Dynamism to promote reproductive medicine and its development, Rihachi Iizuka

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Abstract. Rihachi Iizuka has contributed strong leadership for the remarkable development of reproductive medicine which has undergone a complete transformation in the previous half century. The Keio University Hospital introduced artificial insemination as the first assisted reproductive technology in Japan. As it follows, Iizuka and his colleagues first reported the live birth of a female infant in August 1949 after heterologous insemination: AID. Iizuka and his colleagues were also among the first to successfully inseminate a woman with sperm that had been frozen. He developed the new cryopreservation medium for human semen called "KS Cryo-medium". He also developed semen preparation methods of washing and concentrating sperm counts by centrifugation with Percoll (colloidal silica derivative) solution for oligozoospermic patients. These methods are broadly used in the clinical field. Furthermore, he developed the X-, Y-bearing sperm preseparation method using Percoll which is the so-called "gender selection" procedure for preventing X-linked genetic disorders. The most striking assisted reproductive technology was *in vitro* fertilization first carried out in Britain. Prior to the clinical application in Japan, the Japan Society of Fertilization and Implantation was established as the main organ for the exchange of official scientific information by Iizuka in 1982. As rapid development and spreading of *in vitro* fertilization and its implicated technologies, Iizuka and his colleague of the department had the first success of offspring following embryo freezing and thawing in Japan which was performed at the Tokyo Dental College Ichikawa General Hospital. Already the numbers of offspring following *in vitro* fertilization treatment has risen to approximately 1% of births in Japan. Rihachi Iizuka still undertakes the responsibility for reproductive medicine as he has done so far. (Keio J Med 50 (4): 217–224, December 2001)

Key words: ART, artificial insemination, IVF, cryopreservation, separation of X-, Y-bearing sperm

Introduction

Anyone hearing of Rihachi Iizuka MD must believe they are hearing the history of reproductive medicine itself, not limited to Japan, but world-wide. No-one could possibly deny the truth of this statement.

In an era of striking progress in practically every area of medical science, reproductive medicine, to which Iizuka has contributed strong leadership as his life work, stands out as a field that has undergone a complete transformation in the last two decades. It has achieved major advances in infertility treatment, and it has clinically developed various means of "assisted reproductive technology (ART)" while making remarkable progress toward the prevention and treatment of various pathological conditions.

Iizuka began to study and work on this field immediately after he joined in the Department of Obstetrics and Gynecology, School of Medicine, Keio University, organized by Professor Kakuichi Ando in 1948. The field of reproductive medicine had not been focused in those periods in the medical history, completely in

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Abbreviations: ART: assisted reproductive technology, AIH: artificial insemination with husband's semen, AID: artificial insemination with donor's semen, HIT: histeroscopic insemination into tube, IVF: *in vitro* fertilization.

contrast to the present situation. Reproduction might be considered mysterious and naturally inherited or an untouchable area even if it should be necessary for biological life. Reproductive medicine has developed so rapidly in the previous 50 years and has involved many other fields; biology, endocrinology, veterinary medicine, low temperature science, environmental health, *etc.*

Iizuka has been bearing a central role for development, and built today's remarkable advance of the reproductive medicine from a viewpoint to be always leading. The time when he was concerned with the field of reproductive medicine was with only a few knowledges and techniques for elucidating reproductive functions and mechanisms in the aspects of both physiological and infertile status. At the beginning, he and his colleagues started to elucidate infertile status and to evaluate pathological conditions. Then they have been solving mystery of reproduction wrapped in a veil of mystery one by one and elucidated the infertile status. Various laboratory procedures to determine infertile status and its treatment have been promoted and advanced among the societies concerned with reproductive medicine that was actively cooperated with Iizuka and his colleagues. Iizuka's posture regarding reproductive medicine was to challenge always to be new and to fight without flinching from difficulties. In the development of reproductive technologies to produce new human life, social, ethical and legal considerations have also been required in addition to its medical advantages (Fig. 1).

