A fairy-tale about the wonder of light on human being

Aisaku Fukuda, M.D., Ph.D.
Chairman, IVF Osaka Clinic

Dr. Maiman successfully created laser light oscillation using ruby crystal in 1960. Therefore, he was actually an inventor of laser therapy technology. Since then, laser technology was used for industry, telecommunication and military usage in the United States and Soviet Union. Adoption of laser therapy in the medical field started early in 1961, one year later from Dr. Maiman’s invention. Laser therapy was used for surgery of retinal detachment at first and then, in 1970, laser therapy was used for urinary calculus. Application of laser therapy in Japan started from ophthalmology around 1980. Nowadays many doctors are using laser therapy in many medical fields in Japan. When we see a story about LLLT (Low reactive level laser treatment), as early as the middle of the 1960s, Hungarian surgeon Endre Mester noticed an improvement of wound healing when using LLL. Since then the number of scientific studies about the biological effect of laser light irradiation has been growing steadily. Numerous scientific organizations have been looking into this matter for years in the fields of wound healing, chronic orthopedic problems like osteoarthritis or spinal diseases, acute strains like tenosynovitis, skin problems, allergic reactions and other problems. These studies have encouraged us to enlarge the field from our therapeutic experience in the biological effects of laser therapy. In the 1980th, they were spurred by early reports about the positive effects of laser light on nerve cells and nerve injuries. The perceptible and measurable positive results, getting more and more distinct, effective treatments of diseases with LLLT have encouraged to develop LLLT for the treatment of many other diseases including infertility. Many reports confirmed the fundamental efficacy of laser light which was experienced as subjectively positive and which at the same time was objectively measured. Hippocrates said that the gods had set the diagnosis before any treatment. However, before any therapy by Low-Level-Laser light there should be the effort to understand action mechanisms of this therapy on the patients. Also, we should tell the patients what they know, so that their process of healing is further supported. Light is commonly perceived as positive image on the people for a long time, even from the ancient period. Probably human being sees that light has a perceivable biological effect on our body. The first sunbathing in spring, pleasantly going to sleep in a deckchair in the warm sunshine, the hunger for more and more sunlight from winter to spring and from spring to summer, the disappointment if the weather is worse than we would like, the joy if the sun comes out again, but also the filling of our hunger when we have had enough light. On the other hand, we usually experience lack of light as tiring and unpleasant, and this even more distinctly the longer the lack of light continues. But in spite of everyone perceiving and experiencing the effect of light on one’s body, there is no commonly accepted knowledge about it. Therefore, the question of the biological effect of light on our body is quite justified. Thousands of clinically documented healing successes and patients’ reports give evidence for the biologically positive bio-stimulating effects of light or LLL. On the other hand, some people worry about the dangerousness of light. Only the small range of wavelengths of the ultraviolet (invisible for us) part of natural solar radiation is potentially dangerous for us. The whole of the visible part of natural solar radiation from the visible violet wavelength from 400 nm are all biologically completely harmless. Every living being can live in this quality of light for years without suffering any harm. In millions of years our biosphere has developed under the continuous physical effect of light on the living, but organic structures of all living beings survived without suffering any harm. This is why I would like to introduce you light therapy including LLLT with reference to the history of evolution of human beings under the sun beam.

Laser Reproduction and Other Fields: Development of My Patient-Driven LLLT Technology

Toshio Ohshiro MD. PhD.
Professor of the School of Medicine, Keio University, President of the Ohshiro Clinic.

