

between ovarian responsiveness and this polymorphism despite a relatively small sample size. Further, coupled with other markers this approach may ultimately provide a useful method to predict ovarian response and alter patient management accordingly.

O-079 What do the German population and sterile couples think about PGD? - a comparative study

A. Borkenhagen¹, Y. Stöbel-Richter², C. Fink³, E. Brähler⁴, H. Kentenich⁵
¹Charité Berlin, Medizinische Klinik f. Frauenheilkunde u. Berlin, Germany; ²Universität Leipzig, Abt. f. Med. Psychologie u. Med. Soziologie, Leipzig, Germany; ³Universität Leipzig, Abt. f. Med. Psychologie u. Med. Soziologie, Leipzig, Germany; ⁴Universität Leipzig, Abt. f. Med. Psychologie u. Med. Soziologie, Leipzig, Germany; ⁵Frauen- und Kinderklinik DRK Westend, Fertility Center Berlin, Berlin, Germany

Introduction: PGD is forbidden in Germany at present time. There is a controversial debate about the legalisation and regulation of new methods in reproductive medicine, especially PGD. But what is the opinion of the German population and prospective users? Our study is the first on the attitude of the German population and prospective German patients concerning PGD. **Material and methods:** Data from a representative survey conducted with 2110 persons between 18 and 50 years of age, will be presented. Here, people's general and specific attitudes towards PGD, social sexing and other aspects of modern reproductive medicine, as well as the corresponding knowledge towards this subject, were collected in a interviewer-administered survey and analysed. Results from the general German population will be compared to the quantitative findings raised by the research group Berlin where 100 infertile couples (respondents between 25 to 47 years of age) who attended the Fertility Center Berlin were given a questionnaire that ascertained the attitudes and knowledge about PGD especially the concerns about the different aspects of PGD, the willingness to undertake PGD, and the overall assessment of PGD, sex selection and aneuploidy screening.

Results: The general German population (70%), as well as infertile couples (85%), show an altogether high acceptance of modern reproductive medicine, especially PGD, and plead for a wide application of this procedures in Germany. The degree of acceptance of this practices is higher among infertile couples (85%), when the application is disease-related, than in the general German population. On the other hand, the general German population show a higher acceptance of the application of this techniques in a wide, non-disease-related way.

Infertile couples (85%) plead more for a application of PGD with the goal of diagnosing serious somatic and psychic diseases, than the general population (70%) does. Still, these couples (94%) accept the use of this technique to pursuit social sexing or to screen not disease-related characteristics less than the broad population (89%). The potential demand of PGD is also higher among the infertile couples ((79%) versus general German population (57%)) undergoing an infertility treatment, but only for disease-related reasons. The selection of non-disease-related characteristics is not an issue for these couples (4%) and also just matter in a small way for the general population (9%).

When considering the acceptance of surrogacy ("Leihmutterschaft"), also it is forbidden in Germany, there can be taken in to account of a significant difference between infertile couples (53%) and the general German population (36%).

Reproductive cloning, on the other hand, is still an unaccepted procedure for both groups - infertile couples (92%) and the general population (83%).

Conclusions: The study demonstrate a demand for PGD in Germany. A large proportion of general German population as well as infertile couples find the concept of preimplantation diagnosis more acceptable than that of prenatal diagnosis, when the pregnancy is at high risk. Furthermore, the results of this study indicate, that there is a considerable knowledge deficiency among both, general German population and infertile couples regarding the possibilities of modern reproductive medicine.

O-080 Clinical application of a novel quantitative method for rapid prenatal detection of common chromosome aneuploidies

F. Carvalho¹, E. Garcia², R. Mensink², A. Matias³, S. Dória¹, C. Alves¹, A. Barros^{1,4}

¹Dept Genetics, Faculty of Medicine, University of Porto, Porto, Portugal; ²GDPN-S Castedo, Porto, Portugal; ³Centre for Prenatal Diagnosis, Dept. Obstetrics, Hospital S. João, Porto, Portugal; ⁴Centre for Reproductive Genetics A Barros, Porto, Portugal

Introduction: Early prenatal diagnosis is important to either relieve parental anxiety in at risk pregnancies with normal karyotypes, or to allow timely decisions concerning the outcome of pregnancies when a chromosomal anomaly is detected. Prenatal diagnosis by conventional cytogenetics is reliable but time consuming, due to long culture periods. Fluorescence *in situ* hybridisation (FISH) on uncultured amniocytes has been used as an alternative method for rapid prenatal diagnosis of common aneuploidies. The advantage of Polymerase Chain Reaction (PCR) with fluorescent primers over FISH is that less material is required and the costs are lower. Here we describe the application of a novel quantitative method for rapid prenatal detection of common chromosomal aneuploidies. Multiplex Ligation-dependent Probe Amplification (MLPA) is a new, easy to perform and sensitive method for relative quantification of up to 40 different nucleic acid sequences in a single reaction.

