2.
THE ETHICS OF PRE-IMPLANTATION GENETICS DIAGNOSIS.

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Two procedures are being used in many genetics centers for Pre-Implantation Genetic Diagnosis (PGD). In Embryo Biopsy, a single cell from the 4 - 8 cell blastula is aspirated and tested. This implies a post conceptional diagnosis by which fertilized embryos are selected. Only disease-free ones are transferred for uterine implantation. Thus, it suffers from the same moral, ethical and legal constraints that termination does in this country. The alternate procedure, Polar Body Biopsy, merits closer scrutiny in a community such as ours. The genetic testing by which disease-free oocytes are selected for In Vitro Fertilisation (IVF) is done prior to conception. PGD by Polar Body Biopsy has become a viable alternative for couples at risk of having children effected by hereditary disease but who refuse termination or any post-conceptional manipulation. It also improves the outcome of IVF among women of advanced maternal age. World wide, over 2500 PGD cycles have been conducted in the last decade. Five hundred successful disease-free births have been recorded with an error rate of only 1.8% and the same prevalence of malformations as in the general population.

Of course, PGD by Polar Body Biopsy leads to IVF after selection of disease-free oocytes. However, neither the technology nor the ethics of IVF will be considered in this paper.

Genetic factors account for about one-third to one-half of morbidity and pathogenesis in many disorders. One's health or sickness depends on the interplay of genetics, environment

and life-style choices. In the last few years there has been great progress through molecular biology and genetics to understand physiology, to understand pathology, and to provide powerful diagnostic tools through DNA analysis. We have done extensive studies on a condition known as thalassaemia. It is a hereditary disorder common in Mediterranean and other peoples. In thalassaemia, the production of the red substance in blood cells known as haemoglobin is decreased. The genes and the changes in DNA sequences or 'mutations' responsible for this have been identified, mapped and thoroughly sequenced. The gene defects (mutations) that cause thalassaemia in the Maltese population are well known. A single mutation, so called the beta+;IVS-I,6C in the beta globin gene accounts for over two-thirds of all thalassaemia mutations in Maltese patients. In fact, and as is the case with other genetic disorders, only a small number of DNA mutations cause most cases of thalassaemia among the Maltese as among other populations. For instance, if one looks at the distribution of thalassaemia mutations in the DNA of patients from across the Mediterranean littoral, one finds that as in Malta, in most countries, three or four mutations account for most disease. One can also observe a gradient in the molecular epidemiology with the IVS-I,6C mutation being the commonest one in the west and the IVS-I,110A being the commonest one in the east of the entire Mediterranean basin.

This information, which is concurrently being collected for a variety of genetic diseases is a tool in our hands with which to characterize patients and their relatives. It helps to confirm diagnosis more precisely than other blood tests, to make predictions on the future course of disease or to choose between alternate therapy based on the balance of risks and benefits. DNA analysis helps to identify asymptomatic carriers i.e. healthy heterozygotes and couples at risk in which both parents are heterozygotes. Unfortunately, however, although

we can do extremely well with diagnosis, I think we do extremely poorly when it comes to management or specific treatment of disease. There is, of course, great anticipation that within the next few years, perhaps in five to ten years, that gene therapy techniques finally give us equally powerful tools to do therapy as well as we can do diagnosis. In the meantime, there has been progress in certain diseases such as haemophilia with the production of recombinant products such as coagulation factor VIII to replace those congenitally missing from blood. Correction of certain sequences with specific types of short DNA molecules is making good progress and there is some good progress also with the use of drugs to stimulate foetal or alternative proteins which replace the defective adult proteins in certain diseases. The production of foetal haemoglobin to treat beta thalassaemia is a good example. We have some preliminary satisfying results with this. There is some progress with use of gene transfer in cancer, but in general, gene transfer for hereditary disorders still does not work because safe and effective molecular tools to do it well are not yet available.

Consequently, thus far, the standard of care in genetics has remained that of prevention by counseling or by termination of pregnancy following ante-natal diagnosis. Classically, counseling has been employed to modify the reproductive behaviour of couples at risk if they wished so. On the whole, in most communities, the outcome of this approach has been disappointing. This is the problem that very often confronts us.

It becomes pressing to ask; what are the options available to a couple at risk, i.e. in which both parents are heterozygotes albeit healthy and wanting children ? They are heterozygotes, carriers of a known disease with a mutation which we can identify at the molecular, that is, at the DNA level. Every time they conceive a child together, they run a 25% chance of bearing a homozygote who has inherited both abnormal genes from each parent and is often a sick child.

Up to recently we could offer very little. Even with the best possible use of genomic resources, the best that could be offered was ante-natal diagnosis with or without the option of termination. Knowing what the constraints and thinking in our community are, I will not spend much time on this. However, although often presented together, ante-natal diagnosis and termination of pregnancy are, according to most professional guidelines, actually separate and distinct procedures. It is wrong to assume that one inevitably leads to the other. In fact, quite the opposite is true. At least three-fourths of the time, ante-natal diagnosis saves termination. Furthermore, one has to understand that those couples at risk that have a legitimate access to ante-natal diagnosis and termination end up having a much larger number of healthy babies than those which do not. Admittedly, many couples find termination repelling. They can now turn to alternate procedures such PGD, preferably through Polar Body Biopsy.

