

The 9th World Congress of A-PART

Abstracts

Tuesday, April 21, 2009
CICG Geneva, Salle2
Geneva, Switzerland

The 9th World Congress of A PART

Date: April 21, 2009

Venue: CICG Geneva, Salle2 (Level0)

17 rue de Varembe, CH-1211 Geneva 20, Switzerland

Timetable of the 15th World Congress on IVF

Date: April 19 – 22, 2009

	Sunday April 19	Monday April 20	Tuesday April 21	Wednesday April 22
07:30	Registration Opens	Registration	Registration	Registration
08:30- 10:15	Pre-Congress Courses 1-3	Plenary Lectures	Plenary Lectures	Plenary Lectures
10:15- 10:45		Coffee Break	Coffee Break	Coffee Break
10:45- 12:30		Concurrent Sessions 1&2	A-PART Session (Symposium 1 & 2)	Concurrent Sessions 13&14
12:30- 14:00	Pre-Congress Courses 4-6	Sponsored Symposia Lunch Break Exhibit Poster Viewing	Lunch and Board Meeting	Sponsored Symposia Lunch Break Exhibit Poster Viewing
14:00- 15:45		Concurrent Sessions 3&4 Oral Communications 1	A-PART Session (Symposium 3 & 4)	Concurrent Sessions 15&16 Oral Communications 5
15:45- 16:15		Coffee Break	Coffee Break	Coffee Break
16:15- 18:00		Concurrent Sessions 5&6 Oral Communications 2	Concurrent Sessions 11&12 Oral Communications 4	Concurrent Sessions 17&18 Oral Communications 6
19:00	Opening Ceremony Welcome Reception		General Assembly of A-PART Gala Dinner	

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Program for the A-PART session in the 15th World Congress on IVF

10:45-11:25		Symposium 1: Natural cycle IVF and mild IVF cycle in reproductive medicine	
		Chair:	Wilfried Feichtinger, Vienna, Austria Hirofumi Kamiya, Sapporo, Japan
10:45	1	The role of the mild stimulation in the current IVF practice Artur Bernard, Budapest, Hungary	
10:58	2	Outcome of the recent IVF program transferring single blastocyst retrieved on minimal stimulation cycle and frozen by vitrification method Keiichi Kato, Tokyo, Japan	
11:11	3	Controlled ovarian stimulation, minimal stimulation or no stimulation for in vitro fertilization: is there an optimal stimulation? John Zhang, New York, USA	
11:25-12:30		Symposium 2: Management of PCO patients	
		Chair:	Yona Barak, Herzliya-On-Sea, Israel Yoshiharu Morimoto, Osaka, Japan
11:25	1	The benefit of IVM in PCOs patients Yona Barak, Herzliya-On-Sea, Israel	
11:38	2	Oxygen consumption by Scanning Electrochemical Microscopy (SECM) for human IVM COC & embryo with PCO patients Hiroaki Yoshida, Sendai, Japan	
11:51	3	Clinical data of IVM for PCO patients Yoshiharu Morimoto, Osaka, Japan	
12:04	4	Use of letrozole in PCOS Makio Shozu, Chiba, Japan	
12:17	5	PCOS: Management Strategies in 2009 Frank Yelian, Los Angeles, USA	
14:00-14:52		Symposium 3: ART for fertility preservation	
		Chair:	Steven Kaali, New York, USA Takafumi Utsunomiya, Oita, Japan
14:00	1	Fertility preserving options of reproductive age cancer patients Peter Kovacs, Budapest, Hungary	
14:13	2	The current approach to oocyte vitrification for cancer patients in Japan Takafumi Utsunomiya, Oita, Japan	
14:26	3	Successful vitrification of human oocytes Masashige Kuwayama, Tokyo, Japan	
14:39	4	Cryopreservation of prepubertal testicular tissue Atsumi Yoshida, Tokyo, Japan	
14:52-15:45		Symposium 4: Male infertility	
		Chair:	Herbert Zech, Bregenz, Austria Atsushi Tanaka, Fukuoka, Japan
14:52	1	Does a combination of Chinese herbs and acupuncture treatment affect sperm characteristics in infertile couple? -- A pilot study Ramon Velleman, Tel-Aviv, Israel	
15:05	2	Improvement of a photodynamic system for observation of seminiferous tubules in microdissection-testicular sperm extraction (MD-TESE) Atsushi Tanaka, Fukuoka, Japan	
15:18	3	A novel cryopreservation technique for very few motile sperm from severely infertile men Koichi Kyono, Sendai, Japan	
15:31	4	Arguments to implement the selection of spermatozoa at high magnification before ICSI Herbert Zech, Bregenz, Austria	

Symposium1-1: The role of the mild stimulation in the current IVF practice

Artur Bernard M. D. Ph.D.