Material and methods: A total of 240 amniotic fluids referred in a one year period for prenatal testing were used in the present study. Amniotic fluids were collected from women between 11 to 34 weeks of gestation. Heavily blood stained samples were excluded from the study. The most frequent indications for fetal sampling were advanced maternal age, followed by positive biochemical screening for Down's syndrome and ultrasound abnormalities. DNA extraction was performed using the ReadyAmp Genomic DNA Purification System (Promega), and 5 µl of DNA were used for MLPA. The probe mix included in this kit contains four probes for human chromosome X and Y target sequences, as well as 8 probes specific for each of chromosome 13, 18 and 21 sequences. In addition, 8 probes specific for other chromosomes were included. After ligation and PCR, the multiplex-fluorescent products were denatured and capillary electrophoresis was carried out in an ABI PRISM 310. Analysis of results and calculations of peak areas of both the target sequences (chromosomes 13, 18, 21, X, Y) and control chromosomes were performed using GeneScan Software (Applied Biosystems).

Results: From the 240 amniotic fluids, 1 case showed trisomy 13, 4 showed trisomy 18 and 2 showed trisomy 21. In all cases, conventional karyotyping confirmed MLPA results. No aneuploidies for chromosomes 13, 18, 21, X and Y were missed by MLPA. The efficiency of the method was not influenced by the gestational age.

Conclusions: This method represents a valid diagnostic tool for rapid detection of aneuploidies involving the chromosomes X, Y, 13, 18 and 21, within 24 hours. Together with the low cost of the procedure, the application of this method represents a preliminary tool to reduce prenatal anxiety before completion of cytogenetic analysis.

INVITED SESSION

Session 19 – Single Embryo Transfer: The Ultimate Goal?

Monday 28 June 2004

17:00–18:00

O-081 The cost of single embryo transfer

J. Collins
McMaster University, Hamilton, Canada

Background: Single embryo transfer (SET) is a simple, sensible and available means of preventing most ART twin births. Prevention is important because ART twins are common (20% to 30% of registry births), they are associated

with high rates of pre-term birth (7% < 32 weeks and 48% < 37 weeks), and they encounter more health problems during infancy and childhood (Helmerhorst *et al.*, 2004). Although cost of single embryo transfer is the subject here, the key concern is not cost, but the mortality and morbidity of prematurity associated with multiple birth. Costs are of interest mainly because they serve as a measure of the burden of illness.

Hospital cost of twin births: Reports include average charges per family from a single Boston hospital, based on 1,125 twin gestations (Callahan *et al.*, 1994); costs per family for 111 non-ART twins born at mean gestation of 34.7 weeks in Chicago (Luke *et al.*, 1996); and cost per family for 41 twin births with standard care in central Texas (Ruiz *et al.*, 2001). Due to the need for more intensive care, twin birth costs per family are approximately four-fold higher than singleton birth costs. This accords with the four-fold higher average neonatal cost of pre-term births compared with term birth (Petrou, 2003). Additional hospital admission costs during the first five years of life were three- to ten-fold higher for children from 13,574 pre-term births than for those from 226,120 term births (Petrou *et al.*, 2003). Because these hospital costs reflect higher perinatal, infant and childhood morbidity and mortality, twin births should be avoided whenever possible.

Cost of SET: One model of the costs of SET and double embryo transfer (DET) included pregnancy costs, neonatal care, handicap care and sick leave but not ART procedure or cryopreservation cycle costs (Wolner-Hanssen & Rydhstroem, 1998). DET costs (43,286 SEK) exceeded hypothetical SET costs (10,724 SEK). The large difference was due mainly to neonatal and handicap costs, which totalled 5,268 and 31,409 SEK, respectively, per SET and DET procedure. Another model involved the overall cost of ART procedures, cryopreserved embryo transfer cycles, pregnancy costs and neonatal costs, the latter represented by days of admission in a neonatal intensive care unit, but not handicap costs (De Sutter *et al.*, 2002). SET and DET costs were € 11,803 and 10,966, respectively, and remained similar even when different published outcomes for SET and DET were entered into the model.

Conclusions: SET costs are either far less than or similar to DET costs, depending on the outcomes embraced by the model. It is difficult to imagine a clinical scenario where SET costs could be substantially greater than DET costs. Even so, there should be no imaginable level of SET cost at which cost alone would be a deterrent to using SET as a means of reducing twin births.

