

THE HEMOPHILIA BULLETIN

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(for ASH, Dec 2005, see page)

Preimplantation Genetic Diagnosis (PGD)

Ten years ago, in Bogota, Colombia, the late Dr. Alvaro Robledo and his wife, Maritza, introduced me to a healthy baby boy, born in August, 1995, who had been selected from among the fertilized eggs of his carrier-mother to be implanted into her uterus. The couple had an older son with severe hemophilia A and an inhibitor. The Robledos, founders of the hemophilia society in Colombia, also took me to visit Dr. Elkin Lucena who has an excellent *in vitro* fertilization clinic. He explained pre-implantation genetic diagnosis to me and showed me around his clinic. The DNA studies on the older boy with hemophilia and the fertilized eggs from his mother had been conducted in the United States by Dr. Mark Hughes. A few years later, I learned that Dr. Lucena and Dr. Hughes had helped the mother's cousin, another carrier, to have a healthy baby boy by the same method.

Somehow I had expected to be hearing more about this marvelous technology in the intervening years. I got restless, so I tried to track the story down.

In May, 2005, I went to a meeting in London celebrating the two million children born thanks to *in vitro* fertilization since 1978. Dr. Robert Edwards, one of the obstetricians responsible for helping the first such child to come into existence, recalled the furor with which the early "test-tube babies" were greeted. The doctors were castigated. Families were accosted in the street and harangued about their "un-natural" choice. He recalled, with gratitude, the moral support of the chief ethicist of the Church of England and of the Prime Minister at the time, Margaret Thatcher, as well as the continuing support of the current Prime Minister, Tony Blair.

The first diagnoses of fertilized eggs, or early embryos, *in vitro*, before implantation into the uterus, took place at the end of the 1980's, about a decade after the introduction of *in vitro* fertilization, and were reported in 1990. Since that time, PGD has led to the birth of thousands of children who are not affected by the condition for which the test was done.

In vitro fertilization has been used primarily for infertility, e.g. for women with blocked Fallopian tubes. PGD is used to choose the healthiest fertilized egg to implant in the uterus. The most common use is to make sure that the chromosome count in the embryo is correct. Most women undergoing infertility treatment are in the older age group prone to aneuploidy, an incorrect number of chromo-



Bogota, Colombia: father, elder son, mother, PGD baby boy born in 1995, Dr. Kasper.

somes, in the eggs. A growing use of PGD is to help families with single-gene disorders have unaffected offspring. Cystic fibrosis and thalassemia are among the genetic disorders most often in question, but hemophilia also has been targeted. There may have been as many as 50 unaffected pregnancies in hemophilia carriers thanks to PGD.

Early PGD for sex-linked disorders sometimes stopped at just determining the gender of an embryo, with only female embryos implanted into a carrier. Half of such embryos are also carriers. That just pushes the issue onto the next generation. That type of selection is not ideal; I don't think it's appropriate. In its early years, the expense of PGD was entirely on the shoulders of the parents. Nowadays, in the USA, insurance companies are realizing that it's much cheaper to pay for PGD than to pay for the medical care of a boy with hemophilia. In countries with single-payer systems, such as Canada and the UK, the payers are wising up. A good way to reduce costs is to pay for PGD for genetic disorders requiring costly care. (Hemophilia is not at the head of that list any more, Gaucher's disease and Fabry's disease are more costly.) Some countries, notably Germany and Italy, do not allow diagnostic tests on embryos. Affected families trek to more liberal countries and/or make use of polar body biopsy (see below). PGD is largely taking the place of prenatal diagnosis with selective abortion. It's acceptable to many couples who are not willing to consider abortion and it's acceptable in countries

where abortion is illegal.

My goal is to inform carriers that the option exists and to encourage families to fight to have PGD available to them if they want it. Many adult men with hemophilia have little interest in any aspect of genetic counseling or genetic selection. They may feel that it implies that they should not exist, or would not have existed if their mothers had had choices. They are glad to be alive and often look on their hemophilia as something that molded their characters in a positive way. An occasional man with severe hemophilia has told me that he hoped his grandsons also would have hemophilia so they'd have the same character-building experience. Parents of these men have loved their sons, but recall their own heavy practical burdens and constant anxiety. Of course, treatment for hemophilia is better nowadays and usually paid by third parties, at least in developed countries, so the load on parents has lessened.