Rihachi Iizuka has always been arguing about these aspects with people in the medical and non-medical fields and made his tremendous efforts to prepare the environment for performing these technologies. His powerful and active leadership has been contributing not only to the medical field but also to human society. This strong activity has been based on his warm thoughtfulness to the infertile patients.

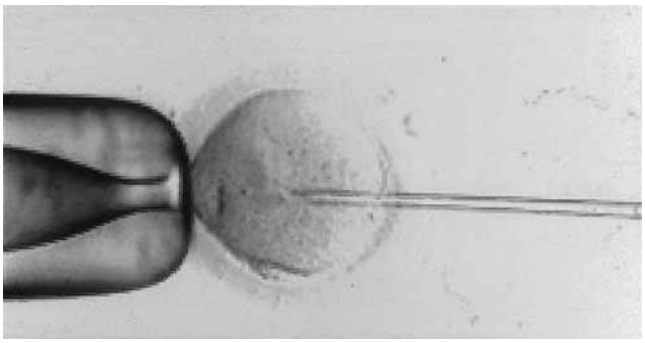

Fig. 1 *In vitro* fertilization promoted by Professor Iizuka dramatically developed reproductive medicine.

Elucidation of Reproductive Physiology and Infertile Status

The science of reproduction is based on elucidation of physiology and pathological status of reproductive function occurring in infertile patients. In fact, the advance of reproductive medicine, to which Iizuka has contributed, began with the elucidation of reproductive physiology. The integrated results of his early achievements are described in the book entitled "The Study of Implantation"[1] in 1972, which has been widely referred as a Bible of reproductive science. Prior to publishing this manuscript, Iizuka reported his enormous scientific outcome at the Annual scientific lecture of the Japan Society of Obstetrics and Gynecology (JSOG) as a representative of this field.

In terms of clinical aspects of infertility, Iizuka established the method of examination to determine the cause of infertility, which is the standard method for the patient at present. His examination protocol is based on the estimation of ovulation timing by basal body temperature and acidophilic index of vaginal cytology, evaluation of tubal function with hysterosalpingography (HSG) and its scoring, Rubin test (kimoinsufflation) and its evaluation, implantation function with endocrinological and histological evaluation, semen analysis, and post coital test, *etc.*[2,3]

In particular, investigation into male infertile factors has lead to the development of assisted reproductive technologies from artificial insemination and sperm selection. Iizuka had focused on spermatogenesis at the human testis,[4,5] and was awarded the degree of Doctor of Medical Science on "the study of testicular biopsy for infertile male patients and consideration of their spermatogenetic ability".[4] The book "Infertility"[2] was written on the whole clinical procedures for infertility by Iizuka, *et al.* and has also been read by most obstetricians and gynecologist as their Bible.

Artificial Insemination

Since Iizuka graduated from the School of Medicine, Keio University, he has devoted his life to reproductive medicine the tremendous future potential of which had already been noted by Dr. Kakuichi Ando, the previous professor of the department. Artificial insemination has grown to occupy a large part of his academic life in the past several decades as well as the clinical management of infertile patients with male and/or female factors.[6] Among numerous therapeutic methodologies for infertility treatments, none has a longer history in the actual practice than artificial insemination in the past hundred years. Iizuka's achievements in the field of artificial insemination have been spread over the wide and deep parts around entire procedures of the treatments: arti-

ficial insemination with donor semen (AID) in Japan and its long-term follow up survey,[7,8] development of a cryo-medium for efficient cryopreservation of sperm,[9] washing and concentrating techniques using density gradient centrifugation,[10] the preconceptional sex selection for prevention of sex-linked hereditary diseases,[11] and the hysteroscopic insemination into fallopian tube (HIT).[12]

The members of the Department of Obstetrics and Gynecology in School of Medicine, Keio University, Iizuka and his colleagues first reported the live birth of a female infant in August 1949 after heterologous insemination; AID.[7] It was shortly after the end of World War II, and Japanese society attached more importance to contraception than to treatments for infertility at that time. Our department practiced, therefore, consultation and education concerning both contraception and infertility.

