Chromosomal aneuploidy in embryos conceived with unstimulated cycle IVF

W. Verpoest1,6, B.C. Fauser2, E. Papanikolaou1,3, C. Staessen4, L. Van Landuyt1, P. Donoso1,5, H. Tournaye1, I. Liebaers4 and P. Devroey1

1Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, 101 Laarbeeklaan, B-1090 Brussels, Belgium; 2Departement of Reproductive Medicine and Gynaecology, University Medical Centre, Utrecht, The Netherlands; 3Centre for Assisted Reproduction ‘Biogenesis’, Genesis Hospital, Thessaloniki, Greece; 4Centre for Medical Genetics, Universitair Ziekenhuis Brussel, Brussels, Belgium; 5Unidad de Medicina Reproductiva, Clinica Alemana, Santiago, Chili

6Correspondence address. Tel: +32-2477-6699; Fax: +32-2477-6649; E-mail: willem.verpoest@uzbrussel.be

There is an ever increasing trend in reproductive medicine to reduce the intensity of ovarian stimulation for in vitro fertilization (IVF) and to restrict the number of embryos that are transferred into the uterine cavity. Recent findings suggest that the magnitude of ovarian stimulation affects the proportion of euploid embryos. As a result of the restriction in the number of embryos transferred, it becomes even more important to select the embryo with optimum implantational and developmental potential. Our aim was to assess the prevalence of numerical chromosomal abnormalities (aneuploidy) in unstimulated cycle IVF embryos. Thirty patients (mean age 31.4 years) underwent oocyte retrieval in a natural cycle without any form of ovarian stimulation, followed by intracytoplasmic sperm injection and Preimplantation genetic aneuploidy screening (PGS) for chromosomes X, Y, 13, 16, 18, 21 and 22. Out of 30 cycles, 21 oocytes were retrieved, 15 of which fertilized successfully. Eleven embryos developed sufficiently in order to undergo the PGS analysis, and four embryos proved to be aneuploid (36.4%; 95% CI: 10.9–69.2%). Six normal embryos were transferred in utero, resulting in three ongoing pregnancies. Two healthy girls were born and one patient miscarried. Numerical chromosomal abnormalities (aneuploidy) are present even in embryos of young women, and in the absence of ovarian stimulation.

Introduction
Many authors have suggested a return to milder ovarian stimulation and even natural cycle IVF in order to avoid potentially negative effects of multifollicular ovarian stimulation on women’s health (Edwards, 1996; Olivennes and Frydman, 1998; Fauser et al., 1999). Reducing the intensity of ovarian stimulation as well as the number of embryos for intrauterine transfer are measures advocated to minimize the impact of ovarian stimulation on endometrial receptivity, hence to improve reproductive outcome, and to reduce the risk of complications such as multiple pregnancies and ovarian hyperstimulation syndrome (Papanikolaou et al., 2006; Heijnen et al., 2007). Ovarian stimulation may also induce numerical chromosomal abnormalities (aneuploidy) in the oocyte, due to errors at different levels, including first and second meiotic oocyte divisions, as well as genomic imprinting disorders (Sato et al., 2007). Recent studies using Preimplantation genetic aneuploidy screening (PGS) have suggested that the proportion of aneuploidy in embryos is reduced by milder ovarian stimulation (Baart et al., 2007).

PGS to detect numerical chromosomal abnormalities (aneuploidy) of embryos is increasingly performed in in vitro fertilization (IVF), aiming to improve the efficacy of reproductive treatment (Goldman, 2007). There is, however, increasing controversy concerning the diagnostic accuracy and clinical efficacy of this technique (Shahine and Cedars, 2006; Twisk et al., 2006; Mastenbroek et al., 2007; Harper et al., 2008).

Here, we report the prevalence of aneuploidy in embryos resulting from unstimulated cycle IVF and PGS, and the ensuing pregnancies including two live births.

Materials and Methods
The study design was a prospective observational study at a tertiary referral University Hospital for intracytoplasmic sperm injection (ICSI) and preimplantation genetic diagnosis (PGD).