For those not acquainted with PGD, here is a synopsis. Ovulation is stimulated and the eggs aspirated from the pelvic cavity. The eggs are placed in a culture dish. At this stage, the egg is not quite mature. It still has 46 chromosomes and they replicate once more. The extras are discarded into a small bubble on the surface of the egg, called the first polar body. Sperm are added to the culture dish (or individual sperm are injected into individual eggs). When the sperm starts to penetrate the outer layer of the egg, that stimulates the egg to get rid of extra chromosomes into a second polar body so that it is left with only 23 chromosomes, ready to join with the sperm's 23 chromosomes. Analysis of the polar bodies tells which chromosomes have been discarded and thus which ones must be left behind in the egg. It's especially useful for sex-linked disorders such as hemophilia transmitted by the mother and not by the father. Polar body analysis can be used alone, for hemophilia diagnosis, if the parents' ethical scruples demand it.

After fertilization, the combined egg and sperm, the "zygote", starts to grow and divide into more cells. At about 48 hours after fertilization, when about eight cells are present, one cell can be taken for genetic analysis. Fertility specialists prefer to take this cell, in addition to the polar bodies, for confirmation, and to make sure the zygote has the right number of chromosomes.

If the woman is at a distance from the diagnostic laboratory, the polar bodies and the cell from the zygote are sent by rapid courier to the diagnostic laboratory. The analysis is completed within one or two days while the zygotes still grow in the culture dish. Then one or two healthy-looking zygotes, or embryos, diagnosed as free of the disorder of concern, are transferred into the uterus.

The diagnostic laboratory has the big responsibility of providing swift and accurate diagnosis. The obstetrician tries to achieve a high rate of pregnancy by choosing healthy-

looking embryos without the disorder. To get a high rate of pregnancy, it's tempting to implant several embryos, because they don't all stick to the endometrium and grow, but that's a temptation best avoided. Implanting more than one or, at the most, two, is frowned upon nowadays. The greatest threat to the baby's well-being is premature birth which is more common with multiple births. The ideal is to achieve a pregnancy with implantation of just one healthy embryo. Practitioners vary in their ability to choose and implant embryos successfully.

A family can access PGD either through a local obstetrician who specializes in "assisted reproduction" or at a full-service clinic with everything on-site, from ovulation-induction to diagnosis to embryo-implantation. The highest rates of success in achieving pregnancy appear to come from full-service clinics. An outstanding full-service clinic is the Reproductive Genetics Institute, 2825 North Halstead Street, Chicago, Illinois, 60657, directed by Drs. Yuri Verlinsky and Anver Kuliev. It also offers diagnostic services to local clinics that do their own ovulation induction, biopsy and implantation (www.reproductivegenetics.com). Another busy and highly reputable diagnostic center is that of Dr. Mark Hughes, Genesis Genetic Institute, 1380 East Jefferson Avenue, Detroit, Michigan 48207; www.genesisgenetics.com. I am grateful to those physicians and to Drs. Alan Handyside and Stuart Lavery of the United Kingdom, all of whom have very patiently and graciously answered my questions.

American Society of Hematology, December

It was a pleasure to visit prosperous Atlanta, with its big modern airport, its squeaky-clean subway and its spacious convention center. Congratulations to ASH on the smooth transfer from New Orleans!

Is prophylaxis justified? Introducing the topic in the plenary session, Catherine Manno briefly described the Cochrane Review of April, 2005, which said that there was "insufficient evidence to assess whether the use of prophylactic clotting factor concentrate is effective in decreasing the frequency of joint bleeds." That declaration had raised fears in the hemophilia community of insurance-company rejection of prophylactic programs. Marilyn Manco-Johnson has been collecting relevant evidence over the past decade. In her multi-center randomized study, children with severe hemophilia A, ages six to 30 months, without inhibitors, and with no more than two prior hemorrhages in any one joint, who had normal joint Xrays and MRIs, were randomized to receive either prophylactic factor VIII concentrate at a dose of 25 IU/kg every other day, or intense on-demand therapy consisting of 40 IU FVIII/kg for an acute joint bleed, followed by 20 IU/kg at 24 and at 72 hours, then every other day until resolution was complete. The outcome was measured at age 6 years, and consisted of physical examination, Xrays and MRIs, with the following results:

	Prophy-laxis	Intensive On Demand
Number of evaluable patients	32	31
Good protocol adherence	86%	95%
Mean number of joint bleeds per year on protocol	0.5	7
Mean concentrate use: IU/kg/year	6000	2300
At study exit, no joint damage on Xray or MRI	93%	58%

The most startling finding was that **in 18% of the joints with damage at the time of the final evaluation, no clinically-recognized hemorrhage had ever been observed!** Subclinical bleeding happens, and it damages joints. Another 25% of joints with damage at the final evaluation had had only one to five recognized hemorrhages. Thus, to preserve joint health, prophylaxis is justified. I wonder whether the dosage ought to be more intensive than that now used. As Dr. Manco Johnson said, ways must be found to help families comply better.

