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Perspectives in human reproduction

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In-vitro fertilization and embryo transfer represent only one of the rapidly emerging applied advances in reproductive medicine beginning in the late 1950s; these include 'the pill' and the IUD for contraception, and hormones for the infertile requiring gonadal stimulation by gonadotrophins, clomiphene citrate or bromocryptine, to mention but a few. But from where and when did the biological basis for these sweeping changes derive? Virtually all the recent applications grew out of imaginative basic research. Fundamental animal studies by pioneers, such as Chang, Thibault and Edwards, taught us nature's axioms for gametogenesis, fertilization, development and differentiation. Millions are now seeking voluntary manipulation of their intrinsic reproductive capabilities to gain quality of life benefits for themselves and their children. Although not universal, the popularity of such options sparked industrial investment, governmental policies and international agencies to promote development of safer, more effective drugs and devices. Increased advocacy of aggressive treatment for infertile couples was a spontaneous outgrowth of this movement. Thus, the right of individuals to procreate, even to pursue the extraordinary means required, arose from the diverse events of the nascent reproductive revolution.

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Human reproduction

We are living in the midst of a most dynamic shift in the modes and means for manipulating (suppression or enhancement) the reproductive potential of men and women. Indeed, the ramifications of the new reproductive technology are indelibly affecting the social, economic, political and ecologic status of mankind across the planet. The drive for quality of human life, threatened for the masses by overpopulation (Figure 1) or for individuals through disadvantages of infertility or heritable defects, is challenging the ancient, inexorable drive to reproduce, as mankind competes for living space, finite resources and choice of life-styles. Effective family planning may be the ultimate solution for starving nations, and among 'developed' countries it has enhanced educational and career opportunities for millions of young women. Reproductive technology is the new hope for progress in antenatal diagnostics and *in utero* treatment for the unborn; now, the fetus, too, is a patient even before achieving legal status as a person. These examples are among the many social and health issues that depend on the new technology. In-vitro fertilization and embryo transfer (IVF–ET) represent only one of the rapidly emerging applied advances in reproductive medicine beginning in the later 1950s; these include 'the pill', the IUD for contraception and hormones for the infertile, requiring gonadal stimulation by gonadotrophins, clomiphene citrate or bromocryptine, to mention but a few.

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IVF–ET and GIFT

The technology of IVF–ET has matured as a treatment to overcome certain problems of infertility. Although exact data are not available, a fair estimate indicates that ~5000 children conceived by IVF–ET will be born by the end of 1987. Given that the human population of the earth is just over five billion persons, ~1/1 000 000 of humanity has been conceived *in vitro*. Using currently prevailing success rates, we can predict this technology will produce ~230 children/1000 treatment cycles and, estimating the activity of IVF–ET clinics worldwide as depicted in Table I, we can surmise ~150 000 attempts at IVF–ET/year. While the impact this technology can have upon infertile couples worldwide is obvious, it is equally important to comprehend the nascent influence of IVF–ET technology on current and future developments in science and medicine more broadly.

IVF–ET has matured rapidly from a technique to a science, drawing strength from numerous established disciplines while simultaneously fostering their diversification. The comprehension of infertility and its treatment cannot be divorced from understandings that lead to new advances in contraceptive technology. Similarly, our understanding of the endocrinology of follicular stimulation has provided us with revelations equal-

ly applicable to menopausal physiology. Furthermore, the techniques of IVF and embryo manipulation in experimental animals have allowed rapid progress in our understanding of developmental biology and molecular genetics. Soon IVF–ET will be sought by patients for reasons other than treatment of infertility. One such application will be prenatal screening of genetic disorders as we learn more about the human genome and the diagnosis of genetic disease.

Embryo biopsy and genetic analysis

The human genome is comprised of ~100 000 genes, consisting of over three billion nucleotides, distributed among 46 chromosomes. There is considerable interest in sequencing the human genome, i.e. determining the exact nucleotide sequences for every human gene; such an endeavour would likely obligate 30 000 person-years and cost over two billion dollars. Why should this deserve priority over other scientific pursuits? And to what avail? Already, we have benefited from limited accomplishments towards sequencing the human genome in the molecular diagnosis of certain genetic diseases.