Kaali Institute, IVF Center

Budapest Hungary

The birth of the first IVF child was achieved from a natural cycle./Steptoe and Edwards 1978/. The aim of the ovarian stimulation has been to produce, retrieve and fertilize more oocytes, and select afterwards the best embryos for transfer. Therefore in the 80's most centers used high dose stimulation to increase oocyte/embryo yield, and therefore to improve pregnancy rates. In 1991 transvaginal ultrasound guided follicle aspiration was introduced, which simplified the retrieval and made it more effective. In 1992 the introduction of ICSI improved dramatically the fertilizations rate. Assisted hatching, the availability of new transfer media and blastocyst stage transfer also improved the implantations rate significantly.. Therefore fewer follicles and oocytes were enough to achieve success in IVF treatment. This brought up the issue of mild stimulation again (R.G. Edwards 1996). The discovery and application the GnRH antagonists enhanced the use of the milder stimulation procedures. Due to the successful application of ICSI in the treatment of the male infertility, the number of the healthy and young IVF patients is increasing. The single embryo transfer-to avoid the multiple pregnancies- is also more widely used. Because of these facts, mild stimulation in the current IVF practice has an increasing importance. Clinical research has to focus on this kind of stimulation, which is safe, effective and cost saving.

Symposium 1-2 : Outcome of the recent IVF program transferring single blastocyst retrieved on minimal stimulation cycle and frozen by vitrification method

Keiichi Kato

Kato Ladies Clinic

Introduction

One of the major concerns of the modern assisted reproductive technology (ART) is to reduce the number of oocytes retrieved and transferred keeping pregnancy rate high. Ovarian hyperstimulation impose physical and financial burden on patients and transfer of multiple embryos cause multiple pregnancy, which cause maternal complications and neonatal morbidity and mortality. To overcome these issues, we have implemented a new IVF program in which small number of oocytes were retrieved under the clomiphene-based minimal stimulation cycle and frozen by vitrification method, and then followed by single blastocyst transfer under hormone-controlled or un-stimulated natural cycle. The purpose of this presentation is to report the most recent results of the program in which more than 10,000 ET cycles were performed in 2007.

Materials and methods

In 2007, 19,305 IVF cycles of minimal stimulation and 10,115 cycles of embryo transfer were performed. All cycles that the IVF protocol specified below were completed included in this study. The mean age of patient was 39.4 ± 4.8 years old. There was no exclusion criterion on cause of infertility.

Oocytes were retrieved on the minimal stimulation cycle: clomiphene citrate (50-100 mg/day) was administered from day 3 up to one days before the oocyte retrieval with or without hMG or rFSH (50-150 IU/every other day, from day 8 or later), to elaborate 1-4 follicles. LH surge was induced by nasal GnRH agonist (Sprecure®, 300 µg) when the size of the leading follicle is more than 21-24mm). 34-36 hr later, oocytes were retrieved and inseminated conventionally or by ICSI cultured to blastocysts. Fertilized oocytes were cultured individually in 20 µl of Cleavage medium (SAGE, USA) from day 1 to day 3, and then in Blastocyst medium (SAGE) from day 4 to day 6. In 4449 cycles, embryo (mostly day 5 blastocysts) were frozen by vitrification method using Cryotop® as described elsewhere. Single ET was performed using thawed embryos on the next cycle or later (4,449 cycles), or using fresh embryo (mostly day2 embryo) (5,666 cycles). For frozen-thaw transfer, uses of hormonal replacement cycle were a choice.

Results

The average number of oocyte picked up per cycle was 1.35 (26,100 / 19,305 cycles). At least one blastocyst was achieved in 54.6% of 19305 cycles. Average number of ET performed was 1.01. Overall survival rate of frozen blastocyst was 95.0%, comparable to that of cleavage stage (98%).

The pregnancy rates achieved frozen-thawed single blastocyst transfer were 41.3% (per ET), which were significantly higher than that obtained by fresh day2-embryo transfer (19.8%). Pregnancy rate showed age-dependent decrease in both frozen-thawed ET as in fresh ET, the positive impact of frozen ET was enhanced in the older age group (≥ 35 y.o.) compared to the younger group (< 34 y.o.).

The overall multiple pregnancy rates were as low as 1.3%, almost comparable to that of normal Japanese population. Ectopic pregnancy rate was 0.3%.

Conclusions

The clomiphene-based minimal stimulation IVF programs gave satisfactory outcomes, in terms of pregnancy rate, comparable to that achieved by standard controlled ovarian hyperstimulation methods in Japan, patient compliance and singleton pregnancy. Use of blastocyst ET also reduced ectopic pregnancy rate to the level of non-IVF pregnancy.

Symposium 1-3 : Controlled ovarian stimulation, minimal stimulation or no stimulation for in vitro fertilization: is there an optimal stimulation?

John Zhang and Osamu Kato

New Hope Fertility Center, New York NY, USA and Kato Ladies Clinic, Tokyo, Japan

The success of IVF treatment is assessed by four criteria consisting of pregnancy outcome, safety, patient comfortableness and cost. With improvement of embryo preservation with vitirfication and promotion of single embryo transfer it is the time to reassess various stimulation protocols in terms of safety and efficiency. In United States of American almost 95% of IVF cycles are performed through daily gonodotrope injection with GnRH analogs or antagonists to control premature ovulation (conventional IVF). Since 2004 we have elected to use primarily a mild stimulation protocol with clomiphene citrate (CC) in combination with a small addition of human menopausal gonadotropin (hMG) in our IVF cycles (minimal stimulation IVF).

