, et al. *Am J Med Genet* 2002;109: 100-115.

Editor's Comment: *This manuscript should be prime reading for those taking care of MFS patients. Space limits the presentation of the multiple figures presented in the manuscript. These growth charts are available in the original manuscript. These types of growth data are extremely important for relatively rare genetic syndromes and can only be accumulated in centers with enormous experience. Not only is the natural history important to elucidate, but understanding how and when to apply various therapies is extremely important.*

Interestingly, the authors point out that some individuals with MFS are taller than others and, surprisingly, that some MFS patients are obese. Secondary genes or other mutations that affect height and weight are being sought. Such studies may be revealing in better understanding the variations of normal stature as well. It is the careful study of rare genetic disorders that helps to provide better therapy of diseased states and better understanding of normal development. We should be very grateful to this group, which has collected these data over many years. I cannot help but note and be dismayed that it is very difficult to find funding for this type of research and, yet, it is so extremely important. Therefore, we should be even more grateful to the authors and hope that they will be reporting similar data obtained in the studies of other rare genetic growth disorders.

Judith G. Hall, OC, MD

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