In 1975, we have established the first private clinic specializing in laser treatment in the world. We have mainly treated pigmented nevi and vascular lesions in the field of dermatology and plastic and reconstructive Surgery. From our experiences of laser treatment of the skin, we established the concept of Ohshiro’s laser apple and classified laser treatments under high reactive-level laser treatment (HLLT) and low-reactive level laser therapy (LLLT). A pivotal point in the application of LLLT for female infertility was reached when we were treating lumbago in a
menopausal with 60 mW, 830 nm GaAlAs diode laser LLLT. To her (and our) surprise, menstruation recommenced, accompanied by vaginal bleeding. Following a further two such cases involving this phenomenon, I reported the case histories to a professor of gynecology who suggested we should try applying LLLT for the treatment of female infertility. This led to our successfully establishing a new project involving LLLT for female infertility, jointly involving the professor’s gynecology department and hospital and my laser clinic. From October 23 1996 to April 6 2000, we treated 74 patients with 60 mW, 830 nm GaAlAs diode laser LLLT. In that period we achieved 16 pregnancies, 11 deliveries, 13 babies (6 boys, 7 girls), 2 gamete intrafallopian transfers (GIFTs), 3 homologous artificial inseminations (AIHs), one spontaneous pregnancy and 10 in vitro fertilizations (IVFs). Only the GaAlAs diode laser LLLT has been used to assist female patients with very severe infertility problems referred from a gynecological hospital. The mean age of the patients we have treated is 39.3 years. The pregnancy rate has been 21.6%, and the delivery rate is 14.9%. In 2006 we established the Japanese Society for Laser Reproduction (JaSLaR) and annual JaSLaR meetings have been successfully organized every year. This history summarizes the birth of LLLT for Chairman Lecture.

**Current Status of PGS in the World**

Atsumi Yoshida

*Director, Kiba Park Clinic*

This lecture will focus on topics such as the current status of preimplantation genetic screening (PGS) in the world and the significance of post-PGS prenatal diagnosis (non-invasive prenatal genetic testing [NIPT], chorionic villus sampling, and amniotic fluid analysis). Indications for PGS in the world include: 1) advanced maternal age; 2) habitual abortion of unknown etiology; 3) implantation failure; 4) severe male infertility; 5) ovarian dysfunction; 6) parents receiving cancer treatments such as chemotherapy and radiotherapy; and 7) prior pregnancies with chromosomal abnormalities. Instead of blastomeres of the cleavage stage embryo, the trophectoderm (cells that will develop into the placenta) of the blastocyst is increasingly used to test the chromosomes of the embryo. Next generation sequencing (NGS) has replaced array comparative genomic hybridization (aCGH) as the major analytical technique. However, NGS allows mosaicism, which remained undetected in the past, to be diagnosed more frequently, making the handling of mosaic embryos a controversial topic.

**First Trimester Screening and Chorionic Villus Sampling**

Tsuyoshi Koga, M.D., Ph.D.

*Assistant Director, Koga Fumitoshi Women’s Clinic*

There are two ways to screen fetal chromosomal aberration in the first trimester of pregnancy. One way is first trimester screening (FTS) and the other is non-invasive prenatal test (NIPT), so called cell free DNA test. FTS is the conventional risk assessment using ultrasound features and biochemical markers which characteristic of fetal chromosomal anomaly. Using risk assessment program offered by Fetal Medicine Foundation (FMF. UK) we holistically consider all the risk variables from the markers multiplying them all the background risk calculated from the mother’s age. NIPT is the latest screening test using pieces of fetal chromosome floating in the maternal blood. Detection accuracy for chromosomal aberration of NIPT with next generation sequencing technology is quite high. For example the detection rate for trisomy 21 is 99% in false-positive rate 0.1%. When the screening returns positive, we confirm the result by using the invasive diagnostic technique, chorionic villus sampling (CVS) or amniocentesis. Thorough genetic counseling is an absolute necessity for the prenatal diagnosis to succeed. Practically FTS screening to CVS diagnosis has been worldwide standard so far, however very few facilities in Japan can be adopting this international standard unfortunately. However FTS does not match NIPT in the respect of the detection accuracy, FTS is cost effective and can detect wider range of chromosomal anomalies with sufficiently high detection rate. Additionally FTS is useful as a clue to diagnosis of fetal morphological anomaly. AS for the invasive diagnostic procedure, amniocentesis has been the first option in this country. However CVS includes around 1% confined placental mosaicism, it enables us to diagnose fetal chromosomal abnormalities at 10-12 weeks’
gestation, earlier than amniocentesis. And procedure related risk of CVS is almost the same as amniocentesis. From our experience, using suitable equipment and technique, trans-abdominal CVS is much safer and useful procedure than amniocentesis.