Clomiphene citrate (50 mg) was initiated orally each day, beginning on Day 3. Subcutaneous administration of 75–150 IU of hMG every 48 hours was begun on Day 5 or 8 depending on Day 3 FSH level. For patients with Day 3 FSH level is more than 15 no Clomiphene citrate will be give and patient will only under natural cycle IVF. A gonadotropin releasing hormone agonist (GnRHa) nasal spray (Synarel) was administered to trigger an endogenous LH surge for final maturation of the oocytes. Oocyte retrieval was performed 34 hours after the administration of the spray

In this presentation we wish to compare various protocols of ovarian stimulation and would like to propose a concept of optimal ovarian stimulation which is based on daily FSH levels. Traditionally daily dosing of stimulation is tailored to patient age, ovarian reserve and how follicular development and estrogen hormone rising. A FSH level based stimulation will provide an opportunity to give the least medicine need to generate certain number eggs in each IVF cycles to minimizing hyperstimulation and save the cost of medications.

Symposium 2-1 : The benefit of IVM in PCOs patients

Yona Barak ,Olga Sikorska, Tomasz Rokicki, Aneta Karwacka, Tomasz Dworniak, Ricardo Faundez

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Objective. In vitro maturation (IVM) was established as a new method for treating infertility. The procedure was applied in our centers in patients showing Polycystic Ovary Syndrome (PCOS).

Design. Rate of maturation, fertilization, embryo scoring and pregnancies were compared in patients with PCOS, undergoing IVM procedure (study group) with patients undergoing IVF due to female mechanical cause of infertility (control group).

Materials and Methods. Twenty nine patients determined as PCOS underwent 32 IVM cycles. The control group (n=28) underwent conventional ICSI procedure. IVM patients were mildly stimulated using 150 I.U. Gonal-F for 3-4 days. IVM was performed according to the procedure of MediCult IVM System (MediCult, Denmark). ICSI was performed 30 hours (Day 0) after IVM medium (MediCult, Denmark). Injected oocytes were then cultured in MediCult ISM1 medium. Patients of control group were stimulated in the short protocol using 75 IU of Fostimon with GnRH antagonist Cetrotide. Ovulation was induced by Ovitrelle. Following ICSI, oocytes cultured identically as PCOS oocytes.

Number of retrieved oocytes, rates of fertilization, cleavage, quality of embryos and pregnancies were compared with PCOS and control group, by Yate's corrected χ^2 test. *Results.* Mean number of aspirated cumulus enclosed oocytes (OCCs) per patient was 8,8 and was significantly lower in comparison to the control (17,0). A significant difference in the rate of OCCs which reached the MII stage was observed after 30h in the PCO group in comparison to the control (138/257 (53,7%) vs 409/509 (79,4%), respectively, $p<0,01$). No differences were observed in fertilization rate (122/156 (78,2%) vs 337/377 (89,4%) however a significant difference was found in cleavage rates (101/119 (84,9%) vs 177/184 (96,2%), $p<0.05$). A higher value of the mean fragmentation grade of 4-5cell stage embryos was observed on day 2 in the PCO group in comparison to the control group (1,6 vs 1,3 respectively; $p<0.01$). Nine pregnancies out of 26 embryo transfers (ETs; 34,6%) were achieved in the PCO group; 23 ETs performed in the control group resulted in 13 pregnancies (46,4%; mean of 2.3 vs 2.0 embryos per patient, respectively).

Conclusions. Although conventional procedure in mechanical infertility patients is still superior, IVM was found to be an efficient method in patients with PCO syndrome. Since our results are based on a small number of patients, further studies are still ongoing.

Symposium 2-2: Oxygen consumption by Scanning Electrochemical Microscopy (SECM) for human IVM COC & embryo with PCO patients.

Dr. Hiroaki Yoshida

Yoshida Ladies Clinic Reproductive Research Center

Introduction: Recently, in vitro maturation (IVM) has been used for human assisted reproductive technology (ART) for women with PCO. IVM-IVF was performed for patients with PCOS in 2008. Among a total of 186 retrieved oocytes, the maturation rate was 60.7%, the pregnancy rate was 29.4%. As the both maturation and pregnancy rates were considered to be unsatisfactory low, the simultaneous use of nuclear and cytoplasmic maturation systems was considered in order to clarify the relationship between cytoplasmic maturation and mitochondrial distribution or function. Furthermore the mitochondrial function of IVM oocytes and the mitochondrial membrane potential were observed using transmission electron microscopy (TEM) and scanning electrochemical microscopy (SECM). As oxygen consumption is an ideal indicator of metabolic activity, SECM measuring systems have been demonstrated to successfully non-invasively measure respiration activity of single embryos from several species. It has been reported that the bovine embryos with higher oxygen consumption are better candidates for further development of good quality embryos and yielded higher pregnancy rates. Despite this apparent correlation between respiration activity and embryo quality, oxygen consumption in human IVM oocytes and embryos has yet to be evaluated.

Material and method: Cumulus oocyte complexes (COC) and oocytes were transferred into a plate filled with HFF99 medium. A microelectrode was used to scan along the z-axis from the edge of the sample and the oxygen consumption rate was calculated based on the spherical diffusion theory using custom software. Measurements of each oocyte were performed rapidly (within 1 min). Subsequently, a sample of each oocyte was collected for observation by TEM.

