Hum. Reprod. Advance Access published February 2, 2010
Human Reproduction, Vol.00, No.0 pp. 1–3, 2010
doi:10.1093/humrep/dep476

human reproduction

ESHRE PAGES

What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium steering committee†

Joyce Harper[1,12], Edith Coonen[2], Martine De Rycke[3], Francesco Fiorentino[4], Joep Geraedts[2], Veerle Goossens[5], Gary Harton[6], Celine Moutou[7], Tugce Pehlivan Budak[8], Pam Renwick[9], Sioban SenGupta[1], Joanne Traeger-Synodinos[10], and Katerina Vesela[11]

[1]UCL Centre for PG&D, Institute for Women's Health, University College London, 86-96 Chenies Mews, WC1E6HX London, UK [2]Department of Clinical Genetics, PGD working group Maastricht, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, The Netherlands [3]Centre for Medical Genetics, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium [4]GENOMA, Molecular Genetics Laboratory, Via Po, 102, 00198 Rome, Italy [5]ESHRE Central Office, Meerstraat 60, 1852 Grimbergen, Belgium [6]Genetics & IVF Institute, 3015 Williams Drive, Fairfax, VA 22031, USA [7]Service de la Biologie de la Reproduction, SIHCUS-CMCO, 19, Rue Louis Pasteur, BP120, 67303 Schiltigheim, France [8]Instituto Valenciano de Infertilidad (IVI), Istanbul, Oya Sokak No:23a, 34387 Mecidiyekoy, Istanbul, Turkey [9]Centre for PGD, Guy's Hospital, Great Maze Pond, SE1 9RT London, UK [10]Department of Medical Genetics, University of Athens, St. Sophia's Children's Hospital, Athens 11527, Greece [11]Sanatorium Repromeda, Vinicni 235, 615 00 Brno, Czech Republic

[12]Correspondence address. E-mail: joyce.harper@ucl.ac.uk

Since 2004, there have been 11 randomized controlled trials (RCTs) mainly for advanced maternal age (AMA), which have shown no benefit of performing preimplantation genetic screening (PGS). Ten of the RCTs have been performed at the cleavage stage and one at the blastocyst stage. It is probable that the high levels of chromosomal mosaicism at cleavage stages, which may result in the tested cell not being representative of the embryo, and the inability to examine all of the chromosomes using fluorescence *in situ* hybridization, have contributed to the lack of positive outcome from the RCTs. We suggest that future RCTs should examine alternative biopsy timing (polar body and/or trophectoderm biopsy), and should apply technologies that allow more comprehensive testing to include all chromosomes (microarray-based testing) to determine if PGS shows an improvement in delivery rate. Currently there is no evidence that routine PGS is beneficial for patients with AMA and conclusive data (RCTs) on repeated miscarriage, implantation failure and severe male factor are missing. To evaluate benefits of PGS, an ESHRE trial has recently been started on patients with AMA using polar body biopsy and array-comparative genomic hybridization, which should bring more information on this patient group in the near future.

Key words: PGS / ESHRE PGD Consortium / randomised controlled trial

The main goal of aneuploidy screening of embryos derived from subfertile patients undergoing IVF is to increase their chance of a healthy pregnancy. Preimplantation genetic screening (PGS) has mainly involved the aspiration of a single cell followed by fluorescence *in situ* hybridization (FISH) using probes for a limited number of chromosomes to determine the ploidy status of the embryo. Subsequently, euploid embryos are selected for transfer and aneuploid embryos are discarded and analysed to provide confirmatory diagnosis, or used for research.

The main indications suggested for PGS are advanced maternal age (AMA; usually defined as maternal age over 37 or 38 years), repeated implantation failure (RIF; usually defined as three or more failed embryo transfer procedures involving high-quality embryos), repeated miscarriage (RM) in patients with normal karyotypes (usually at least three previous miscarriages) and severe male factor (SMF) infertility

†Not externally peer-reviewed.

(usually defined as abnormal semen parameters; Goossens *et al*., 2009). In addition, PGS has been used for a variety of 'other' indications including a previous sporadic genetically abnormal pregnancy, poor embryo quality, previous radiotherapy and single embryo transfer (ESHRE PGD Consortium, unpublished data).