From a simplistic viewpoint, genetic diseases can be categorized into three classes: (i) cytogenetic errors with additions, deletions or rearrangements of chromosomes (e.g. Down's syndrome); (ii) multigene defects where many aberrations occur simultaneously producing a recognizable syndrome (e.g. childhood diabetes); and (iii) single gene defects, including point mutations in single nucleotides (e.g. sickle cell anemia, among 3000 others). How can these genetic errors be identified before their expression? Enzymes that cleave DNA at very specific places, called endonucleases or restriction enzymes, can be used to cut DNA into fragments that vary in length depending on nucleotide sequences and the enzyme(s) used. By knowing the specific nucleotide sequence of a particular gene and the cleavage site of a particular endonuclease, one can predict with great accuracy the size(s) of fragments that should result from digestion of the normal DNA; such is the case with the gene for sickle cell anemia. The endonuclease *Mst*II produces 'nicks' wherever the nucleotide sequence 'CCTGAGG' appears, thus cleaving DNA at that point. Three such sites appear in the normal β-globin gene, producing two DNA fragments of ~1150 and 200 bp, respectively. The β-globin gene with the point mutation resulting in sickle cell anemia lacks the internal 'CCTGAGG' sequence, thus only a single DNA fragment (~1350 bp) results from digesting this gene with *Mst*II (Figure 2). Such restriction fragment length polymorphism (RFLP) is diagnostic of sickle cell anemia without the need for this gene to be expressed (Wilson *et al.*, 1982). Similarly, as more normal and abnormal gene sequences are elucidated, the use of different endonucleases to fragment these genes can be diagnostic of various genetic diseases. Such diagnostic tools are already available, or are under intense investigation for several disorders, including Huntington's chorea, Alzheimer's disease and cystic fibrosis.

It would be incredibly naive to imply we could foretell all of the future uses of IVF–ET to address clinical problems. However, we would like to delineate one important use here:

WORLD POPULATION

YEAR	PERSONS
1830	1 Billion
1930	2 Billion
1960	3 Billion
1975	4 Billion
1986	5 Billion
2001	6.5 Billion

Fig. 1. World population.

Table I. Estimated scope of IVF–ET and GIFT clinics worldwide[a]

Number of clinics	Cycles/year for clinic	Total number of cycles/year
20	500	10 000
50	200	10 000
50	100	5000
100	50	5000
Total 220		30 000

About 30 000 treatment cycles/year
About five oocytes and inseminations/cycle
About 150 000 attempts/year total

[a]By the end of 1987, ~5000 children worldwide will be delivered as a result of IVF–ET and GIFT therapy.

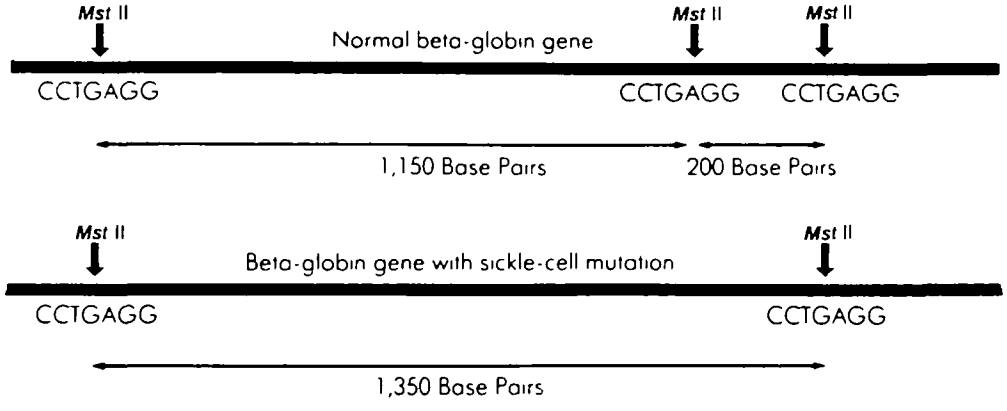

Fig. 2. Use of the endonuclease *Mst*II in the diagnosis of sickle cell anemia by RFLP.

the potential of IVF–ET in screening genetic disorders. Currently available technologies for prenatal screening of genetic disorders include amniocentesis or chorionic villus biopsy; the clinical intervention after positive diagnosis is abortion. The ability to detect genetic disorders in a 'pre-embryo' prior to replacement into the uterus for implantation may be a socially desirable alternative to other outcomes, yet not acceptable to some viewpoints.