**Invited lecture**

**Trophectoderm biopsy using OCTAX laser**

Klaus Rink, Ph.D.

*OCTAX Microscience, Director*

The presentation will give a short overview about the development of lasers in IVF based on different tissue interaction processes. After some safety study aspects I will present a short overview about different applications. The main part will be focused on the current topic using lasers in IVF: day 5 respectively trophectoderm biopsy in different development stages of an embryo addressing the different strategies for a minimal invasive biopsy.

**Invited lecture**

**Polarization microscopy: Application in ART**

Markus Montag, Ph.D.

*iiabcomm GmbH, CEO*

Polarization microscopy was first used in the 30s of the last century to study the architecture of animal cells. Later it was applied for the visualization of microtubule-dependent structures, like the mitotic spindle in living cells. In 1995 real-time visualization of meiotic spindle became available due to the advances in computer technology. This allowed for the first time to visualize spindle properties in large cells like human oocytes. Within the field of assisted reproductive technologies (ART) polarization microscopy was used to study the presence and location of the spindle in human metaphase-II-oocytes. It was shown that the first polar body is not a reliable predictor of the location of the metaphase-II-spindle. The impact of deviations of the spindle from the position of the first polar body was intensively discussed in the literature. Another topic was the presence of the metaphase-II-spindle, as in some oocytes a spindle was not detectable at the time of investigation. An explanation was given by studies on the dynamic nature of the spindle during the course of meiosis, which is a reflection of the nuclear maturity of human oocytes. This in turn has an impact on the outcome of the results obtained by intracytoplasmic sperm injection (ICSI). Several reports found a correlation between spindle presence at the time of ICSI and fertilization rates and embryo development. While investigation spindle birefringence it became apparent, that the zona pellucida surrounding the oocyte has birefringence properties, too. Several studies reported independently that zona birefringence assessment of the inner layer of human oocytes may serve as a non-invasive prognostic marker for oocyte quality and has an impact on the clinical results. Part of this may be linked to the fact that the different zona birefringent properties correlate with expression profiles of certain candidate genes I subpopulations of the cumulus-oophorus complex. In summary, polarization microscopy opens up a new way of looking non-invasively on properties of human (and animal) oocytes that can be used as a prognostic marker. Furthermore, spindle imaging can detect problems in ovarian response during stimulation and at the same time is a useful tool for quality assessment of laboratory parameters like suboptimal pH or temperature.
Men’s health clinic - How to care delicate male patients with male infertility and sexual dysfunction?

Toshiyasu Amano, M.D., Ph.D.

Department of Urology,
Nagano Red Cross Hospital

According to the WHO report, 24% of infertility couple were found only male infertility factors, and both male and female factors exist in 24%. Thus, 48% of infertility couple are considered to have male infertility factors. We should recognize the importance of male factors in infertility clinics. The causes of male infertility include testicular spermatogenesis dysfunction, obstruction of semen tract and sexual dysfunction. In our men’s health clinic, we have to take care of these delicate patients with male infertility, sexual dysfunction, late-onset hypogonadism (LOH) syndrome, etc.

#1 Sexual dysfunction; Sexual dysfunction is consist of sexual desire dysfunction, erectile dysfunction (ED), ejaculation dysfunction (EjD) and orgasmic dysfunction. After launch of phosphodiesterase type 5 (PDE 5) inhibitors, ED treatments dramatically have made progress, and male infertility caused by ED also improved. Even so, some delicate ED patients with infertility are not able to complete sexual intercourse just under psychological pressure for pregnancy. We also need to listen attentively and counseling for these patients besides PDE 5 inhibitors.