Since the publication of the first articles on PGS using cleavage-stage embryos (Gianaroli *et al*., 1997) and polar bodies (Munné *et al*., 1995a,b; Verlinsky *et al*., 1995), there have been numerous publications on this topic and PGS has been established in many IVF centres worldwide. There has been a steady increase in the number of PGS cycles reported to the ESHRE PGD Consortium, from 116 cycles in the data collection from 1997 to 1998 to 3900 cycles in 2007 (Goossens *et al*., 2009).

Until recently, the majority of studies on PGS were non-randomized studies with poor experimental design and inadequate control groups. Only a few of these studies reported delivery rate as the end-point, some involved small numbers of patients, some used 2-cell biopsies and some used low numbers or random sets of FISH probes (Twisk *et al*., 2006).

In 2007, Mastenbroek *et al*. published their RCT involving 200 patients per arm (control and treatment group) which showed a significant lowering of the delivery rate in patients who had undergone PGS. The article was highly criticized for its poor efficiency, low pregnancy rate in the control group, etc. (Cohen *et al*., 2007; Donoso *et al*., 2007). Meanwhile, the American Society of Reproductive Medicine (The practice committee of the Society of Assisted Reproductive Technology and the American Society of Reproductive Medicine, 2008), American College of Obstetricians and Gynaecologists (2008) and the British Fertility Society (Anderson and Pickering, 2008) have all issued statements that PGS should not be performed for any indication. In 2007, the ESHRE PGD Consortium was asked to write a position statement in reply to the Mastenbroek paper, but at that time there was insufficient data to write such a statement. Instead, a comment was written by some of the members of the steering committee (Harper *et al*., 2008). We did not feel that we could say that PGS should no longer be performed for AMA as there were many criticisms of the three randomized controlled trials (RCTs) published at that time (Staessen *et al*., 2004; Stevens *et al*., 2004; Mastenbroek *et al*., 2007). Instead our conclusion was that 'the most effective way to resolve the debate about the usefulness of PGS is to perform well-designed and well-executed randomized clinical trials'.

There are now 11 RCTs published on PGS, 10 using cleavage-stage biopsy (Staessen *et al*., 2004, 2008; Stevens *et al*., 2004; Mastenbroek *et al*., 2007; Blockeel, 2008; Hardarson *et al*., 2008; Mersereau *et al*., 2008; Debrock *et al*., 2009; Meyer *et al*., 2009; Schoolcraft *et al*., 2009) and one using blastocyst biopsy (Jansen *et al*., 2008). All have used FISH testing of a limited number of chromosomes and none have shown an improvement in delivery rates, with some showing a significant decrease in delivery rates after PGS. Most of the RCTs have been for patients with AMA (Staessens *et al*., 2004; Stevens *et al*., 2004; Mastenbroek *et al*., 2007; Hardarson *et al*., 2008; Debrock *et al*., 2009; Schoolcraft *et al*., 2009).

The lack of positive outcome from these RCTs can be explained by the likelihood that the tested blastomere is not representative for the whole embryo (Vanneste *et al*., 2009a). Indeed, high levels of chromosomal mosaicism have been observed in blastomeres from cleavage-stage embryos evaluated by FISH for a limited number of chromosomes in infertile women (Harper *et al*., 1995; Munne *et al*., 1995a, b) or by array technology for all chromosomes in fertile women (Vanneste *et al*., 2009b). Therefore, future work in this area should explore different timing for biopsy (polar body and trophectoderm biopsy) and the use of new technology that allows for more comprehensive screening of chromosomes (array-based technology). Already, clinics are applying array-comparative genomic hybridization (a-CGH) at the cleavage stage for PGS (Hellani *et al*., 2008). Before these procedures are used routinely, the array platforms need to be validated (Le Caignec *et al*., 2006; Fiegler *et al*., 2007, Mamas *et al*., submitted) and RCTs are needed to prove that use of this procedure will result in a significant increase in delivery rates (Harper and Harton, submitted).

We also said in our comment (Harper *et al*., 2008) that ESHRE was investigating setting up a multicentre RCT for PGS. This study has been set up and is currently undergoing initial trials to assess the technology (Geraedts *et al*., 2009). The trial will be performed on patients with AMA using polar body biopsy and a-CGH. To fully explore the PGS question, a similar trial will need to be conducted on blastocyst biopsy.