A very real potential for early detection of genetic disorders is through analysis of cells taken from the pre-embryo. At least three scenarios can be envisioned: (i) removal of one or more blastomeres from a two-to-eight cell embryo; (ii) biopsy of the trophectoderm, leaving the inner cell mass untouched; or (iii) splitting of the blastocyst, where a portion of the trophoblast and inner cell mass are collected for analysis. Early mitotic activity in the embryo, certainly prior to blastocyst formation, is indeterminative cleavage; there is an increase in cell number, but each blastomere remains equally as totipotent as another. Mitotic activity in the embryo eventually becomes determinative, where differentiative events accompany or follow cytokinesis, and gene expression in the resulting two cells is no longer identical; totipotentiality has been lost (Alberts *et al.*, 1983). An obvious example of this is the initial differentiation between cells of the embryoblast (inner cell mass) and the trophoblast during blastocyst formation. The point at which mitotic activity of the human inner cell mass becomes determinative is not clear. However, embryos derived from splitting the inner cell mass after this differentiation begin to seem more likely to exhibit developmental irregularities. Therefore, until we undersand more completely the regulatory processes involved in preimplantation growth and differentiation, the last alternative presented above seems less likely for clinical use due to any potential uncontrolled risks involved.

The former two alternatives are more attractive, but present additional technical problems. First, we must consider the time involved to implement this form of genetic analysis; decisions must be made concerning the dispensation of pre-embryos that are continuing to grow and a uterus that is continuing to mature. An obvious and attractive way to address these concerns is through continued development of cryopreservation technology; selected embryos may then be replaced at the appropriate time of subsequent cycles. Secondly, we have the problem of genetic analysis of one, or a very limited number, of cells. Current molecular genetic technology, and that of the foreseeable future, does not permit us to perform techniques such as RFLP analysis on single cells. Thus, to analyse blastomeres or trophoblast cell biopsies from pre-embryos, we will either have to amplify the amount of DNA in those cells or amplify the number of cells. Although the use of plasmids, etc. will allow us to amplify select portions of DNA isolated from thousands of identical cells (Cohen, 1975) there is no available way to amplify the total DNA content of a single cell to facilitate reliable genetic analysis. Thus, we focus on increasing the number of cells. This can be accomplished by: (i) the immortalization of cells through transformation with a virus such as SV40; (ii) the formation of hybridomas by fusing embryonic cells with a rapidly growing cell line, similar to techniques used in the formation of monoclonal antibodies; or (iii) the induction or augmentation of mitotic activity in isolated cells. The first two choices are unattractive for clinical diagnostic use since the consequence of inserting viral genetic sequences into cells, or forming heterokaryons in hybridomas, could be incomplete retention or inaccurrate replication of the genome of the original pre-embryo cell (Ringertz and Savage, 1975). The last alternative seems the most likely; to this end, it is fortuitous that blastomeres, and particularly trophoblastic cells, are naturally active mitotically.

When the technologies of molecular genetics and cryopreservation are refined to more standardized clinical use, those couples who are carriers of sickle cell anemia (cystic fibrosis, Huntington's chorea, Lesch–Nyhan or Tay Sach's syndrome or a variety of genetic disorders) may avail themselves of IVF–ET clinic services. They may select pre-embryo(s) for transfer that are free of those genetic diseases.

Stewardship of science to society

The vast array of new reproductive technologies brings simultaneously the increased responsibility for scientists and physicians to communicate with the public at a level seldom realized. This means making a major investment in time and talent for listening to public concerns and needs, as well as explaining scientific objectives and possible clinical advances that are sought. In short, the public trust is the foundation upon which biomedical research must reside.