The physiological process of female gametogenesis leads to the production of a mature oocyte which is then fertilized by a mature spermatocyte. The precursor oocyte with a normal, diploid, quantity of DNA (2N) first duplicates its DNA (4N). It then goes through two reductive divisions, during which the quantity of the DNA and the number of chromosomes is successively halved and assorted in equal amounts to one or other daughter cells which end up with 1N of DNA each. Only one of the products of these cellular divisions continues down the path of development into a mature oocyte. The others are expelled as polar bodies. The first polar body is the result of the first reduction division, and the second polar body is the result of the second reduction division. In the heterozygous oocyte, with one of the two parental genes being normal and the other one being not, both are first duplicated. Then, the two copies of both the normal and the abnormal genes are assorted to the daughter oocyte or polar bodies. After the first reductive division, the abnormal alleles may be both

distributed to the oocyte or both to the first polar body or one into each. If both copies are detected by DNA gene testing in the first polar body, then one can infer that the primary oocyte has retained only the two normal alleles and one may proceed to IVF with husband's sperm. The outcome of this fertilization may be a healthy foetus with only normal genes or a healthy heterozygote like its parents. It can be seen that, if one knew beforehand the particular DNA mutation in the mother, by testing the first polar body and, ideally from a technical point of view also the second polar body, one can particularly select oocytes with which to proceed. The chances of a couple at risk such as these of having any sick babies practically decreases to zero from the theoretical 25%. They can be rewarded with the joy of a healthy baby in conditions of genetic risk.

Both chromosomal abnormalities such as trisomy 21 and DNA sequence alterations such as those of thalassaemia and many others can be tested with advanced molecular biology techniques. Fluorescent In situ Hybridization (FISH) on chromosomes or DNA sequencing with fully automated methodology are currently available in state of the art facilities. Most major single gene disorders whether autosomal recessive or dominant, mitochondrial disorders, and those due to tri-nucleotide instabilities that result in conditions like Huntington's disease can be done. The only ones that are not suitable to be approached in this way would be the ones that are male-linked. The latter are extremely few and extremely rare.

One technical drawback that has precluded most laboratories working in this field from using exclusively test analysis of the first of the two polar bodies is known as "allele dropout". Only minute quantities of DNA can be obtained from a single cell such as the first polar body. One can imagine that it is possible for only one of the two alleles to be detected and this could result in false negative judgements. The problem can be

overcome by including analyses of the second polar body, which increases the accuracy, and by testing for additional DNA sequences flanking the known mutation.

Having considered the procedures involved, I think that there are two main issues to discuss with respect to PGD. It seems that the procedure, as such, down to the stage of the first polar body, does not raise any moral or ethical dilemma of any great significance. I mean, there is a continuity of life. The two cells, the oocyte and the fertilizing spermatocyte originated in a prior human organism. They are a sort of vehicle in the generation of a new life and subsequently a new human person. If one is ascertained of the stringency or the reliability of the diagnosis based on the DNA of the first polar body, then, in my way of thinking, there ought not be any major issue to consider. However, most workers in this field are not happy stopping at the first polar body. One would like to have the comfort or the assurance of a confirmatory diagnosis from the DNA analysis of the second polar body. This raises a different issue because, unlike other organisms, the second polar body in the human is most commonly produced only after fertilization. This brings up questions about the beginning of a new human life and human personhood. There are various views on the transition of human life to the establishment of a new human person. The main issue with regard to the good conduct of PGD would be to ask and hopefully answer the question "when, during the process that starts with the fertilization of a single oocyte by a single spermatocyte, and ends with the fusion of both parenteral pronuclei with the formation of a new diploid genome does a new human life begin ?"

At what stage can one determine, can one ask, a new human being has begun to exist ?

I think it would be nearly irrational to assume that this can happen ever before one has acquired a new diploid genome. By diploid I mean, the DNA that has been derived from both parents; one half from the mother, and one half from the father. Although there is some evidence that some of the separate DNAs may be active, I think this is trivial. For a period of time after fertilization they are separate, and then they have to come together to form a new diploid genome and conceive a new human being.

The second issue is perhaps less demanding intellectually or philosophically, but there is in PGD a tool with which couples could, if so wanting, design a baby for particular means. Couples may seek offspring with certain tissue markers suitable for the donation of organs in transplantation. Consider a family that has a sick child who needs an organ transplantation for a cure of a condition such as a leukemia or a thalassaemia. Possibly, there is within the concept of Christian sacrifice a legitimate way to reconcile the couple and their sick child with the possibility of deriving curative tissue from a loved "designer baby"

These are not easy questions. One tends to look at them from different perspectives especially when one is closely involved in practice with these problems. These, I think, are the two questions that need reflection with respect to the good conduct of PGD. Otherwise, PGD offers suitable means with which couples at genetic risk can seek to have healthy offspring.