Since the first case was succeeded, artificial insemination has been performed actively in one section of our out-patient department called ''The Family Planning Division'' (Kazoku Keikaku Soudanjo) to which numerous clinicians and scientists have contributed. AID has been carried out at a very limited number of facilities in Japan since its first application at Keio University Hospital in 1948. More than ten thousand babies have been already delivered by AID in 50 years. Furthermore, as the implicated technology of artificial insemination, Iizuka reported the first case of pregnancy in Japan by artificial insemination with the frozen-thawed semen, in 1958.[7] He also developed the instruments for artificial insemination. The 10cm long needle is called ''Keio's Insemination Needle'' and is still used widely all over Japan. His studies were further developed resulting in preconceptional sex selection using Percoll solution in 1983,[11] and in Japan's first cases of pregnancy obtained from cryopreserved human embryos in an IVF program in 1989.[13]

Cryopreservation of Donor Semen

In Iizuka's medical achievements, the first biggest impact to the world was the success of pregnancy using cryopreserved semen.

Iizuka and his colleagues were among the first to successfully inseminate a woman with sperm that had been frozen. Historically, Japan played a pioneering role in the development of this technique. He developed the new cryopreservation medium called ''KS Cryo-medium'' which was mainly composed of glycerol and egg yolk. Semen is mixed with an equal volume of KS Cryo-medium, placed in a glass ampule or plastic straw (0.5 ml), exposed to liquid nitrogen vapor or immersion in a dry-ice ethanol bath and then stored in a liquid nitrogen container at −197 °C. Thawing of the cryopreserved semen is performed by immersing the ampule or straw into prewarmed water at 30 °C. In 1958, Iizuka reported the first offspring case after artificial insemination with the frozen-thawed semen. This cryopreservation method offered the basic technique for cryopreservation of semen and is still used in the clinical scene.

Sperm Preparation and Selection Technology

About one half of infertility factors is considered to occur in the male. Although the development of treatment for males is extremely important, most medical treatments have not been efficient enough. Iizuka developed semen preparation methods of washing and concentrating sperm counts by centrifugation with Percoll solution which is a colloidal silica derivative. These Percoll methods currently became standard sperm preparation procedures and are broadly used in the clinical field. These methods basically have two advantages of removing seminal plasma and concentrating sperm counts. In contrast to the capable volume of insemination to uterine cavity, 0.3–0.8 ml, ejaculated semen volume used to be more than 2.0 ml. The sperm count can be concentrated by separation procedures. The seminal plasma, which is sometimes the reason for the induction of lower abdominal pain, contains many components; prostaglandins, bacteria or debris. Removing the seminal plasma can not only concentrate sperm counts but also removes seminal plasma to avoid the side effects of artificial insemination.[14,15]

Furthermore, Percoll density gradient methods have many developmental potentials by these points beyond the pretreatment for oligozoospermia. The typical technology developed from the basic procedure of the Percoll method was the X-, Y-bearing sperm preseparation method.[11] In other words, it is the so-called ''gender selection'' procedure. This technology further developed into the method to remove HIV virus from semen HIV.

The monolayer density gradient in 20% Percoll was first adopted to sperm preparation. The discontinuous Percoll density gradient of 4 steps was secondly developed for the selective concentration of progressively motile sperm for use of AIH. However, the discontinuous Percoll density gradient is too delicate and time consuming. In order to obtain high practical utility, Iizuka developed a new simple procedure, *i.e.* a continuous-step density gradient, for the selective concentration of progressively motile sperm.