As for PGS for other indications, such as RIF (Blockeel *et al*., 2008), RM and SMF, there is a lack of data. Like the use of PGS for AMA, it would stand to reason that a different stage of biopsy and array-based technology would be needed to assess these indications. Centres that have already begun RCTs using 'older' methods (cleavage-stage biopsy with FISH testing for a limited set of chromosomes) are encouraged to finish the trial and report the results to add to the literature. Different subgroups of each indication and different age ranges of the patients chosen for the RCTs and culture methods could affect the results of the RCTs (Beyer *et al*., 2009). We strongly recommend that clinics interested in these indications should perform RCTs to validate the use of PGS for these patients using delivery rate as the standard outcome measure.

Conclusion

The widespread use of PGS without evidence of its ability to improve delivery rates has been a problem in the field of IVF. We must learn from this experience and ensure that techniques are brought into our treatment programmes only when there is scientific data to support their use. It is hoped that other centres will undertake rigorous RCTs to validate the use of PGS so that in future only proven techniques are applied in clinical practice.

There is now ample evidence that PGS for AMA using cleavage stage biopsy and FISH testing of a limited number of chromosomes is not a valid procedure and should be replaced by more appropriate approaches. Until results of RCTs using a different biopsy stage and arrays can demonstrate a significant increase in delivery rates, there is no evidence that routine PGS is beneficial for patients with AMA. Currently there is a lack of scientific data on the use of PGS applied for RM, implantation failure and SMF and so RCTs are also required for these indications. Multicentre studies such as the one now launched by ESHRE should be encouraged to obtain information on best approaches and eventually establish valid techniques for PGS in routine practice in the benefit of patients.

References

American College of Obstetricians and Gynaecologists. ACOG Committee Opinion No. 430: preimplantation genetic screening for aneuploidy. *Obstet Gynecol* 2008;**113**:766–767.

Anderson RA, Pickering S. The current status of preimplantation genetic screening: British fertility society policy and practice guidelines. *Hum Fertil* 2008;**11**:71–75.

Beyer CE, Osianlis T, Boekel K, Osborne E, Rombauts L, Catt J, Kralevski V, Aali BS, Gras L. Preimplantation genetic screening outcomes are associated with culture conditions. *Hum Reprod* 2009; **24**:1212–1220.

Blockeel C, Schutyser V, De Vos A, Verpoest W, De Vos M, Staessen C, Haentjens P, Van der Elst J, Devroey P. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. *Reprod Biomed Online* 2008;**17**:848–854.

Cohen J, Wells D, Munné S. Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. *Fertil Steril* 2007;**87**:496–503.

Debrock S, Melotte C, Spiessens C, Peeraer K, Vanneste E, Meeuwis L, Meuleman C, Frijns JP, Vermeesch JR, D'Hooghe TM. Preimplantation genetic screening for aneuploidy of embryos after in vitro fertilization in women aged at least 35 years: a prospective randomized trial. *Fertil Steril* 2009; [Epub ahead of print February 25].

Donoso P, Staessen C, Fauser BCJM, Devroey P. Current value of preimplantation genetic aneuploidy screening in IVF. *Hum Reprod* 2007;**13**:15–25.

Fiegler H, Geigl JB, Langer S, Rigler D, Porter K, Unger K, Carter NP, Speicher MR. High resolution array-CGH analysis of single cells. *Nucleic Acids Res* 2007;**35**:e15.

Geraedts J, Collins J, Gianaroli L, Goossens V, Handyside A, Harper J, Montag M, Repping S, Schmutzler A. What next for preimplantation genetic screening? A polar body approach!. *Hum Reprod* 2009; [Epub ahead of print December 23].

Gianaroli L, Magli MC, Munné S, Fiorentino A, Montanaro N, Ferraretti AP. Will preimplantation genetic diagnosis assist patients with a poor prognosis to achieve pregnancy? *Hum Reprod* 1997; **12**:1762–1767.

Goossens V, Harton G, Moutou C, Traeger-Synodinos J, Van Rij M, Harper JC. ESHRE PGD Consortium data collection IX: cycles from January to December 2006 with pregnancy follow-up to October 2007. *Hum Reprod* 2009;**24**:1786–1810.

Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, Reismer E, Borg K, Wikland M, Bergh C. Preimplantation genetic screening in women of advance maternal age decrease in clinical pregnancy rate: a randomized controlled trial. *Hum Reprod* 2008; **23**:2806–2812.

Harper JC, Coonen E, Handyside AH, Winston RM, Hopman AH, Delhanty JD. Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. *Prenat Diagn* 1995;**15**:41–49.

Harper JC, Sermon K, Geraedts J, Vesela K, Harton G, Thornhill A, Pehlivan T, Fiorentino F, SenGupta S, de Die-Smulders C *et al*. What next for preimplantation genetic screening (PGS)? *Hum Reprod* 2008; **23**:478–480.

Hellani A, Abu-Amero K, Azouri J, El-Akoum S. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. *Reprod Biomed Online* 2008;**17**:841–847.

Jansen RP, Bowman MC, de Boer KA, Leigh DA, Lieberman DB, McArthur SJ. What next for preimplantation screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy. *Hum Reprod* 2008;**23**:1476–1478.

Le Caignec C, Spits C, Sermon K, De Rycke M, Thienpont B, Debrock S, Staessen C, Moreau Y, Fryns JP, Van Steirteghem A *et al*. Single cell aneuploidy detection by array CGH. *Nucleic Acids Res* 2006; **34**:e68.

Mastenbroek S, Twisk M, Van Echten-arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NEA, Arts EGJM, De Vries JWA, Bossuyt PM *et al*. *In vitro* fertilization with preimplantation genetic screening. *N Engl J Med* 2007;**357**:9–17.

Mersereau JE, Pergament E, Zhang X, Milad MP. Preimplantation genetic screening to improve in vitro fertilization pregnancy rates: a prospective randomized controlled trial. *Fertil Steril* 2008; **90**:1287–1289.

Meyer L, Klipstein S, Hazlett W, Nasta T, Mangan P, Karande VC. A prospective randomized controlled trial of preimplantation genetic screening in the 'good prognosis' patient. *Fertil Steril* 2009; **91**:1731–1738.

Munné S, Dailey T, Sultan KM, Grifo J, Cohen J. The use of first polar bodies for preimpantation diagnosis of aneuploidy. *Hum Reprod* 1995a;**10**:1015–1021.

Munné S, Sultan KM, Weier HU, Grifo JA, Cohen J, Rosenwaks Z. Assessment of numeric abnormalities of X, Y, 18, and 16 chromosomes in preimplantation human embryos before transfer. *Am J Obstet Gynecol* 1995b;**172**:1191–1199.

Schoolcraft WB, Katz-Jaffe MG, Stevens J, Rawlins M, Munne S. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. *Fertil Steril* 2009; **92**:157–162.

Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I, Van Steirteghem A. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004; **19**:2849–2858.

Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, Devroey P. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. *Hum Reprod* 2008;**23**:2818–2825.

Stevens J, Wale P, Surrey ES, Schoolcraft WB. Is aneuploidy screening for patients aged 35 or over beneficial? A prospective randomized trial. *Fertil Steril* 2004;**82**:249.

The practice committee of the Society of Assisted Reproductive Technology and the American Society of Reproductive Medicine. Preimplantation genetic testing: a practice committee opinion. *Fertil Steril* 2008;**90**:S136–S143.

Twisk M, Mastenbroek S, van Wely M, Heineman MJ, van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in *in vitro* fertilisation or intracytoplasmic sperm injection. *Cochrane Database Syst Rev* 2006;**1**:CD005291.

Vanneste E, Voet T, Melotte C, Debrock S, Sermon K, Staessen C, Liebaers I, Fryns JP, D'Hooghe T, Vermeesch JR. What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for the low success rate. *Hum Reprod* 2009a;**24**:2679–2682.

Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F *et al*. Chromosome instability is common in human cleavage-stage embryos. *Nat Med* 2009b;**15**:577–583.

Verlinsky Y, Cieslak J, Freidine M, Ivakhnenko V, Wolf G, Kovalinskaya L, White M, Lifchez A, Kaplan B, Moise J *et al*. Pregnancies following preconception diagnosis of common aneuploidies by fluorescent *in-situ* hybridization. *Hum Reprod* 1995;**10**:1923–1927.