In EjD, premature ejaculation (PE) is rather important issue especially in abroad. However, few PE visit urological clinic in Japan and there are no problems for infertility in PE patients. On the other hand, ejaculation difficulty especially intra-vaginal ejaculation insufficiency is important medical issue, because this condition is rather difficult to cure and accompany with male infertility. When a couple with EjD would like to have child, assisted reproductive technique (ART) should be recommended to prevent time consuming.

#2 Hypogonadism; LOH syndrome is treated by counseling, Japanese herbal medicine (Kampo) and testosterone replacement therapy. In general, infertility is not concerned for LOH syndrome. However, some conditions in male hypogonadism are very important diseases for infertility. Although treatments for infertility in some of male hypogonadism are quite difficult, hormonal treatments are very effective in a few of them such as male hypogonadotropic hypogonadism (MHH). Thus, it is important to diagnose hypogonadism condition correctly.

#3 Photobiology in male infertility; In the field of urological photobiology, we previously investigated photo dynamic therapy (PDT) for bladder cancer, ultra-weak photon detection from urological cancer. In current clinical practice, laser treatments for urolithiasis are widely applied with fiber scope. We also revealed the attractive relationship between semen, sperm parameters and Japanese traditional herbal medicine (Kampo) with fluorescence spectra study. Application of photobiology is promising strategy in the field of urology and infertility.

Men’s health clinic is required high privacy of patients, and many of them often hesitate to visit our clinic. Special consideration and specific knowledge and technique are indispensable for the doctors in charge of men’s health clinic.

Laparoscopic surgery prior to IVF in infertile patients.

Shigeo Akira, MD, PhD

Department of Obstetrics and Gynecology, Nippon Medical School

Recently, IVF-ET has become very popular and its indications have been extended to infertility of unknown origin. Laparoscopic surgery has been proven to be efficient with a high evidence level for examination of unexplained infertility and improvement of the pregnancy rate in patients with endometriosis. In addition, laparoscopic cystectomy of ovarian endometrioma reduces the risk of infection, rupture, and malignant transformation. Furthermore, laparoscopic adhesiolysis and excision of deep infiltrating lesions can be expected to reduce chronic pelvic pain and dyspareunia.

Currently, many ART clinics readily perform IVF. However, laparoscopic surgery prior to IVF often results in improvements in the pregnancy rate and QOL of patients. In addition, laparoscopic surgery has been reported to be effective in patients with repeated IVF failure.

In this seminar, evidence for the usefulness of laparoscopic surgery prior to ART, removal of endometriotic foci and tuboplasty as examples, will be discussed.
Introduction of PIEZO ICSI for improving ICSI performance

Tetsunori Mukaida 1, Tomoko Yorita 2
1: Clinical Director of Hiroshima HART Clinic, 2: ART labo director of Hiroshima HART Clinic,

Since 1992, G. Palermo introduced intracytoplasmic sperm injection (ICSI) technique as an assisted fertilization approach to overcome the fertilization failures of several reasons, ICSI has been established and widely utilized for severe male factor as well as fertilization failures of IVF. Conventional ICSI technique itself always require fine capillary pipette with sharp spike at the tip of pipette and well trained technical skills. It usually takes half to one year for new embryologists to get used to perform ICSI with acceptable results. However, no matter how the performance would be excellent, around 80% fertilization and 5-10% degeneration rate can be happened, due to the difference of characteristics of plasma membrane elasticity on each individual oocyte and nature of the procedures itself (puncturing the egg and aspiration of cytoplasm). PIEZO apparatus create forward rapid pulse force, these multiple pluses can break the zona pellucida without deformity of oocyte and a single pulse can cause the gentle rupture of plasma membrane of oocyte enable to put the single spermatozoon inside the cytoplasm. Technical damage to oocyte during the step of ICSI can be minimized. Since PIEZO ICSI introduced in our ART Labo as a routine procedures, fertilization rate has been increased and degeneration rate has been reduced statistically. Also, since PIEZO ICSI itself is very simple technique compared with conventional ICSI, training periods for new embryologists to learn PIEZO ICSI has been dramatically reduced to a couple of months. Those technical improvements can create the increased possibility to have more embryos available either for transfer or for cryopreservation. We believe in future PIEZO ICSI technique will wide spread to ART clinic.

