

A-PART 日本支部学術講演会 2005 要旨集

平成 17 年 7 月 23 日
都市センターホテル

A-PART 日本支部学術講演会 2005

1. 日時 2005年7月23日 13:45~17:15
2. 会場 都市センターホテル「コスモスホール」(3階)
〒102-0093 東京都千代田区平河町2丁目4番1号、TEL 03 (3265) 8211
3. プログラム
 - 12:30~ 受付
 - 13:45 開会の辞 宇津宮 隆史 (セントルカ産婦人科)
 - 13:50 Session 1: 座長 宇津宮 隆史 (セントルカ産婦人科)
「Single embryo transfer」
メッセージ紹介、
Pia Saldeen, MD, PhD.(IVF Clinic CURA, Malmo, Sweden)
 - 13:55 「Current status and future aspects of ART in United States」
John Zhang, MD, PhD. (New Hope Fertility Center, New York, USA)
 - 14:45 「もっとも有効な胚移植を目指して」
-加藤レディースクリニックにおけるSingle embryo transfer-
寺元章吉 (加藤レディースクリニック)
 - 15:35 Coffee break
 - 15:55 Session 2: 座長 長田 尚夫 (日本大学医学部産婦人科)
「移植胚卵管回帰説に基づく胚移植法の適応」
加藤 修 (加藤レディースクリニック)
 - 16:45 A-PART 日本支部総会
議案 1. 平成16年度事業報告ならびに決算報告
議案 2. 平成17年度事業計画案ならびに予算案
その他報告事項
 - ・理事会報告
 - ・6th World Conference of A-PART (New York) 開催のご案内
 - 17:15 閉会の辞: 加藤 修 (加藤レディースクリニック)
 - 17:45~ 懇親会 (都市センター1階アイリスにて)

以上

Reduction of twinning with remained pregnancy rate

Pia Saldeen, MD, PhD.

IVF Clinic CURA, Malmo, Sweden

Multiple pregnancy is considered to be a major complication after IVF treatment and is associated with increased risks for both mother and child. However, multiple pregnancy has until recently been regarded as almost inevitable in assisted reproductive techniques. Most neonatal morbidity after IVF treatment is related to the increased incidence of multiples and there is a widely accepted consensus in the IVF community that the proportion of multiple pregnancies after IVF must be reduced.

Elective single embryo transfer (eSET) is usually defined as transfer of one good quality embryo when at least two good quality embryos are available. In Sweden, a debate ended with legislative measures on January 1st, 2003: The Swedish National Board of Health and Welfare stated that all IVF treatments should be performed as SET, but two embryos may be transferred if the risk of twinning is considered low. eSET by legislation regarded necessary since no voluntary agreement had been reached.

In an article in Human Reproduction, 2005, we presented a retrospective study on the effects of the eSET legislation. The study comprised three periods, depending on which transfer policy was in force. Period I: Double embryo transfer policy (DET). Period II: Transitional period (public patients = SET, private = DET). Period III: SET in all. All women undergoing fresh ET were included in the study. Although a SET policy was in force during Period III, under certain circumstances DET was allowed also after 2003 (indications were poor embryo quality, female age > 39 years, > 3 previous failed IVF cycles). The study showed that it was possible to implement SET in 73% of all cycles, with a maintained viable pregnancy rate of 34 % and a reduction of twinning from 23% to 6%.

In a multicenter randomized controlled trial from Scandinavia [Thurin et al., 2004], 661 women were randomized to elective SET or DET. The inclusion criteria were: female age < 36 years, 1st and 2nd cycle and at least two good quality embryos available. The aim was to show that one fresh SET and (if pregnancy was not achieved) one additional frozen SET would give a similar birth rate as one fresh DET. The study showed that when frozen cycles were used in the SET group, the live birth rate was not substantially lower than in the DET group (38.8% and 42.9%, respectively). The authors concluded that these results support the introduction of eSET as an effective method for women less than 36 years to reduce multiple births due to DET.

The key factors in proceeding to eSET are embryo selection and patient selection.

Strandell et al. [2000] showed in a retrospective study that the variables predictive of birth and multiple birth, adjusted for the number of previous IVF cycles were: female age, number of good quality embryos, tubal infertility, and dose of FSH.

There is a decline in implantation rate with increasing age and women ≥ 38 years might not be the target group for eSET. In our unit, women ≥ 40 years are routinely offered DET although we are considering to decrease the cut off age for DET to 38. The rank of IVF cycle in a couple influences the outcome. In most studies eSET has been used in the 1st or 2nd cycle. At our clinic, eSET policy is applied in the first three cycles. The infertility diagnosis has been suggested to influence the results, with decreased chances in tubal infertility. However, we have not routinely applied DET in these cases.