The thrust for new knowledge, while typically not on a wholly predictable course, cannot be muted if we expect to continue to improve medical care. Yet, the public has every right to scrutinize the intentions and means invoked by biomedical investigators. Indeed, patients clamor evermore to physicians for skills, medications and devices that can alleviate the ailments that limit the quality of their lives, or life itself. Continued progress in biomedical research must be predicted on the successful balance of the investigator's pursuit of knowledge and the public's willingness to support that endeavor, both harboring the conviction of ultimate benefit to mankind.

The young physicians and scientists who will lead biomedical research into the 21st century are in training now in our clinics and laboratories. Superimposed on the obligation to instruct in clinical care and research methodologies is the duty to teach responsible social behavior. Herein lies the route to a fuller discourse between investigators and the public. We must treasure and conserve the public trust above personal ambitions or short-term research goals so pressing in the rich competition of scientific discovery. Thus, we become stewards of a process few are privileged to experience.

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Discussion

Schaison

As I told you, I have observed exactly the same results, i.e. I could obtain ovulation with pure FSH in hypophysectomized women.

I have checked the amount of LH in the ampoules of pure FSH and there is only 0.9% of LH. Do you think this tiny amount of LH is able to increase the androgen production by the theca cells and subsequently the oestrogen production?

Hodgen

Yes, I think so. Our studies in monkeys, as I said, were titrated. We tested different additions of LH, starting with a 1/10 000 dilution of 10 IUs per dose: we have showed that, with a ratio of FSH:LH of 88:1, we had a completely normal response. At doses where the ratio was about 120/1, we saw partial effects. I think it may be that this very tiny amount of LH can be efficient with respect to oestrogen secretion. Also the oestrogen rise is affected. So treatment with pure FSH associated with a potent GnRH antagonist must be much longer than normal. The surprising thing is that there must still apparently be cell division going on among the granulosa layers. Otherwise, I do not think it would be possible to see production of large quantities of progesterone after HCG, because I would suppose the cellular machinery would be missing. Probably, it is that tiny amount of LH, or the patient has some in her body. Did this patient have Kallman's syndrome?

Schaison

They all have a negative LHRH test. Some of them had pituitary tumours and I studied them after surgery.

Hodgen

With almost zero endogenous LH?

Schaison

Yes.

Sureau

I am very impressed, of course, by your slides on contraception and the comparison you have made concerning Nigeria and South Korea. What would be your personal suggestions in order to promote contraception in this developing world?

Hodgen

I think we must find the financial means to augment the introduction of preparations there, until they are understood and demanded by people there.

Sureau

Do you mean also to use the traditional birth attendant for these people in order to involve them in this project?

Hodgen

I think so. I believe there is an analogy here with vaccination: we have the techniques to vaccinate people all around the world against smallpox, polio and other diseases, but look at all the people in the world who had never received this technology. It is the same with contraception: it does not get there, and the primary reason why it does not get there is that economic status will not permit its introduction.

Sureau

May I ask you a very provocative question? It has been suggested over the last months or years, at least in France, that there should be a form of moratorium concerning research into this type of subject because of the possible misuse of technology. What would be your own personal position concerning this point—in favour or against any kind of moratorium in research in this area?

Hodgen

I am not in favour of a moratorium because I think a moratorium is, in some ways, only an excuse for not facing the problem responsibly. Organon, our sponsor for this meeting, produce Marvelon, for example, which is a very good and safe contraceptive, with none of the problems that we all had 20 years ago with too high doses of steroids. We must get this technology into the people's hands and I don't think there is any question of whether it is right or not. The question is how to get it there.

van Keep

I will just make a statement. I am pessimistic as regards the development of contraceptives in the future, particularly if you think that there are only three pharmaceutical industries willing to spend their money in this field. We will certainly not make the same progress in the next 25 years as we have made in the 5 years between 1960 and 1965.

Hodgen

I certainly agree and I think the point is that, before we introduced the contraceptives which have evolved in the past 30 years, there was a big demand from the developed world. We have begun to saturate a part of that demand. But there is also a new opportunity and this is why I showed South Korea as an example of a country in transition and I think our responsibility is to find those particular countries, and to be very careful in the selection, and to encourage the introduction of, new Family Planning methods there.