Results: We classified morphologically the COC and oocyte size from grade 1 to 5. Our observation suggested that oocyte maturation rate was decreased from grade 1 to 5. The consumption for a single oocyte was as follows: GV 0.49, MI 0.47, MII 0.41 $\text{F} \times 10^{14}/\text{mols}^{-1}$. Weak correlation with COC size and oxygen consumption rate before and after 26 hrs culture was observed; however there was no significant difference between oocytes before and after culture. These findings suggested that the oxygen consumption rate is representative of the COC respiration activity and that it can be used to indicate adequate oxygen consumption. There were no significant difference in the mean of oxygen consumption at each cleavage in embryos ($0.26 \sim 0.56 \times 10^{14}/\text{mols}^{-1}$).

Conclusion: The COC mitochondria which were enlarged and showed elongated morphology. Good quality oocytes have rich mitochondria and have gap junction between cumulus cells. IVM COC was found to be important for oocyte maturation and embryo development and it is strongly related to oocytes via gap junctions. There was no significant difference embryo consumption which moderate respiration rate of embryos showed high developmental rate to the blast cyst. Further study of cytoplasmic maturation and mitochondrial function from IVM oocytes and embryos is required.

Symposium 2-3 : Clinical data of IVM for PCO patients

Yoshiharu Morimoto, MD, PhD

CEO & Chairman

The Centre of Reproductive Medicine and Infertility

IVF JAPAN

Polycystic ovary syndrome (PCO) is a disease including ovulation dysfunction of ovaries or amenorrhea, hirsutism and obesity. It is one of the most important origins of infertility and is difficult to be treated. In the treatments of PCO, ovulation induction, birth control pills, operative procedures such as wedge resection of ovary and ovarian drilling under laparoscopy have been applied. Recently insulin sensitizing medications such as Metformin, Pioglitazone and Rosiglitazone have been added as an option.

In Vitro Maturation (IVM) has been first reported by Cha et al. (1991) as one of treatments for PCO and nowadays spread worldwide. In the initial stage, the clinical success rate of IVM was low, however, it has been improved remarkably by the development of media specified for IVM and puncture needle. Hence, IVM may be a preferable option to be selected in terms of prevention of ovarian hyperstimulation syndrome (OHSS) and accuracy.

We had the first success in IVM procedure in 1999 in Japan. The first baby was born next year, and first baby by frozen embryo transfer by IVM was born in 2001.

Our indication for IVM procedure is mainly for PCO. But we apply this procedure for the patients with normal menstrual cycles, rarely for the cases of poor quality embryo.

We start follicle monitoring from Day 7 of the menstrual cycle. At the day we can recognize at least two follicles of the diameter of over 7mm, immature oocytes are collected. The program should be cancelled when we see a dominant follicle or a ovarian cyst. HCG priming by 10000 iu is done 36 hours before oocyte retrieval. Follicles are aspirated by IVF OSAKA IVM needle (Kitazato Biopharma Co.ltd, Tokyo, Japan). The IVF OSAKA IVM needle is designed by ourselves and is composed of two segments of puncture needle and holding needle. MediCult IVM medium is arranged with 10% patient's serum and HCG and FSH. Retrieved oocytes are cultured for maturation for 24 hours and all matured oocytes are inseminated by ICSI.

Up to 2007, we had 705 IVM cases and 91 pregnancies. The live birth and on-going pregnancy rate was 78% and the rate of abortion and stillbirth was 23.1%. We had only one case of malformation which was Goldenhar syndrome.

From these data, we strongly recommend to select IVM option for the treatments of PCO patients as a first choice.

Symposium 2-4 : Use of letrozole in polycystic ovary syndrome (PCOS)

Makio Shozu, Tomoya Segawa*, Satoshi Kawachiya*, Osamu Kato*

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An aromatase inhibitor, letrozole, has recently been introduced into variable situations of ovarian stimulation. The major application of the drug is superovulation in IVF or IUI protocols, in which letrozole is administered combined with gonadotropins to increase the number of oocytes ovulated and the resulting pregnancy rate. Letrozole is also used for PCOS in an attempt to achieve single ovulation and singleton pregnancy. This is because, in contrast to clomiphene citrate (CC), letrozole potentially allows natural selection of a dominant follicle. An additional advantage of theoretical interest is seen with letrozole use for PCOS compared to CC. The shorter half-life of letrozole favors rapid recovery of endometrial thickening, which may enhance implantation rate. Ovarian hyperstimulation syndrome may also be avoided by reducing the number of oocytes. These advantages of letrozole over CC have been postulated based on clinical studies in infertile women with heterogeneous etiologies, including PCOS, and the advantages in PCOS remain inconclusive. The effectiveness of letrozole in infertile patients with PCOS (2003 ESHRE/ASRM criteria) was thus assessed. Over 400 PCOS patients treated with or without letrozole were included and reviewed for this analysis. With concomitant use of FSH (75 or 150 IU/every other day, days 8 - 12), letrozole (2.5 mg/day, days 3 - 7) was as successful for ovulation induction as CC (100 mg/day, days 3 - 7), with ovulatory rates of 89% and 83%, respectively. Total dose of FSH was significantly lower in the letrozole+FSH group (mean, 242 IU; range, 75 - 1050 IU) compared to the letrozole+CC group (mean, 406 IU; range, 100 - 1200 IU). Single ovulation rate was significantly higher in the letrozole+FSH group (68%) compared in the CC+FSH group (38%). Clinical pregnancy rate tended to be higher in the letrozole+FSH group (44%) than in the CC+FSH group (34%), although this difference was not significant. Letrozole is thus as effective as CC in terms of ovulation induction and may be superior to CC in terms of single ovulation even when administered with FSH.