The ovarian reserve has also been suggested to influence the results. At the ESHRE congress in Copenhagen in June 2005, we presented a paper named 'Is it reasonable proceeding to ovum pick up in elderly poor ovarian responders?' and reported on the astonishing poor results in poor responders ≥ 37 years: The pregnancy rate was only 3% per ovum pick-up (OPU). Poor response was defined as ≤ 5 follicles at the time of OPU. Furthermore, young poor responders also had a significantly lower pregnancy rate/OPU compared to young normo-responders (14% and 34.5% respectively, $P < 0.005$). The following variables were related to poor outcome (no pregnancy): number of oocytes retrieved, female age, FSH dose, number of oocytes/total FSH dose. We are now considering to include poor ovarian responders as candidates for DET.

Selecting the best embryo is of crucial importance when moving to eSET. The legislation of eSET are forcing IVF units to focus on their ability to select the embryo with the highest implantation potential for transfer. Most scoring systems are based on morphological assessments of zygotes or preembryos. With regards to early cleavage stage, the definition of top quality varies among different authors.

In our clinic we define a top quality embryo on day 2 as, 4 cells, $< 30\%$ fragmentation, equally sized and symmetrically arranged blastomeres, and no signs of multinucleation. Embryos cultured to day 3 are scored as top quality when 7-8 equally sized blastomeres with $< 30\%$ fragmentation are found. At the ASRM congress in Philadelphia 2004, we presented a paper showing that the pregnancy rate is significantly higher after SET with one day 2 preembryo with 4 cells and with all blastomeres being mononucleated (4 visible mononucleated blastomeres, MNBs) compared with SET with a preembryo having 4 cells but with 0-3 visible MNBs. In summary, this study showed that the number of MNBs in a day 2 four-cell preembryo is a strong predictor of the implantation potential, and the presence of 4 MNBs predicts a significantly higher implantation rate compared to other markers, such as degree of equality of blastomeres and rate of fragmentation. Furthermore, visualization of 4 MNBs predicted almost twice as high an implantation rate as a finding with not all blastomeres showing a nucleus. We therefore no longer consider a day 2 four-cell preembryo as a top quality embryo unless all four blastomeres

are mononucleated. However, caution should also be observed when considering DET with two 'four cell/0-3 nuclei' preembryos, since such preembryos still have a fairly high potential to implant (22 %).

In our setting the physician in charge is performing the embryo grading and selection. The grading is simple and done under a light microscope at X 200 magnification. The number of nuclei in each blastomere is routinely checked for on day 2. Until now, we have in the eSET group (< 40 years, rank of IVF cycle \leq 3) performed 1257 eSETs with an overall implantation rate of 37%. During the last 2,5 years, the proportion of SETs was 75%, the majority of these being eSET.

Intuitively, by reducing the number of multiple pregnancies the overall neonatal morbidity in children conceived with IVF technology should be reduced, but this remains to be demonstrated. The only solution to reduce the number of twins is by a substantial increase in SETs. Sweden undertook legislative measures for the introduction of eSET. The implementation of the SET policy turned out to be easier than expected. Swedish national data from the first year of SET policy indicate an overall increase in SET to 55% of all fresh IVF cycles, and a reduction in the multiple birth rate to 12% in 2003.

Current status and future aspects of ART in United States

John Zhang, MD, PhD

New Hope Fertility Center, New York, USA

Treatment of human infertility with assisted reproductive technology in USA has become a very big business and every year more than \$ 1,4 billion are spent in in vitro fertilization treatment. It also appears that the average pregnancy rate in USA is higher than many parts of the world. However, USA has also produced high multiple pregnancy rates in the world .

In 2002, a total of 115,392 IVF procedures were registrated with CDC and from those procedures 33, 141 live-birth deliveries and 45,751 infants have resulted. Noticeably the risk for multiple pregnancy in patients with fresh embryo transfer were 42% (donor egg IVF) and 35% (autologous IVF), respectively. While most of IVF centers still try to give patients high dose of FSH/hMG possible with hope to generate a large number of eggs. Indeed, it is a common practice in USA that an IVF attempt will be canceled for egg retrieval if less than 6 dominant follicles are produced. Generally doctors in USA believe that to improve IVF outcome need to produce a large number of eggs from each patient. Sometimes patients are misled to assess the competence of a doctor by check how many eggs are obtained from their egg collection procedures. It is the time to rethink IVF practice. The statement which needs to be promoted today in USA is use less medicine to produce a few good egg and perform single embryo transfer.