There were several interesting reports on **inhibitors**. Jan Astermark looked for correlations between polymorphisms in various interleukins and found a **strong association between inhibitor development in hemophilia A and an interleukin 10 allele** with 134 bp in one of the CA repeat microsatellites, IL-10G, in the promoter region. The 10G allele was found in 32/77 (41.6%) of inhibitor patients, in 12/87 (13.8%) of non-inhibitor hemophilia patients and in only 7.8% of the general Swedish population. This is the first genetic indicator of inhibitor susceptibility, other than the hemophilia mutation type..

The efficacy of FEIBA® and of NovoSeven® were compared. Erik Berntorp described a multi-center study using either FEIBA® (in one dose of 75-100 U/kg, as recommended in the package insert) and Novo-Seven® (in two doses, of 90-120 micrograms/kg, at intervals of 2.5-3 hours, as recommended in the package insert) in an open-label cross-over trial for joint hemorrhages in patients with hemophilia and inhibitors. They evaluated only those patients treated an equal number of times with each agent, for a total of 96 joint hemorrhages. The primary endpoint was apparent hemostasis evaluated at six hours after the initial injection. At that time, 80.9% of hemarthroses treated with one dose of FEIBA® and 78.7% of those treated with two doses of NovoSeven® had improved.

Elena Santagostino described a **randomized crossover study of two doses of NovoSeven® in the treatment of hemarthroses**. The standard dose was 90 micrograms/kg repeated as necessary every three hours, and the high dose was 270 micrograms/sults were as follows:

	Single high dose	Repeated standard dose
At 9 hours: successful	25%	31%
At 9 hours: partially successful	56%	59%
At 24 hours: successful	50%	53%
At 24 hours: partially successful	33%	34%

With repeated standard doses, the median number of doses needed for hemostasis was 3 (range two to 16) and with the high dose the median number was one, but outliers required up to 10 doses. Why was the median number of standard doses needed for hemostasis higher, at three doses, than the two doses reported in an earlier study? Dr. Santagostino believes that more target joints had been included in this series. It's notable that **the total amount of NovoSeven® used in the two protocols was identical**. It seems much easier to give the necessary dose all at the beginning than to give repeated injections. On the other hand, some individual patients, and perhaps some non-target joints, may respond to lower doses.

Peter Collins reported a prospective survey in the United Kingdom to get **an accurate incidence of acquired factor VIII inhibitors**. Between May 2001 and April 2003, all 256 U.K. hematology departments were asked to report all acquired factor VIII inhibitors, and 255 complied. Based on their reports, **an annual incidence of 1.5 cases per million population** was calculated. The incidence is strongly age-related, reaching 7 cases/million population at ages 65-89 and 10.3 cases/million population at ages of 90 and above. As populations in developed countries age, more and more acquired cases will be seen. Patients were treated, or not, according to local protocols. Remission rates were similar in 41 patients who had been given steroids only (77% remission) versus 88 patients treated with steroids and concurrent cyclophosphamide (76%), and, the relapse rates in these two groups were similar (22% and 24%, respectively) after stopping the drugs. Only nine patients were treated with cyclophosphamide alone and three achieved remission. The chance of remission was not related to age or to inhibitor level. Not surprisingly, death rates were higher in older patients but death was not always due to bleeding.

Frits Rosendaal gave the Ham-Wasserman Lecture on the **role of genes, environment and behavior on venous thrombosis**. The risk of thrombosis after long-distance travel is of particular interest to me because I've had such a thrombosis and now take low-molecular-weight heparin prophylactically before long flights, al-