Symposium 2-5 : PCOS: Management Strategies in 2009

Frank Yelian, MD, PhD, FACOG

LIFE Clinic, Los Angeles, California, USA

Polycystic ovarian syndrome is one of the most common endocrine disorders in reproductive women. It often presents with oligomenorrhea, androgen excess, insulin resistance, and obesity. The short and long term consequences of PCOS include ovulatory dysfunction and infertility; metabolic syndrome and type II diabetes; endometrial hyperplasia and cancer; dyslipidemia and cardiovascular diseases. PCOS is now considered to be a complex genetic disorder. It has been suggested that the disorder is a result of one of many intrinsic variant genetic traits that interact with other congenital or environmental factors to cause the endocrine dysfunction. The pathophysiology and clinical symptoms of the condition are caused by abnormal pituitary function; abnormal steroidogenesis in ovary and adrenal gland; and abnormal tissue response to various hormones. Due to the complexity of the disorder, the management strategies should be designed to tailor individual patient. Based on each patient's presentation and the goal of seeking for medical care, we should form a short and long term treatment plan. This presentation will address the current management strategies on androgen excess; ovulatory dysfunction; insulin resistance; endometrial protection; obesity and metabolic syndrome. Various options in ovulation induction and ART in management of PCOS will also be discussed.

Symposium 3-1 : Fertility preserving options of reproductive age cancer patients

Peter Kovacs MD, Steven G Kaali MD

Kaali Institute IVF Center, Budapest Hungary

Over the past few years the number of reproductive age survivors of cancer treatment has increased as a result of newer surgical methods and improved chemotherapeutic and radiation therapy regimens. Survival rates of hematologic malignancies (most lymphomas, leukemias are diagnosed in patients under the age of 40) have increased to 50-90%. Ten to forty percent of gynecologic malignancies are also diagnosed in reproductive age women. Less destructive surgical options, a wider availability of medical options and improved assisted reproductive technology all offer hope to a significant proportion of these women.

Because of these developments the fertility concerns of these women have to be addressed. As most treatment methods will still severely compromise or even destroy ovarian function the pre cancer treatment and post cancer treatment options need to be reviewed with the patient when appropriate. There are surgical, medical and ART options that may be utilized to maintain fertility.

Surgical options should be considered for patients diagnosed with gynecologic malignancies. They include conservative surgery for certain type and stage ovarian cancers, radical trachelectomy for early stages of cervical cancer and adnexal lateral position in case abdominal radiation is planned.

Medical options (GnRH agonist use) may help to reduce the toxic effects of chemotherapy. Progestin therapy is an alternative to surgery when well-differentiated, localized, early stage endometrial cancer is diagnosed.

ART is playing an increasingly important role in the management of these patients. For a long time embryo cryopreservation meant the only option. While it is still considered the most effective technique it cannot be used in all cases. In recent years oocyte freezing and ovarian tissue cryopreservation offer further options.

Finally, alternative options like donor egg use or adoption need to be mentioned to complete the list of choices these patients have.

During the presentation the role of the above-mentioned surgical, medical, ART methods will be reviewed including our own cases as examples. We wish to stress the importance of appropriate counseling of these patients which often requires a multidisciplinary approach including, psychiatrists, internists, surgeons, gynecologist, oncologists and fertility experts to address the problem.

Symposium 3-2 : The current approach to oocytes vitrification for cancer patients in Japan

Takafumi Utsunomiya¹, Osamu Kato², Hirofumi Kamiya³, Hiroaki Yoshida⁴, Yasuhito Michikura⁵, Masanori Ochi⁶, Yuji Fujino⁷, Kohji Yano⁸, Yasuyuki Mio⁹, Shokichi Teramoto¹⁰.

1 St Luke Clinic, Oita, 2 Kato Ladies Clinic, Tokyo, 3 Kamiya ladies clinic, Sapporo, 4 Yoshida Ladies Clinic, Sendai, 5 Towako ladies' clinic, Komatsu, 6 Yume clinic Nagoya, Nagoya, 7 Fujino Ladies Clinic, Osaka, 8 Yano maternity clinic, Matsuyama, 9 Mio fertility clinic, Yonago, 10 Shinbashi yume clinic, Tokyo, Japan.

OBJECTIVE:

Aggressive chemotherapy and radiotherapy have greatly enhanced the life expectancy of young cancer patients, but these treatments cause massive destruction of the ovarian reserve resulting in infertility or sterility. However, oocyte cryopreservation can preserve their fertility of these patients after cancer treatment. We applied a minimal ovarian stimulation protocol using clomiphene citrate (CC) to retrieve mature oocytes, and the cryotop vitrification method to cryopreserve them in Japan.