It increasingly becomes popular to add preimplantation genetics diagnosis (PGD) to daily IVF. Currently 10% of IVF cases are performed together with PGD and the number of PGD cases is still increasing. Studies from Munes group has showed that PGD with 9 DNA probes can improve implantation rate by 20%. Meanwhile the miscarriage rate reduces by 30%. Ideally, all embryos before transfer should be biopsied for aneuploidy screen to improve IVF outcome and reduce pregnancy loss. Eventhough it adds extra cost to IVF procedure ultimately it minimizes nonviable embryo transfer and pregnancy loss.

Infertility treatment technology has advanced dramatically in treating male infertility through intracytoplasmic sperm injection. Same progress has also been made in long term storage of human oocytes through vitrification techniques. However, there is hardly any progress in overcoming female infertility due to poor quality eggs. Currently the only solution is USA is through third party reproduction, donor egg IVF. Research has been carried out through nuclear transfer technique to reconstruct oocyte with hope to rescue the incapacity of cell machinery in human oocytes. Nuclear transfer is a technique through which a cell nucleus is removed and transferred into another cell whose nucleus has been removed in a similar manner.

Through nuclear transfer the nucleus from patient oocyte will undergo meiotic divisions in healthy cytoplasmic environment, thereby improve the quality of oocyte. Oocyte reconstruction through nuclear transfer has great potential in human reproduction. It can become a unique egg donation by using third party cytoplasm but retain patient own nuclear genetic materials. It also provides a new approach to have healthy children for female patients with mitochondrial diseases.

「もっとも有効な胚移植を目指して」

加藤レディースクリニックにおける Single embryo transfer

寺元 章吉

加藤レディースクリニック

緒言: 多胎妊娠は体外受精胚移植における深刻な問題であるが、SETによりその予防は完全に可能である。本研究では採卵周期当たりの妊娠率という視点から、ガラス化保存法による胚凍結を利用した反復SETを行い、多胎妊娠の予防と着床率の向上という相反する目的の解決を試みた。

対象と方法: 2004年1月より2005年4月の期間にhMG併用クロミフェン周期により採卵し分割胚を得た2160症例(37.4±3.6才)が対象である。卵巣刺激は、d3からのクロミフェン(50mg)連日投与(投与日数9.8±1.9日)とd8からのhMG(150IU)隔日投与(投与総量347±157IU)の併用により行い、卵子成熟化をGnRHa 300µgの鼻腔内噴霧により誘起し33:53±1:15後に採卵した。新鮮胚SETは最良4細胞期胚で行い、新鮮・余剰胚の扱いは移植歴により以下の如く分類した。

移植歴	新鮮胚の扱い	余剰胚の扱い
≦1回	4細胞期胚SET	4細胞期凍結+胚盤胞凍結(余剰杯≧2個)
2回	4細胞期胚SET	胚盤胞凍結
≧3回	移植(-)	全て胚盤胞凍結(→融解胚SET)

胚盤胞培養はBlastocyst-medium (Irvine Scientific)により胚盤胞径≧Φ180µmまで培養した後、クライオトップ法(桑山ら, 2000)によりガラス化保存した。

初回SET不成功時には、凍結4細胞期胚の融解SETを自然排卵後1日目に、また胚盤胞の融解SETを自然排卵後4.7±0.4日目にE2=153.5±53.5pg/ml、P4=16.0±6.0ng/mlのホルモン環境下で行った。

結果: 胚状況別に1胚移植の着床率を示す。表中非選択的とは分割胚が1個のみであった場合を意味する。

	非選択的新鮮	選択的新鮮		凍結胚盤胞1胚移植
	4細胞期1胚移植	4細胞期1胚移植	凍結4細胞期1胚移植	凍結胚盤胞1胚移植
周期数	498	406	169	848
GS陽性率	24.7%	40.5%	33.8%	41.9%
年齢	36.5±3.7	34.7±3.8	34.8±3.6	36.2±3.8

余剰胚、非余剰胚別の凍結成功率を示す。

	選択的 1 胚移植余剰胚	非余剰胚
周期数	406	1438
4 細胞期胚凍結率	130 周期、97.8% (42 歳未満 : 100.0%)	37 周期、100%
胚盤胞凍結率	276 周期、86.0% (40 歳未満 : 94.9%)	1401 周期、60.5% (40 歳未満 : 64.8%)