The washing and concentration of poor quality semen (discontinuous Percoll density gradient method)

When sperm precipitation by the monolayer Percoll method is used, the sperm with various modes of

movements as well as other cellular components are condensed together in the sediment. Most of the specimens exhibited an increase in sperm concentration with a mean of 4.3 ± 3.7 times, and was over 3 fold in over half of the cases. Nearly half of the cases showed improved motility, with the mean increase being 1.9 ± 1.1 times. Although the ejaculate contains heterogeneous sperm populations, only mature sperm with progressive motility are considered to contribute to fertilization. The discontinuous Percoll density gradient of 4 steps is used for the selective isolation of progressively motile sperm. For the selective isolation of mature sperm with progressive motility, the bottom layer of the density gradient of 4 steps was determined to be 1.10 g/ml, which corresponded to 80% Percoll. The progressively motile sperm were mainly recovered in the sediment, whereas immotile sperm, immature sperm with cytoplasmic droplets, and other cellular components remained at the upper density interfaces.

Almost all of the specimens demonstrated the improvement in sperm qualities with averages of 65.3 ± 31.1 × 10^6/ml and 76.3 ± 11.0%.

The selective concentration of progressively motile sperm (continuous-step density gradinet centrifugation method)

The continuous-step density gradient technique is the preparation procedure using isotonic 80% Percoll solution. The whole ejaculated semen was placed on 80% Percoll solution, and the continuous density gradient was made in a centrifuge tube by mixing the semen and subjacent Percoll layer with a L-shaped rod.

The specimens with more than 30% motility had improved motilities of 81% through 97% regardless of the original motilities. Overall improvement of sperm motility was 4.3 ± 3.5 times, with an average of 1.9 ± 1.0 times. Consequently, the increases in sperm concentration and motility lead to a dramatic improvement in the fertility index (7.3 ± 3.7 times higher).

Separation of human X- and Y-bearing sperm (discontinuous Percoll density gradients method)

As described above, the use of the Percoll density gradients brought not only a better clinical outcome in AIH, but also a coincidental alteration of the sex ratio which was noted in the delivered cases; females predominated over males in significant numbers. This fact led us to the assumption that density gradient centrifugation might result in the separation of human X- and Y-bearing sperm. The original purpose of this technology is for the carrier female with X-linked genetic disorders to avoid an affected male baby. A discontinuous Percoll density gradient of 6 steps or 12 steps among 1.06–1.11 g/ml in 0.01 g/ml or 0.05 g/dl steps, respectively and each 1.0 ml was layered successively. Semen specimens were loaded on the gradients and centrifuged. The proportion of X- and Y-bearing sperm in each fraction differs gradually according to the density increment. The purity of X-bearing sperm recovered in the sediment ranged from 87 to 94%, whereas that of Y-bearing sperm in the lighter fractions was below 60%. Purification of X-bearing sperm was much improved by increasing the number of steps in the Percoll density gradient from 6 to 12, although this did not improve the separation of Y-bearing sperm.

In previous several years, the another technology of the diagnosis from embryo, preimplantation genetic diagnosis, has been developed and ethically approved to be performed as clinical research by JSOG in Japan.

Gender determination from an embryo is already technically available as an established procedure. However, single gene disorders should be examined on the mutation gene instead of gender determination from the ethical point of view of the JSOG. The problem is that the gene mutation is not completely diagnosed yet according to the type of mutation. Therefore, Iizuka's developed X- and Y-bearing sperm preseparation procedure is less invasive and still the only available procedure for sexing.

This technology has come into the limelight in the field of animal industry especially for cattle. It was such a sensational achievement that journalists also payed great attention. However, the apprehensions were that it might be used only for family planning by people's desire. There was a history that the ethical committee in the School of Medicine, Keio University was founded in the face of this argument.