Technical point to perform PIEZO ICSI

1. Setting of PIEZO apparatus
   - piezo pipette positioning,
   - loading the fluorine inside the piezo pipette
   - concentration of PVP solution (less than 7%)
   - visual confirmation of active pulse force at the tip of piezo pipette

2. Location of puncturing site of zona pellucida at the oocyte
   - choosing the area that has an enough perivitelline cavity.
   - continuous pulses to create a hole at the zona pellucida
   - insertion of piezo pipette inside the cytoplasm very gently
   - moving the piezo pipette forward until 2/3 diameter of oocyte
   - single pulse to break the oolemma (plasma membrane)
   - waiting few seconds to adjust the plasma membrane tuning back to stretched position, releasing the sperm without aspiration of cytoplasm and minimizing the introducing PVP solution inside cytoplasm.

3. Trouble Shooting
   - no actual pulse available ➔ adjust the Piezo apparatus and setting the piezo pipette
   - spontaneous breakage of oolemma prior to the point of piezo pulse introduction ➔ changing the position of puncturing site without releasing the sperm.

4. Fundamental advice; Consult the expert of Piezo ICSI to get the important technical steps on site.
   - If possible, invite the experts to your clinic to have them perform on your own piezo equipment.

Effectiveness of Acupuncture and Moxibustion in Conjunction with Laser Treatment

Kazunori Nakamura

General Director, Kyoto Nakamura No. 2 Acupuncture Clinic / Shiga Kusatsu Ritto Acupuncture Clinic
Chairman, Infertility Acupuncture Network

Recently, couples who wish to conceive use various alternative therapies in hope of increasing their chance of concep-
tion. However, the effectiveness of acupuncture and moxibustion to patients undergoing reproductive medicine is difficult to verify. Therefore, in our clinic, we attempt to verify whether acupuncture and moxibustion is truly contributing to reproductive improvement. I’d like to take this opportunity to share with you the results of two finding which verifies the effectiveness of laser treatment used in our clinic (linear polarized infrared irradiation instrument, Super Lizer (SL) manufactured by Tokyo Medical Laboratory Co., Ltd.) when used in conjunction with acupuncture.

1) Blastocyst Reaching Rate (BRR)

The cleavage progression of fertilized ovum in ART greatly influences the availability of embryo for implantation and is directly linked to live birth rate. Therefore, in the past, a comparison of BRR before and after the intervention of acupuncture and moxibustion was observed in retrospect, and found significant difference. (two-sided T test, P < 0.05). Additionally, the comparison of BRR of 59 patients before the start of acupuncture and moxibustion (total ovum retrieval cycle 199, number of ovum retrieval 764) and after (174, 667 respectively) was observed in retrospect and found correlation between maturation period of ovum and BRR. In other words, the use of acupuncture and moxibustion in conjunction with laser therapy (AMLT) for a repetitive, continued period of 3 to 6 months saw an increase of BBR by twofold. This coincides with the period in which ovum prepare for ovulation, which is 120 to 150 days, including the period before ovum acquires FSH sensitivity.