MATERIALS AND METHODS:

Thirty four unmarried hematopoietic defect patients with informed consent who underwent the CC cycle from January, 2007 to October, 2008. Fifty mg CC was administered from cycle day 3 and 75 IU recombinant FSH was administered every other day from day 8 until the leading follicle developed to 18 mm in diameter. Administration of CC was then stopped, and 300µg GnRH-agonist (buserelin) was given as a maturation trigger. Oocytes were retrieved from 30 to 36 hrs following the administration of the GnRH-agonist using a 22 gauge needle with local anesthesia (Teramoto, 2007). The retrieved oocytes were denuded before vitrification. The cryotop method (Kuwayama 2005) was used to vitrify the oocytes. The oocytes were equilibrated in 7.5% ethylene glycol and 7.5% DMSO in modified medium 199 (M-199) for 15min before being transferred into the vitrification solution (VS) for 30 sec. Oocytes were then transferred into onto the cryotop with minimum volume, and immediately submerged into liquid nitrogen.

RESULTS:

Oocyte retrieval and cryopreservation was successful in 89% (54/61) of the patients. No patients had any adverse side effects using the minimal ovarian stimulation protocol and aspiration with a 22 gauge needle under local anesthesia. The mean age of the patients was 26.0 (±4.8, S.D.). The mean numbers of oocyte retrieval cycles per patient was 1.79, and the mean number of retrieved oocytes per patient was 7.7, and per cycle was 4.3. The mean number of morphologically normal cryopreserved oocytes per patient was 6.4, and per cycle was 3.5. The type of cancers included acute and chronic leukemia, malignant lymphoma, aplastic anemia, and myelodysplastic syndrome.

CONCLUSION:

Our data showed that the minimal ovarian stimulation protocol using CC for oocyte retrieval with a 22 gauge needle and with local anesthesia was a safe, simple, effective method of preserving fertility for unmarried cancer patients.

Symposium 3-3 : Successful vitrification of human oocytes

Masashige Kuwayama

Advanced Medical Research Institute of Reproduction,
Kato Ladies' Clinic, Tokyo, Japan

Abstract

Recent drastic advances in cryobiology have made it possible to preserve various types of reproductive cells with relatively little loss of viability. Ultra-rapid vitrification, the alternative cryopreservation method seems to be a powerful tool to any biological specimens, which cannot be preserved by the conventional slow freezing and previous vitrification. Ultra-rapid vitrification realized the successful clinical use of vitrification not only for human PN zygotes, cleavage stage embryos and blastocysts but also for oocytes.

The purpose of this lecture is first to introduce the history of vitrification of human IVF, and the mechanism of vitrification of the cells, how to maintain the high viability of the oocytes and embryos under liquid nitrogen temperature, for your basic knowledge.

To provide evidence for the potential significance of vitrification, achievements with the Cryotop technology, an advanced version of the "minimal volume approaches" is analyzed. This technology alone has resulted in more healthy babies after cryopreservation of any stages of embryos than any other cryopreservation technique, and more successful human oocyte vitrification resulting in normal births than any other cryopreservation method. The value of this method is also demonstrated by achievements in the field of domestic animal embryology.

Cryotop method has been applied to more than 100,000 clinical cases of oocytes and embryo cryopreservation for these 8 years, and is now used in more than 800 IVF faculties in 12 countries producing excellent clinical results (nearly 100 % of post-thaw survival rates for PN zygotes, 4-cells stage embryos, blastocysts and oocytes) especially the method actually realized the oocytes cryopreservation as an effective protocol in Human IVF. More than 450 of healthy babies have been already delivered from Cryotop oocytes vitrification.

Human oocytes banks for unmarried young cancer patients and for egg donation program have also established and been giving dream and courage of life for the patients to improve their quality of lives as women.

Symposium 3-4 : Cryopreservation of prepubertal testicular tissue

Atsumi Yoshida, Yoko Kurotaki, Miho Tanaka, Hiroki Suzuki

Kiba Park Clinic

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For the progress of recent advances in cancer treatments, cure rates of childhood cancers are very high. However, these treatments may prove toxic to the testis. Fertility preservation is becoming an important issue in the management of the quality of life of prepubertal boys undergoing gonadotoxic treatment. As these patients do not yet produce spermatozoa for freezing, the only theoretical option for preservation of fertility in these boys is the preservation of the spermatogonial stem cells for autologous intratesticular stem cell transplantation or in vitro culture of spermatogonia. Currently, spermatogonial stem cell transplantation is considered to be the most promising tool for fertility restoration in young cancer patients. The spermatogonial stem cells are the male germline stem cells. They can self renew to maintain the stem cell population and produce large numbers of differentiating cells of the spermatogenic line. Spermatogonial stem cell transplantation was firstly introduced in the mouse by Brinster et al. In animals, the initial method for transplantation was intratubular microinjection in the seminiferous tubules. In human, the most common infusion technique for germ cells is ultrasound guided injection into the rete testis. Most studies on cryopreservation of testicular tissue aimed at preserving sperms for intracytoplasmic sperm injection. Testicular sperm freezing is carried out using in many instances. The simple rapid method of suspending straws in the uncirculated liquid nitrogen, whereas in freezing of spermatogonial stem cells, it is necessary to accomplish freezing by lowering slowly the temperature by means of a programmed freezer as is the case with round spermatids and late spermatids. At this institution, we make it a rule at present to perform freezing of testicular tissues in a programmed freezer, using a cryoprotectant solution containing sucrose and ethylene glycol. However, for prepubertal testicular tissues, it is considered necessary to re-evaluate the cryoprotectant and the program of freezing, taking account of their being unlike adult testicular tissues. Moreover, testicular tissue from cancer patients may be contaminated with cancerous cells. It would be of importance to restore, with discretion in various respects, testicular cells collected from a pediatric cancer patient into that patient after completion of cancer treatment.