though there's no study confirming its efficacy. Dr. Rosendaal said that there is no difference in risk between air and surface (train or car) travel, but air travel may cause mild hypoxia, postulated to add to the risk. (I suspect that dehydration is also commonplace.) Women on oral contraceptives have a four-fold risk of DVT, when not traveling, over those not taking The Pill, and with long trips, their risk increases dramatically. Thrombophilic genes, especially factor V Leiden, also contribute to travel risk. Persons of non-standard sizes, including the obese, the taller-than-average and the shorter-than-average are at greater risk; none of them may fit well into standard seats. Dr. Rosendaal thinks it isn't cost-effective to screen the general population for thrombophilic mutations, for there aren't clear guidelines on what to do for persons with such genes, most of whom never have a thrombosis. On the other hand, he's concerned about women with factor V Leiden or prothrombin 20210A because they have a 15 to 30-fold increased thrombosis risk on oral contraceptives. He suggests that women might be screened for those thrombophilic genes before going on The Pill or on postmenopausal hormones. My own comment is that cost-benefit analyses for the general population, the public-health approach, may not satisfy the individual person, who may prefer to know his or her thrombophilic genetic profile, before considering hormone therapy or long-distance travel. There may be no controlled double-blind trials of the efficacy of any potentially risk-lowering therapy, but if medicine were limited to evidence-based therapies, we'd have little to work with. While awaiting the gold-standard trials we'd all like to see, we often must go with our best guess.

In the past two months, I've been on two domestic flights in economy class. I measured my kneecap-to-seat-back room. On one airline (American) there was four inches. A nearby extra-large passenger, appearing to be about 6-and-a-half feet tall, fit into his seat without touching his kneecaps to the seat in front of him. On the other airline, Northwest, I had about a half-inch of kneecap room and my neighbor, about six feet tall, had to slew his legs on a diagonal to fit into the allocated space. A height of six feet is not exceptional for American males. That airline's spacing policy looks inherently unsafe, as well as inhumane.

What is an adequate coag lab work-up for a patient with a mild bleeding history? I liked the presentation of Catherine P.M. Hayward, of McMaster University, in the Education Program (it's published in that ASH book.) I agree with her that as much as possible should be done on the first visit, for more than two visits are not really practical. Her panel of first-tests include the complete blood count and differential; ABO blood group; ferritin (because Iron deficiency is common especially in women with bleeding problems); the PT and APTT with specific assays of factors VIII, IX and XI; thrombin clotting time (= thrombin time) and clottable fibrinogen measurement (because fibrinogen deficiencies and abnormalities may

not show up in other tests); a screen for VWD consisting of FVIII, VWF:RCo and VWF:Ag, and if any abnormality is found, also multimer analysis; platelet aggregation with an array of agonists and, if possible, platelet secretion (as with the Lumi-Aggregometer); evaluation for platelet dense granule deficiency (which is about as common as VWD in her population, and for which she screens with electron microscopy); and, if there's any question of the patient's general health, tests for renal, liver or thyroid disease. She suggests further tests for a second visit, and, very sensibly, says that any abnormal test should be confirmed by repeating it. I fear that good diagnostic coagulation laboratories are disappearing. Ideally, the doctor seeing the patient clinically should be in very close contact with the laboratory, and, the laboratory scientists should consider the patient's history and consult with the doctor. I do not like the growing practice of routinely sending samples to distant reference laboratories without close consultation.

Let me call your attention to an excellent new educational booklet called "**Playing it Safe**", written by physiotherapists Alice Anderson and Angela Forsyth, based on a prior publication by Marvin Gilbert, and published by the National Hemophilia Foundation. It describes conditioning, fitness training and various sports, with their risks and benefits. It suggests how to prepare to take part in sports and how to stay safe. It rates sports according to the level of danger encountered. Some sports have a high risk of injury and are inherently dangerous for anybody. The authors frankly recommend certain sports, offer caution about others, and strongly disapprove of some. The strongly-disapproved sports are dangerous for all participants, but, the consequences of an injury are worse for a person with a bleeding disorder than for a person with normal coagulation. I find this booklet eminently sensible. Boys and young men with hemophilia have been subtly encourage to think they can do anything with impunity. It's just not so. (The booklet is available from NHF at 116 West 32nd Street, 11th Floor, New York, NY 10001; 1-800-42-HANDI.)

The 2006 Registry of Clotting Factor Concentrates can be found on the WFH website, the ISTH website and my own website. A printed international registry will be mailed to readers outside the USA with this Bulletin. Those in the USA will get a list of products licensed in the USA with this Bulletin and, later, when it has been printed, a handy little pocket guide highlighting US-licensed concentrates but also listing those available in other countries. US readers should also refer to the concentrate list of the Medical and Scientific Advisory Committee of the National Hemophilia Foundation, and to Craig Kessler's excellent article in the ASH Education Program Book, 2005.