2) Clinical pregnancy rate after blastocyst implantation (CPR)

Attempting to verify CPR after blastocyst implantation and the effectiveness of AMLT can be difficult because of the different IVF clinics visited by patients and also the difference in the implantation protocol and guidelines used (fresh embryo vs frozen and freezing standards) which differ from clinic to clinic. However, when SL treatment was added to 48 of the 146 blastocyst implantation patients, we saw an increase of CPR by 7% (59.6 - 66.6%) Furthermore, with patients who have undergone PGD, we obtained CPR of 81.2% (9/11 ET) and 90% (9/10 patients) in euploidy embryo. We hope to verify the effectiveness of AMLT in fostering blastocyst implantation in the near future as the number of patients increases.

Based on these two observations, we believe that combined therapy with acupuncture/moxibustion and laser can contribute to reproductive wellness and contribute to better treatment results. We also believe that from the results seen from these two observations, AMLT to be effective in fostering natural pregnancy also. In this presentation, I would like to explain the theoretical evidence and interaction of AMLT from the perspective of cell biology, immunology and somatic autonomic nerve reflection.

---

**Nutritional Therapy for Fertility Patients -Focused on the Intestinal Flora-**

Makiko Komatsu

*Registered dietitian, IVF Osaka Clinic*

Bacterial flora is the collective bacteria and other microorganisms in the human body. It has been reviewed that intestinal bacterial flora existing in the intestine are deeply involved in biological functions and have various physiological influence maintaining a symbiotic relationship with the human host. Abnormal changes in the intestinal flora are related to the onset of several diseases. Recently, the association between reproductive function and intestinal flora have been focused on. Analytical outcomes of intestinal flora could be a health index, therefore it is expected they are available in early detection of diseases and determination of therapeutic effect by comparing with healthy subjects. And they could also be useful parameters in infertility treatment as the association with reproductive function is revealed.

Our hospital conducts nutritional counseling for infertile patients to acquire high-quality embryos for IVF-ET. We inquire the diet and lifestyle of patients and then propose nutritional therapy that patients can practice themselves for improvement of embryo quality. However, the reality is that nutritional counseling especially focused on infertile patients is not much different from standard nutritional counseling. Therefore, we are aiming to reveal the relationship between embryonic quality and intestinal flora and to establish a new nutritional guidance regarding the diet which gives patients the intestinal flora having a beneficial influence on embryo quality.

We focus on dietary fiber, especially water-soluble fiber. The physiological influence of dietary fiber on increase of stool has long been known, and recently its involvement in carbohydrate and lipid metabolism have been revealed. The intake of dietary fiber in Japanese women is decreasing year by year, therefore nutritional counseling concerning dietary fiber is expected to work. We set a goal for the future of performing truly effective reproductive nutritional counseling.
The Clinical Use of LLLT (Low reactive-Level Laser Therapy) and Acupuncture and Moxibustion in Treatment of Infertile Patients

Shoji Kokeguchi
Hanabusa Women’s Clinic

Integrated medicine has been used for health care, and recently, it was used for refractory patients who have difficulty conceiving even with ART. Many clinics use supplements and Chinese medicine together as integrative medicine in ART treatment. We reported how many clinics implement LLLT and Acupuncture/Moxibustion treatment (A/M) from this research. Out of 280 ART clinics, only 16 clinics have adopted LLLT, and 19 clinics have adopted A/M. The A/M treatment is included in traditional medicine. It is a type of complementary and alternative medicine and has been used for infertile patients for a long time. Recently, LLLT has been attracting the attention of infertility specialists. Low reactive-Level Laser Therapy has been used for infertile patients because of the beneficial effects of increasing circulation of the reproductive organs and activation of mitochondrial function. We compared the medical effects of LLLT and A/M using a questionnaire. The results indicated that LLLT and A/M have the same curative effects on sensitivity to cold and poor circulation. The number of retrieval oocytes and embryos’ grade are improved slightly more using LLLT than A/M. Implantation rates are improved more using A/M than LLLT. Using both methods together for intractable infertile patients leads to higher pregnancy rates than using each method individually. From this research, we can show that LLLT and A/M might have distinctive and different curative effects on the reproductive system. We should continue the effort to prove the details and effective mechanisms of LLLT and A/M.