In Vitro Fertilization and Its Associated Technologies

Japan was quick to embrace the *in vitro* fertilization technology first carried out in Britain in 1978.[16] Japan began applying the procedure in 1982, the same year the Japan Society of Fertilization and Implantation (JSFI) was established as the main organ for the exchange of official scientific information in the field. The central figure of these actions had been always the person Iizuka. The first proposal of this Society was promoted at the Kitasato Lecture Hall of the School of Medicine, Keio University on November 15, 1982. There had been a wide variety of discussions for the enforcement of *in vitro* fertilization in Japan especially at the beginning, while the technical or medical problems needed to be resolved. The technical and scientific discussion had mainly been settled in the JSFI. On the other hand, starting with the *in vitro* fertilization in 1982, the JSOG has continuously reviewed such technologies as they passed through the various

Prof. Campos da Paz (Brazil)
Prof. Iizuka
Prof. Insler (Israel)
Prof. Steptoe (Great Britain)

Fig. 2 Professors Campos da Paz (Brazil), Insler (Israel), Iizuka and Steptoe (Great Britain) at the International Federation of Gynecology and Obstetrics in 1979, Tokyo [from left]. Professor Campos discovered crystallization of cervical mucus and founded the World Congress on Human Reproduction. Dr. Steptoe succeeded with the first *in vitro* fertilization.

stages of development and clinical application, formulating opinions on their ethical repercussions and issuing guidelines to its members accordingly. After five years of research and preparation and additional time spent awaiting clinical guidelines from the JSOG in 1992, Japanese doctors carried out their first microfertilizations for couples who cannot be helped by *in vitro* fertilization (Figs. 2, 3).

Since *in vitro* fertilization began in Japan, the organizations responsible for self-regulation of specialists in reproductive medicine – the JSOG, the JSFI, and the Japan Society of Fertility and Sterility (JSFS) – have become extremely cautious regarding new reproductive technologies more strictly than that of the United States or Britain. The main reason is that patient privacy, one of the major issues of concern from the beginning, was seriously compromised by the callous behavior of the press, which pursued the story zealously, even tracking down families and disrupting the lives of patients in conservative rural communities. Some journalists insisted that the fault lay with the medical community for failing to disclose enough information to begin with. The medical community, for its part, recognized that the press had its own agenda and was not in the mode for a fair and sensible exchange of views. As a result, the two sides became increasingly polarized, and there was little evidence in the newspapers or on television of any productive debate on the subject. Iizuka and other members of the societies had contributed passionately for the agreement.

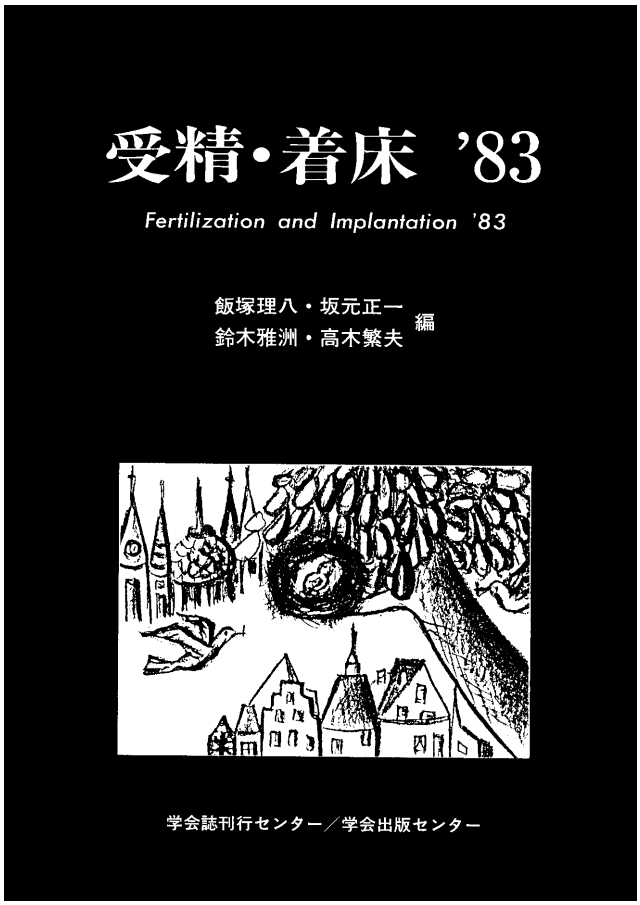

Fig. 3 The proceedings of Japan Society of Fertilization and Implantation; "Fertilization and Implantation (Vol. 1)" was first published in 1983 edited by R. Iizuka, S. Sakamoto, M. Suzuki and S. Takagi.