Patients
Thirty consecutive patients were included from 1 October 2005 until 30 April 2006. Inclusion criteria for participation in the study were: (i) maternal age ≤36 years of age, (ii) regular menstrual cycle (25–35 days), (iii) less than three failed IVF cycles, (iv) normal intrauterine cavity after pretreatment assessment and (v) consent to undergo oocyte retrieval in an unstimulated cycle. Exclusion criteria were: (i) the use of testicular sperm (ejaculated sperm only),
(ii) early (Day 3) follicular phase follicle stimulating hormone (FSH) levels $\geq 15$ IU/l, (iii) American Fertility Society (AFS) grades III and IV for endometriosis, (iv) IVF/ICSI with PGD, (v) body mass index (BMI) $\geq 28$, (vi) karyotype abnormalities in either patient or partner, as well as either one of the partners being carrier of a known genetic disorder. Baseline investigations were performed according to the unit guidelines. Transvaginal ultrasound was performed in all patients prior to recruitment. Patients could participate in the study only once. The study was conducted from 1 October 2005 until 30 April 2006. Approval of the local Ethics Committee was obtained. The patients were requested to sign informed consent prior to the treatment procedure.

**Procedures**

In a natural, unstimulated menstrual cycle, the patient underwent transvaginal ultrasound and serum hormone analysis for FSH levels, luteinizing hormone levels, estradiol and progesterone. Final oocyte maturation was triggered with 5000 IU urinary hCG as the sole medical intervention, as soon as a follicle of 16 mm was seen at ultrasound. In the case of drop of estradiol and concomitant rise of progesterone $>1.5$ nmol/l, ovulation had to be assumed and oocyte retrieval had to be cancelled. The oocyte retrieval (OPU) was scheduled at 32 h after hCG administration. The oocyte retrieval was performed under local anesthesia using Scandicaine 2%, without premedication, and the patients were allowed to leave the unit 1 h after the procedure.

ICSI was used in all cycles in order to reduce the risk of non-fertilization. The details of the IVF and ICSI procedure have been described previously (Van Landuyt et al., 2005). Fertilization was assessed after 18 h and embryo development was further evaluated on Days 2 and 3 prior to the biopsy. Embryo biopsy was performed on embryos at a 6-cell or later stage of development. A hole was made in the zona pellucida using two or three laser pulses of 5–7 ms of a non-melting 1.5 nm diode laser system (Fertilase; Octax, Herbron, Germany) coupled to a micromanipulator on an inverted microscope. One blastomere containing a nucleus was gently aspirated through the opening. The blastomere was fixed on a slide using the HCl/Tween 20 method. A two-round FISH procedure was performed, which allowed for the analysis of chromosomes X, Y, 13, 18 and 21 (Multivision PGT Probe Panel; Vysis, Downers Grove, IL, USA) in the first round, and chromosomes 16 and 22 in the second round.

The embryo transfer was performed at the blastocyst stage on Day 5 after oocyte retrieval. Luteal support was not given.

**Results**

The mean age of the patients was 31.4 years. Indications for reproductive treatment in the study population were unexplained infertility in 12 patients (40%), oligoasthenoteratozoospermia in 17 patients (56.7%) and tubal block in one patient (3.3%).

In twenty cycles, one oocyte was successfully retrieved, 19 of which were at metaphase II and therefore suitable for ICSI. Fifteen oocytes fertilized successfully, and 11 embryos developed adequately to the cleavage stage with the presence of at least six blastomeres. These embryos were considered suitable for single blastomere biopsy. Four embryos were aneuploid at screening, i.e. one embryo was diagnosed with monosomy 16, one with trisomy 22, one with trisomy 16 and the fourth aneuploid embryo showed combined abnormalities. Of the embryos diagnosed as aneuploid, results of single blastomere biopsy were confirmed at re-analysis in two embryos, whereas of the two remaining aneuploid embryos, one appeared to be mosaic and the other embryo euploid. On this basis the %aneuploidy in this sample was 36.4% (95% CI: 10.9–69.2). In one embryo, no diagnosis could be made due to signal absence. Six euploid embryos were transferred. Three ongoing pregnancies were achieved, two healthy girls were born, with a birthweight of 3960 and 3110 g, and one patient miscarried.