Symposium 4-1 : Does a combination of Chinese herbs and acupuncture treatment affect sperm characteristics in infertile couple? -- A pilot study

Ramon Velleman¹, Tal Belo¹, Ilya Barr, Guy Cassuto³, Shai Davids¹, Yona Barak⁴

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Objective:

Classic therapies are known to have a limited effect in the treatment of patients with poor sperm characteristics. The aim of this study was to determine the effect of the combination of Chinese herbs and acupuncture on these males, who failed to conceive in their previous IVF-ICSI attempts

Design:

Preliminary prospective analysis

Material and Methods:

The study consisted of 12 couples who failed to conceive in at least 3 previous IVF-ICSI attempts. Patients were analyzed according to the "8 principles" of the Chinese Medicine. Herbs formulas were daily administrated. Acupuncture treatments, were done weekly. Sperm analyses were performed by light microscope, according to WHO criteria, before and 22 to 57 days (mean of 39.58±9.3 days) after the treatments started. A comparison of the following sperm parameters: volume of ejaculate, PH, sperm concentration, sperm motility and morphology, was performed before and during the study. These parameters were also compared with sperm criteria of 19 patients, who underwent 2 sperm analyses within a 4 months period of time, taking antibiotics in conventional treatments".

Results:

Out of the 12 couples, 6 pregnancies were achieved following our treatment and ART, 2 of which resulted in successful deliveries, 3 ongoing pregnancy and one ectopic. A higher rate of normal morphology was noticed in a comparison of rates of normal forms before and after 1 month of treatments using a paired T-Test (14.17±6.4 vs 26.58%±11.7, respectively; (F(29)=13.594, p<.001)). No change was noticed in the rate of normal forms in the control group (21.9±10.4 vs 25.26%±10.29).

Conclusion:

The combination of acupuncture and Chinese herbs may be a useful, no traumatic supporting treatment for males of couples which failed to conceive in IVF, and intend to undergo further fertility treatments. Since our pilot study is based on a small number of patients further investigation is still taking place.

Symposium 4-2: Improvement of a photodynamic system for observation of seminiferous tubules in microdissection-testicular sperm extraction (MD-TESE)

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Objective: Operating microscopes have long been used for microdissection-testicular sperm extraction (MD-TESE). Although “white, thick, and twisted” seminiferous tubules have been targeted in TESE, it is necessary to observe the inside of the seminiferous tubules through the walls for more accurate identification of sperm and sperm cells. From this point of view, we here investigated the potential of a newly modified photodynamic system.

Methods: (1) Ophthalmology contact type lenses (2.0~3.0X, Blumenthal Suturelysis lens, VOLK OPTICAL INC. USA) were placed at the focus on the light axis of the microscope. The light axis and focus distance were appropriately fine-tuned and it was possible to firmly fix the lenses placed at the front with an additional flexible arm for the surgical microscope. Magnification was improved by attaching a contact-type lens flexible arm for ophthalmology use to a surgical microscope. In addition, it was possible to clearly observe inner structure of the testicular tube by obtaining an appropriate lighting angle and height with an external light source (fiber-type). To fix the operational distance, “the direct contact method” was employed, and three-dimensional views were obtained by binocular vision. (2) By attaching ophthalmology lenses as an adaptor, magnification and stability of the attachment were improved. (3) An appropriate lighting angle and proper luminous intensity were obtained with an external fiber light source.

Results: 1. Sperm or spermatid were found totally in 35.3% (54/153) of non obstructive azoospermic men using this optic system. 2. In 85% (54/64) of TESE trials, spermatids and sperm were found when thick seminiferous tubules were filled with homogenous fine and whitish granules that did not move so much within. 3. No spermatogenic cells (primary spermatocyte, spermatid) were found and sperms were absent (0/25) when seminiferous tubules were filled with lower density, yellowish, coarse granules. Yellowish substances were debris. 4. Sperm or spermatogenic cells were likely to be found more frequently when the whitish granules in the seminiferous tubules were not moving so much compared to those when they were moving more. 5. Pregnancy rates and miscarriage rates after microfertilization using testicular sperm and spermatid were [38.9 % (7/18), 28.6% (2/7)], [22.2% (8/36), 37.5% (3/8)] respectively.

Conclusions: With this system, it was possible to three-dimensionally visualize the structure of seminiferous tubules and objectively evaluate the degree of attachment of sperm cells, cellular density, cellular sizes, and color from the wall thickness of the seminiferous tubules. Clinical results with this new photo dynamic system will be improved.

Symposium 4-3 : A novel cryopreservation technique for very few motile sperm from severely infertile men

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ABSTRACT:

Sperm of severe oligozoospermia and azoospermia frequently demand freezing for future fecundity. However, no reliable protocol for freezing very low numbers of sperm has been established. Therefore, we evaluated two new freezing procedures for sampling very low numbers of sperm.