Enlisting the agreement of the other major professional societies, the JSOG in October 1983 issued its first set of guidelines on reproductive technology, titled "Opinion on *In Vitro* Fertilization and Embryo Transfer". Under these guidelines the society expressed its approval for the procedure on the condition that it be provided only to legally married couples unable to bear children, that the couple give their assent after a complete explanation of the procedure, that the privacy of the patient and the infant be respected, and that no attempt be made at genetic engineering. There is no doubt that Iizuka exhibited leadership in forming the decision of JSOG, JSFI and JSFS.

The significant development of *in vitro* fertilization has established the status as not only an infertility treatment but also the population control of this country. Already the numbers of offspring following *in vitro* fertilization treatment has risen to occupy approximately 1% of births in Japan. As *in vitro* fertilization has spread rapidly world-wide, there has been a progressive development of associated reproductive tech-

nologies. The combination of fertilization *in vitro* with subsequent zygote intrafallopian transfer (TEST, embryo intrafallopian transfer) was introduced with the expectation of a higher pregnancy outcome. The assumed advantages of gametes intrafallopian transfer (GIFT),[17,18] zygote intra fallopian transfer (ZIFT),[19] and TEST are that the gametes or embryos are placed in an environment which is expected to be optimal for fertilization and early embryo growth. In addition, a more accurate synchronization of embryo development and endometrial receptivity would be expected, since the *in vitro* fertilized or intrafallopian transferred embryos probably reach the uterine cavity at the morula/blastocyst stage.

Achieving fertilization *in vitro* is compromised in patients suffering from severe oligozoospermia, asthenozoospermia, teratozoospermia, or combinations of the above. Although insemination of oocytes with increased concentration of motile sperm may result in fertilization in less impaired cases, gamete micromanipulation has become increasingly applied to overcome the protective barriers of the zona pellucida and ooplasmic membrane. In addition, the creation of gaps in the zona pellucida with acidic solutions, laser, or glass microneedles may increase the likelihood of a blastocyst hatching through a zona which has become hardened from *in vitro* culture.

The first successful pregnancy following *in vitro* fertilization of human oocytes was made possible by innovations in laboratory and clinical practice. Establishment of the metabolic requirements of embryos led to the creation of suitable culture media. New techniques of semen processing permitted the extraction of capacitated sperm. Experience with the effects of administration of exogenous gonadotoropins allowed the scheduling of follicular aspiration at the time of optimal oocyte maturity. Continued advances in our understanding of gametogenesis, embryology, cryobiology, genetics, and molecular biology have dramatically broadened the scope of laboratory procedures which are relevant to human *in vitro* fertilization therapy. Recent results achieved by this technology suggest that only the truly azoospermic semen specimen precludes a trial of microsurgical insemination. Furthermore, embryo cryopreservation has also become an integral feature of many successful *in vitro* fertilization programs.[20]

Embryo Freezing

As current ovarian stimulation protocols yield high numbers of oocytes which can be fertilized, the option of cryopreservation of the resulting embryos provides several clinical advantages. These include, (1) reducing the risk of multifetal gestation by limiting the number of embryos replaced in the initial stimulating cycle, (2) increasing the number of potential replacement cycles without additional egg retrievals resulting in an improvement in the overall pregnancy rate, (3) decreasing the potential risk of severe ovarian hyperstimulation syndrome by substituting embryo transfer in the stimulation cycle at risk with transfer of frozen-thawed embryos in a subsequent unstimulated cycle, and (4) creating the potential for pregnancy following the birth of a child resulting from *in vitro* fertilization – embryo transfer without additional oocyte retrieval.

The cellular effects of cooling are presented as well as the steps taken to minimize the damaging sequelae of intracellular crystal formation. Progressive, slow cooling and ultrarapid freezing protocols have been developed.