**Discussion**

The results of this study illustrate in the first place that absence of exogenous ovarian stimulation does not rule out the occurrence of numerical chromosomal abnormalities of embryos. A number of studies both in animal as well as in human oocytes have suggested increased aneuploidy rates after ovulation induction (Boué and Boué, 1973; Gras et al., 1992; Van Blerkom and Henry, 1992). More recent studies (Sato et al., 2007) have shown an increased incidence of genomic imprinting disorders in oocytes following ovulation induction and *in vitro* maturation. This may not only be due to maternal background factors such as age, but also to external factors such as suboptimal or abnormal follicular fluid biochemistry and cytoplasmic development. Moreover, aneuploidy rates in human oocytes are known to be substantially higher compared with animal species oocytes, which, as far can be assessed, could be due to ovarian stimulation, as this is the only setting in which aneuploidy in oocytes was determined. Other factors such as sperm quality (Obajasu et al., 1999; Pfeffer et al., 1999; Vegetti et al., 2000) and embryo culture conditions (Van Blerkom and Henry, 1992) can affect the chromosomal constitution of an embryo, giving rise to post-zygotic abnormalities such as mosaicism. These abnormalities will not be eliminated by omission of ovarian stimulation. Baart et al. (2007), however, were able to demonstrate that the proportion of chromosomal abnormalities can be reduced by reducing the dosage of gonadotrophins for ovarian stimulation.

Secondly, our study confirms that aneuploidy is present in single blastomeres of embryos of young women under 36 years of age. Although we know that aneuploidy above 36 increases, little is known about the aneuploidy rate in embryos of young women, as PGS is uncommonly carried out in this group. Baart et al. reported a numerical chromosomal abnormality rate of 64% (aneuploidy 14%, mosaicism 50%) using FISH probes for eight autosomal and two sex chromosome pairs and double blastomere biopsy in Day 3 embryos of patients under 38 years without a specific indication for PGS. It should be noted that only half of the mosaic embryos were confirmed to be aneuploid at re-analysis (Baart et al., 2006).

Although the current study design enables us to assess the embryo in conditions close to the natural cycle in terms of absence of exogenous gonadotrophin administration during folliculogenesis and an optimum time frame for oocyte retrieval, we recognize that the administration of exogenous hCG to induce the completion of the first meiotic division and extrusion of the first polar body is a potential bias, theoretically increasing the risk of spindle misalignment and chromosomal malsegregation (Hodges et al., 2002). Recognizing the limitations of this
study in terms of sample size, it is also important to realize that PGS, being the only means available to assess chromosomal competence of an embryo, may not be the optimal tool to assess the influence of ovarian stimulation on embryonic ploidy status. PGS assesses only one or two blastomeres of an embryo that has at least six blastomeres, giving rise to potentially confusing results including mosaicism, as illustrated in our (Baart et al., 2007) and other studies. Other techniques such as polar body analysis may therefore be more suitable to assess the effect of ovarian stimulation, and larger studies are needed to identify the patient groups that may benefit from PGS.

Conclusion
The data of the current study illustrate that omission of exogenous ovarian stimulation in young women does not exclude numerical chromosomal abnormalities. The presence of mosaicism indicates that an aneuploid blastomere may not provide sufficient information on the ploidy status of a developing embryo at the blastocyst stage. The potential value of PGS in assessing the impact of ovarian stimulation on embryo quality, as well as in preventing viable aneuploid pregnancies and reducing miscarriage rates, needs to be substantiated in larger studies.

Acknowledgements
We thank the patients who participated in the study, and without whom this investigation would not have been possible. We thank Mr Julian Mitchell for kindly reviewing the spelling and syntax in this manuscript. The authors also thank the clinical, laboratory, technical, nursing and secretarial staff at the Centres for Reproductive Medicine and Medical Genetics at UZ Brussel.

Funding
This study was supported by the Fund for Scientific Research (FWO), Flanders and the Onderzoeksraad, Vrije Universiteit Brussel.

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Submitted on April 1, 2008; resubmitted on May 21, 2008, accepted on May 28, 2008

What next for preimplantation genetic screening?