References

- [1] Callahan,T.L., Hall,J.E., Ettner,S.L., Christiansen,C.L., Greene,M.F., & Crowley,W.F. (1994) The economic impact of multiple gestation pregnancies and the contribution of assisted reproduction. *New England Journal of Medicine*, **331**, 244-249.
- [2] De Sutter,P., Gerris,J., & Dhont,M. (2002) A health-economic decision-analytic model comparing double with single embryo transfer in IVF/ICSI. *Human Reproduction*, **17**, 2891-2896.
- [3] Helmerhorst,F.M., Perquin,D.A.M., Donker,D., & Keirse,M.J.N.C. (2004) Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *British Medical Journal*, **328**, 261-265.
- [4] Luke,B., Bigger,H.R., Leurgans,S., & Sietsema,D. (1996) The cost of prematurity: a case-control study of twins vs singletons. *American Journal of Public Health*, **86**, 809-814.
- [5] Petrou,S. (2003) Economic consequences of preterm birth and low birthweight. *BJ Obstet Gynecol*, **110**, 17-23.
- [6] Petrou,S., Mehta,Z., Hockley,C., Cook-Mozaffari,P., Henderson,J., & Goldacre,M. (2003) The impact of preterm birth on hospital inpatient admissions and costs during the first 5 years of life. *Pediatrics*, **112**, 1290-1297.
- [7] Ruiz,R.J., Brown,C.E., Peters,M.T., & Johnston,A.B. (2001) Specialized care for twin gestations: improving newborn outcomes and reducing costs. *JOGNN*, **30**, 52-60.
- [8] Wolner-Hanssen,P. & Rydhstroem,H. (1998) Costeffectiveness analysis of invitro fertilization: estimated costs per successful pregnancy after transfer of one or two embryos. *Human Reproduction*, **13**, 88-94.

O-082 Single embryo transfer. What does it offer?

A. Tiitinen

Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, PO Box 140, 00029 HUS, Finland

One of the main challenges in assisted reproductive treatment (ART) programmes is to avoid twin pregnancies without there being a significant decrease in the overall pregnancy rate (PR). In many countries more than 2% of new-borns are born after ART. Traditionally, ART has been associated with a 20-fold rate of multiple pregnancies compared with spontaneous twin pregnancies. This means that as many as half of the children born after assisted reproduction may have originated from multiple pregnancies. Neonatal outcome after IVF is worse than that in the general population of similar maternal age, parity and social standing, but this is mainly due to the large proportion of multiple births after IVF. However, an excess risk of low birth weight among singletons conceived with ART has been reported in a large population-based materials. In our hands, when comparing singleton IVF pregnancies with carefully chosen controls, the overall outcome has been identical.

The following recommendations were formulated during the ESHRE Campus Course (2001): A twin PR of 25% or more is not acceptable and the aim should be to reduce the incidence to perhaps around 10%. At the same time, an ongoing PR of 30% or more per started treatment cycle is very acceptable. We have shown in our clinic that this strategy can be introduced into IVF programmes. Indeed, the multiple pregnancy rate in our IVF programme in 2001 was only 7.5% and the multiple delivery rate very low, 5%. At the same time the overall ongoing PR had fallen slightly, but was still above 30%, which is even more than the natural conception rate. The policy of limiting the number of embryos transferred is not possible without a good cryopreservation programme. Elective SET combined with embryo cryopreservation can result in a cumulative pregnancy rate near to 50% per cycle.

Laboratory expertise is highly important in an eSET programme, especially in terms of embryo culture, embryo selection, and freezing and thawing techniques. Even though embryo quality is the most important single factor predicting pregnancy and birth, other factors should be remembered when individualising the transfer strategy. Endometrial receptivity is of importance, as well as the age of the woman. The quality and outcome of ovarian stimulation seems to be predictive: a good ovarian response to FSH stimulation leads to several mature oocytes, and could be a marker of good reproductive function as such. This leads to more numerous embryos and allows more choice for selection of one good embryo for transfer. Correct counselling is very important, as infertile couples are known sometimes to desire multiple pregnancies. It is likely that such couples are not really aware of the real risks associated with multiple pregnancies.

The need to prevent twin pregnancies is widely accepted, although transfer of two embryos is standard policy in many IVF centres. Our eSET programme shows that multiple PR under 10% can be achieved with acceptable delivery rates. The additional impact of a well-functioning cryopreservation programme on the overall cumulative PR per ovum pick-up is very important to bear in mind. The Finnish experience confirms the significance of elective SET. The effect of the increased use of this policy has already been observed as reduction in the proportion of twin deliveries following ART in Finland, as well as in a reduction of the proportion of multiple births in the Finnish Medical Birth Register. All this should arouse more interest in eSET in all countries.