We used some ejaculated semen from infertile men. Sperm was retrieved by putting a few sperm into a 2 µl droplet of cryoprotectant using a manipulator under a reverse microscope. The droplet was diluted by mixing 0.7 of Sperm Freeze™ (FertiPro, N.V., Belgium)(SF) and 1.0 of HTF (Irvine, USA). Each included some sperm a 2 µl droplet placed on 60 mm petri dish (Falcon1006, USA) was covered with mineral oil and wrapped in Saran wrap. It was left for 10 minutes at room temperature(RT), for 15 minutes at 4 °C, for 30 minutes on liquid nitrogen vapor, and immersed into LN₂.

On the other hand, in the tip group, 2 µl droplets were aspirated into a Cryotip^R (Kitasato Biopharma, Tokyo, Japan). It was left for 10 minutes at RT after closing the tip point, for 5 minutes on LN₂ vapor, and respectively and immediately immersed into LN₂.

In thawing procedure of the Dish group, the dish was thawed at RT until droplets with sperm were completely thawed, and sperm was retrieved by a manipulator. In thawing procedure of the Tip group, the cryotip was incubated in water of 37 °C for 20 seconds, and sperm was retrieved by a manipulator. We compared retrieval rates of sperm (RR), survival rates (SR) and available useful rates (AUR) for ICSI between the Dish group and the Tip group.

In the Dish group, RR was 97.3% (299/309), and SR was 30.0% (82/273), and motility rate after thawing was 2.9% (8/275), and reaction of hypo osmotic swelling test (Host), positive was 27.9% (74/265), and AUR was 26.5% (82/309). In comparison, in the Tip group, RR was 67.7% (239/353), and SR was 15.9% (36/226), and motility rate after thawing was 3.0% (7/232), and reaction of Host, positive was 13.2% (29/219), and AUR was 10.2% (36/353). RR, SR, and AUR were significantly different between the two groups (P<0.01).

In conclusion, it has been suggested that the freezing methods using a dish is a highly useful protocol for very low numbers of sperm. However, to raise the collection rate and the survival rate we need to try other kinds of cryoprotectant and improve the cryopreservation.

Symposium 4-4 : Arguments to implement the selection of spermatozoa at high magnification before ICSI.

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TEXT:

Selection of spermatozoa during ICSI procedure is performed at a rather low magnification at 200-400X with Hoffman Modulation interferential contrast (HMC) optics. Using such optical tools, selection of spermatozoa has severe limitations. This has been one of the major concerns related to ICSI as the sperm selection process, occurring during normal IVF in presence of cumulus cells, is totally bypassed. The changing of HMC by Nomarski differential interferential contrast and examination at high magnification, has allowed a better observation of sperm cells in real time. Using "motile-sperm organelle-morphology examination" MSOME, "normal spermatozoa" exhibit a large panel of nucleus malformations in terms of shape, size and presence of vacuoles are detected and normally not selected. However, a more acute selection of spermatozoa means that it is not always possible to find spermatozoa morphologically completely normal. Several studies reported that the existence of large vacuoles in the nuclei of spermatozoa dramatically reduces the proportion of good quality embryos reaching the blastocyst stage and logically the pregnancy (PR) and implantation (IR) rates. More over higher rates of abortion is also noticed.

As a consequence, the pending and crucial question concerns their meaning, origin and the further impact on embryo quality and downstream consequences on embryo development and more concerning, the progeny.

Can we assume that there is a relationship between chromatin defect - DNA damage and the presence of nuclear vacuoles? Two recent papers (Garolla 2008 and Franco 2008), reinforce the concept that an association between large vacuoles and secondary and tertiary DNA structure damages in sperm nucleus exists.

What are the consequences of DNA damage? In the light of the reports of Aitken (2007), the negative effect of sperm DNA fragmentation may affect the next generation (Aitken, 2007).

Furthermore a recent work of Fernandez-Gonzalez (2008) in the mouse model demonstrated that DNA-fragmented spermatozoa in ICSI can generate effects that emerge during later life, such as aberrant growth, premature aging, abnormal behaviour, and mesenchymal tumors.

Even though there is a strong "in vivo" selection after embryo transfer. We must be cautious, in order to lower the potential risks mentioned here.

In fine, the one of the most frequent questions regarding IMSI relates to its indications: should we perform IMSI to all the patients? Another debate raises the question of what should we do for patients carrying 100% large vacuoles in their sperm samples. Even though there is no real proof in the human species on the abnormal outcome generated by spermatozoa carrying vacuoles, this ultra-morphology technique has to be added as an additional tool for ICSI knowing the consequence of possible DNA damage for offspring (Carrell, 2008). Shall we continue the attempt with his sperm or propose the option of sperm donor? The establishment of new classification criteria, based on an

assessment system, seems a valuable approach to determine a threshold limit for making the right therapeutic decision. In conclusion, sperm selection before ICSI seems more and more unavoidable whatever the screening technique (hyaluronique binding test), in order to lower the potential risks detailed here. Analysis of semen samples on an ultramorphologic scale for the presence of vacuoles should therefore be recommended to patients before ICSI.