Reproductive Acupuncture for Conception Enhancement

Taiken Jo, OMD
Director, ACURA Acupuncture Clinic
Executive Director, Japan Institution for Standardizing Reproductive Acupuncture and Moxibustion (JISRAM)

Number of children born through ART is increasing rapidly. Nevertheless, ART is not a perfect cure for everyone. Providing adjunct treatment or solution when ART seem futile provides hope for patients. Most patients who seek help from acupuncture usually experience recurrent failed ART cycles or are told by their doctors that nothing further can be done. Some may seek help from acupuncture as a last resort in helping them conceive.

With this in mind, our treatment focuses on these key physiological reaction.

1) Blood flow improvement to the ovary
   Microvasculature of ovary is such that it requires abundant blood circulation for optimum folliculogenesis. Therefore, our technique strives to augment the blood flow to the ovarian blood vessels.
2) Blood flow improvement to the endometrium
   Low vascularity flow index and endometrial volume can be attributed to thin endometrium. Implantation capability could be jeopardized by insufficient blood flow. Therefore, our technique also strives to augment blood flow to the endometrium.
3) Regulation of the Hypothalamic–Pituitary–Gonadal axis (HPG axis)
   Acupuncture regulates dysfunction of HPG in several ways, and influence some gene expression of brain, thereby, normalizing secretion of endogenous hormones, such as GnRH, FSH, LH and E2
4) Regulation of the immune system
   Absence of a maternal immune response against the fetus and placenta during pregnancy is vital for the prevention of spontaneous abortion and transplant rejection. Immune system also plays an important role in follicular growth and ovulation. We believe acupuncture, in conjunction with LLLT, can be beneficial in the regulation of the immune system.
Acupuncture is generally intended to bring out the body’s potential to heal itself and balance the autonomic nervous system, thereby improving blood circulation and providing symptomatic relief. My goal in acupuncture for infertility is to improve the body’s inner balance and make it easier for women to become pregnant.

Acupuncture brings about a balanced nervous system, which improves the hormonal balance and allows the uterus and ovaries to function better.

Improved blood circulation reduces coldness in the abdomen and around the trunk.

Relief of muscle tightness and pain helps eliminate stress.

With these benefits in mind, I provide acupuncture to help women become pregnant.

In 1975, we have established both Ohshiro Clinic and Japan Medical Laser Laboratory (JMLL) as the first private clinic specializing laser medicine in the world and the first medical laser research and development center in Japan. In 1980, JMLL invented prototype device of low-power laser system (GaAlAs diode laser system) for pain relief, and we started pain clinic with this. In 1982, in collaboration with Matsushita Electric Industrial Corporation (Panasonic), we have developed Panalas 4000 system which was the first made-in Japan medical diode laser system approved by Minister of Health, Labor and Welfare. After 1992, JMLL independently developed the laser treatment devices, OhLase-3D1(Console type) and HT 2001(Handy type), both of which were got approved by the Ministry of Health, Labor and Welfare as medical laser devices in Japan. Ohshiro named a non-thermal laser-tissue interaction using low power laser LLLT (Low reactive Level Laser Therapy). From our clinical experience of LLLT, we have found that the menstrual cycle could be normalized and the menopausal symptoms could be alleviated by LLLT, in 1996 we started to apply the LLLT to infertility treatment.

In this presentation, we would like to introduce the Proximal Priority Theory in LLLT suggested by Ohshiro, and demonstrate the way how to treat the female infertile patient by LLLT.

In the management of patients in whom multiple attempts with IVF-ET have not resulted in pregnancy and childbirth (repeated failure patients), low reactive level laser therapy(LLLT) is expected to be effective as part of integrated medicine. And it’s important to consider the patient’s feelings while LLLT is being performed.