Iizuka and his colleague of the department had the first successful offspring following embryo freezing and thawing in Japan which was performed at the Tokyo Dental College Ichikawa General Hospital. They cultivated the human cell freezing method also for the embryo following that for semen. At this moment, the technical success and subsequent successful pregnancy outcomes resulting from cryopreservation of preimplantation embryos have contributed to its widespread incorporation into *in vitro* fertilization programs.

Implication and Administration of the Societies

As mentioned above, Iizuka was concerned with most of the major Society in the reproductive field not only concerning medical but also animal research. The JSFS which was founded by a former Professor of Keio University Kakuichi Ando and other members in the field, is one of the major responsible societies. The scientists based on the three specialties of Obstetrics and Gynecology, Andrology (Urology), and animal science join this Society and work with each other. Iizuka has been organizing the activities of JSFS for a long time and served as the president of the Society from 1988 through 1992.

The JSOG is also the major society for our field throughout entire categories of clinical and research affairs. Iizuka was the executive director from 1974 through 1989 and was the president of the society from 1986 through 1987. He had established the ethical guidelines of the Society especially for *in vitro* fertilization. These guidelines formed the basis of the Society which was not established at that time.

The JSFI was founded in 1982 by Iizuka for the development of *in vitro* fertilization and involved technologies. The annual meeting had already been held 19 times by 2001.

This society has promoted not only the development of technologies and scientific assets but also the rapid spread and growth of ART so far. As a result,

Japan is evaluated as one of the leading countries of this field at this moment. This significant meaning of this society has been extremely important to prevent any decrease in the birth rate in Japan.

In addition to the societies already mentioned, Iizuka has taken a great interest in other societies, both domestic and international. Especially, relations with the United States have been grown strongly to take part in the development. The biggest society for the field of infertility is the American Fertility Society (AFS). In 1986, Iizuka became the honorary vice president of the AFS. The annual meeting was held in Toronto in November, 1986. In fact he became not only a representative of Japan but also in the United States in the field of reproductive medicine. The AFS has been developed according to the marverous progress of reproductive medicine. Thus in 1996, the AFS was renamed the American Society for Reproductive Medicine (ASRM). The field of reproductive medicine has come into usage in recent years in keeping with the changing realities of this area of medical science. Although the treatment of infertility has been the driving force behind most advances in the field, the science has expanded beyond the original objective of facilitating conception and now concerned itself also with such matters as the storage and genetic diagnosis of reproductive cells.

In 1987, Iizuka became the president of the World Congress on Human Reproduction. This was the evidential achievement that his previous activity has been well evaluated world-wide. He organized this World Congress in Tokyo in June, 1987 as the president of this world organization. The Crown Prince and Princess (Present Emperor and Empress) attended this World Congress to express the encouragement to the attendants from Japan and from overseas. He was president up to 1990 when he passed the baton to a new president in Helsinki. He has contributed to receive many fellows from foreign countries; Taiwan, Korea, Indonesia, Brazil, Paraguay, Argentina, *etc.* The fellows have established the reproductive medicine and its application to the clinical treatment in their countries after they finished the training at Keio University (Fig. 4).

Although we may recognize the extremely rapid and marvelous current achievements of reproductive technologies, the progress has led to a broad array of secondary problems. These include the legal and ethical dilemmas surrounding ownership and disposal of frozen embryos, pregnancies resulting from the use of donor sperm or eggs, and the rights of surrogate mothers, as well as the economic issue of the high cost of fertility treatment to health insurance systems strapped for funds.[21] The application and role of reproductive technology needs to evolve continually as the technology itself develops, and a good understanding of the current state of reproductive technology is essential to the formulation of sound principles of treatment.[22–24] Iizuka has been and is still strongly concerned with these social, economical, legal and ethical problems which successively appeared as the technology progress. He has always proposed insatiable inquiries and has never forgotten his affectionate consideration for less fortunate patients.

Fig. 4 Professor Iizuka at his laboratory in 1988.

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