凍結胚盤胞の融解後生存率は 100% であった。

結論: クロミフェン周期における獲得卵子数は 2.7 ± 1.9 個と少なく全周期の 47% は必然的に単一胚移植となるが、複数分割胚が得られた周期では選択的 1 胚移植にガラス化保存法による胚凍結を利用した反復 S E T を併用することにより、胚を最大限に有効利用した単一胚移植が可能となる。また患者の既往歴・移植歴次第では全胚の胚盤胞凍結、融解 1 胚移植を行うことも多く、実際当院では 61.9% がこの凍結移植法の適応となっている。これら 1 胚移植の移植周期当たりの着床率は 36.1% であるが、反復試行後の積算妊娠率は選択的 1 胚移植では 85%、凍結胚盤胞では 68% であり、本法の高い有用性が証明される結果となった。

移植胚における卵管回帰説
(マウスモデル実証試験と臨床応用戦略)

加藤 修

加藤レディスクリニック

卵管閉塞患者における初期胚子宮移植後の妊娠率は胚盤胞移植により有意に改善されること、初期胚子宮移植後に約7-8%の子宮外妊娠が発生する（当院データ）ことから、子宮内に移植された初期胚は、本来あるべき卵管内へ移動するという仮説（子宮移植胚卵管回帰説：F.E.R）が提唱された（加藤修,2004 不妊学会）。本研究では、この仮説の真偽を明らかにするため、マウスを用いて子宮内へ移植された胚が卵管内へ輸送されるかどうかを検証するとともに、この事実を踏まえた体外受精胚の移植方法(移植胚ステージと移植日)の適応を考察した。

マウス実験の結果、子宮内移植した胚が、移植10分後では子宮内に、24時間後には卵管内に存在していたことから、胚が受動的に卵管内へ輸送されることが明らかとなった。すなわち、子宮へ移植された初期胚はそのステージでの発育に最適な環境である卵管膨大部まで輸送され、その後は自然妊娠と同様に卵管間質部で発育し胚盤胞となって再び子宮へ回帰、着床すると考えられる。これらの結果から、臨床においては、卵管因子不妊患者へは胚盤胞移植が、卵管正常患者へは初回移植は初期胚のDay2移植を行い、良好胚移植にもかかわらず妊娠不成立の場合には、潜在的卵管因子を踏まえて、胚盤胞移植の適応が適切と考えられる（SET）。

参加者の皆様

Pia Saldeen 先生、急病欠席によるプログラム変更のお詫び

謹啓

本日は、ご多忙のところ A-PART 日本支部学術講演会 2005 にご臨席を賜りまして誠にありがとうございました。

さて、Session 1 「Single embryo transfer」におきまして講演を予定しておりました、Pia Saldeen 先生方より 7 月 21 日に急病のため来日できないとの急報が入りました。御講演を楽しみにされていた皆様には大変申し訳ございませんが、やむを得ずプログラムの一部を急遽変更させて頂きました。

学会開催直前の出来事でもあり、参加者の皆様にご案内が間に合いませんでしたことを重ねてお詫び申し上げます。

尚、講演要旨につきましては、先生のご了解のもと、ご本人様からのお詫びのメッセージも添え、皆様に配布させていただきますので、ご一読くださいますようお願いいたします。

謹白

平成 17 年 7 月 23 日

A-PART 日本支部事務局

Malmö on July 21, 2005

Dear Colleagues,

I am very sorry I could not come and give my lecture on elective single embryo transfer and my opinion on how to select the optimal quality embryos for transfer. Since long I had looked forward for the journey and I was excited to discuss with you these important issues. I was really looking forward to visit your beautiful and exciting country.

But mother Nature had other plans for me. Three days before departure I fell ill with high fever and severe abdominal pain. I was admitted to the Malmö University Hospital yesterday and transferred to the Surgical Department. Last night I underwent emergency laparoscopy due to peritonitis.

I am still in the hospital (Thursday) and on intravenous antibiotics. The physician in charge strongly discourages me from travelling for another week, and are planning to keep me in hospital for at least 2-3 additional days.

Elective single embryo transfer is, in my opinion, the most attractive way to fight the problems and complications due to multiple pregnancy. We have been able to maintain the same pregnancy rate with single embryo transfer as we had with transfer of two embryos, at approximately 35%. Elective single embryo transfer is now performed in about 75 % of cases at our clinic. Our Obstetricians and Neonatologists are happy with this development and progress. However, double embryo transfer is still performed in selected cases such as elderly women, couples with poor embryo quality, etc.

With these words I wish you a successful meeting and fruitful discussions. Again, I am very sorry I could not join you.

With my warmest regards

Pia Saldeen