We evaluated the clinical results after LLLT, psychological and physical symptoms before and after LLLT, and its effects in patients who had experienced repeated IVF-ET failures. In our previous study, in patients who had not become pregnant by the first thaw-blastocyst transfer (T-BT) on a hormone replacement therapy (HRT) cycle, and were scheduled to undergo selected blastocyst transfer on a HRT cycle again between December 2011 and February 2014, LLLT was performed once a week until the day before T-BT (LLLT group), or T-BT was performed without LLLT (no-LLLT group).
between April 2009 and October 2012, and the pregnancy rates were compared (Evaluation 1). Psychological and physical symptoms were studied using our original questionnaire, and their changes after LLLT were evaluated (Evaluation 2). As a result, in Evaluation 1, the pregnancy rate was significantly improved by LLLT performed before T-BT. In Evaluation 2, beneficial effects other than pregnancy, such as alleviation of morbid sensitivity to cold, shoulder stiffness, and low back pain were observed after LLLT. [Matsuura D, Furui K et al. Journal of Japan Society of Assisted Reproduction, 2015, 18(2): 6-9]

This lecture will discuss and demonstrate the practical skill of LLLT, the communication between the patients and nurse, and the method of creating a relaxed environment for the patient. In addition, we will evaluate the effects and application methods of LLLT to cater to the patients' needs, communication and stress (counseling).

**Key words:** IVF-ET • patients with repeated failures • Low reaction level laser therapy (LLLT) • practical skill • counseling

### Hands-On II

#### LLLT in IVF Japan Group

**Yuki Tamura**, **Yoshiharu Nakaoka**, **Yoshiharu Morimoto**

1: **IVF Namba Clinic**  
2: **HORAC Grand Front Osaka Clinic**

LLLT in IVF Namba was started in 2008. My technique can be classified under two main procedures: the first irradiation is applied on to any area around the neck in contact laser therapy, and the second on to the 4 abdominal area. I instruct stretching and massage neck and shoulder of patient to improve blood flow. I also check the change of patient body temperature with thermography camera. I introduce my therapy. I would like to share my experience with audience.

### Hands-On IV

#### Piezo-ICSI micromanipulation for human oocytes

**Kenichiro Hiraoka PhD**

*Kameda Medical Center, Kameda IVF Clinic Makuhari, Tokyo Medical and Dental University*

Kameda Medical Center opened a branch clinic Kameda IVF Clinic Makuhari in Chiba city. In this clinic, the intracytoplasmic sperm injection (ICSI) is performed by using Piezo-ICSI technique for all oocytes. In our Piezo-ICSI technique, membrane breakage is performed by applying a Piezo pulse which produced ultra-fast submicron forward momentum using uniquely shaped flat-tipped microneedles with no bevel or spike. In our Piezo-ICSI technique, the wall thickness of the microneedle used is as thin as 0.45 micro meter, the sperm selection is performed under 1,200x magnifications, the sperm immobilization procedure is performed by applying two piezo pulses on the sperm's tail while the sperm's tail is attaching to the edge of the microneedle; the spindle status of the oocyte is observed whether the spindle can be visualize or not before sperm injection and the sperm injection is performed from the tail first into the cytoplasm. I performed Piezo-ICSI for 137 mature oocytes retrieved from 22 patients (27 cycles) between May 2016 and January 2017. The 2PN rate was 91.2%, the lysed rate was 2.6%, the 0PN rate was 2.6%, the 1PN rate was 1.8%, the 3PN rate was 1.8%, the good quality Day-3 embryo (≥ 7 cell with ≤ 15% fragments on Day-3) rate per 2PN oocytes was 70.9%, the blastocyst rate was 56.2% and the good quality blastocyst (scoring B or higher both ICM and trophectoderm grades by Gardner's criteria) rate was 29.2%. I will introduce the excellence of the Piezo-ICSI to you.