Is chromosome analysis mandatory in the initial investigation of normovulatory women seeking infertility treatment?

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BACKGROUND: There is no agreement about the frequency of chromosomal abnormalities (CAs) in the female partner of an infertile couple and therefore there is no evidence base for determining whether karyotype analysis is mandatory before the initiation of infertility treatment. The aim of this prospective study was to estimate the prevalence of karyotype abnormalities in normovulatory women attending an infertility clinic and compare it to that known to be present in the newborn female population. METHODS: Cytogenetic testing was performed in 1206 women with normal ovulatory cycle seeking infertility treatment. At least 15 GTG-banded metaphases were analysed in each case. In the case of a structural abnormality, fluorescent in situ hybridization (FISH) analysis and high resolution banding (HRB) were performed on a new blood sample to elucidate the aberration. When mosaicism was suspected, the number of analysed metaphases was increased to a total of 115 and an additional analysis of 200 metaphases was done on a second blood sample. RESULTS: A chromosomal abnormality was demonstrated in 0.58% (95% CI: 0.28–1.19) of cases which did not differ significantly from that reported in female newborns (0.79%; 95% CI: 0.68–0.94). Balanced reciprocal translocation was observed in 0.4% of patients (n = 5), paracentric inversion of chromosome X in 0.08% (n = 1) and gonosomal mosaicism in 0.08% (n = 1). However, chromosomal aberrations were less common among females with primary infertility compared to those with secondary infertility (0.25 versus 1.25%, P = 0.04). CONCLUSIONS: The present study suggests that routine cytogenetic analysis cannot be advocated in normovulatory infertile women. Nevertheless, the relatively higher frequency of abnormal karyotypes in women with secondary infertility indicates that this subgroup of patients might benefit from a routine karyotype analysis.

Key words: chromosome abnormalities/infertility/normovulatory/ICSI/karyotype/IVF

Introduction

About 15% of all couples will experience primary or secondary infertility during their reproductive life (Healy et al., 1994). Chromosome abnormalities (CAs) may cause infertility in both men and women, and they are most common among infertile males (Chandley, 1998a). Nowadays, although genetic tests are available to explore the cause of infertility even at the molecular level, peripheral blood karyotype analysis remains the first step in evaluating the genetic characteristics of an infertile couple.

The indications for genetic screening of the male partner of an infertile couple are well established (Van Assche et al., 1996; Chandley, 1998b). Karyotype analysis is also performed in women presenting with primary amenorrhoea, premature menopause, and recurrent pregnancy loss (Hens et al., 1989; ESHRE Capri workshop group, 2000). However, controversy exists whether karyotyping should be performed in every female partner of an infertile couple (ESHRE Capri workshop group, 2000; Foresta et al., 2002).

Several studies have reported an increased frequency of chromosome aberrations in infertile women. A 2.01% frequency was observed in patients undergoing artificial insemination (IUI) (Mattei et al., 1980), while in patients undergoing IVF the range was 1.8–2.4% (Hens et al., 1988; Schreurs et al., 2000). Cytogenetic studies of female patients enrolled in an ICSI programme reviewed by Gekas et al. (2001) have shown an unexpectedly increased incidence of abnormal karyotypes, ranging from 1.1 to 9.8% when cases with low level sex chromosome mosaicism were included. The above prevalences of chromosome abnormalities are significantly higher than those reported (<1%) in the newborn population (Nielsen and Wohlert, 1991).

Based on the above findings, the routine karyotype evaluation in infertile women who have failed to conceive after 1 year of sexual intercourse has been advocated prior to the initiation of assisted reproductive techniques (Foresta et al., 2002). Similarly, cytogenetic screening has been recommended for the female partner of ICSI couples (Gekas et al., 2001).
However, assessing the prevalence of CAs in specific groups of patients according to the treatment administered (IUI/IVF/ICSI) cannot lead to solid estimates. This is due to the fact that a patient might need to undergo different forms of treatment over time, whereas the decision to follow a specific treatment is not always based on the same criteria (Bhattacharya et al., 2001; Tournaye et al., 2002). On the other hand, the validity of the aforementioned high prevalence of CAs is debatable. The inclusion of low-level mosaicism (Gardner and Sutherland, 1996) and the analysis of heterogeneous patient populations might be confounding factors.

Currently there are no data on the incidence of chromosomal abnormalities in normovulatory subfertile women, who represent -80% of the females encountering infertility (Jones and Toner, 1993). There is therefore no solid basis for recommending routine karyotyping in these women. The aims of this prospective study were to estimate the prevalence of karyotype abnormalities in normovulatory women attending an infertility clinic irrespective of the cause of infertility diagnosed for the couple and the proposed infertility treatment, and to compare it to the prevalence known to be present in the general female population. This would provide evidence whether or not normovulatory women should be routinely karyotyped before the initiation of assisted reproductive treatment.

Materials and methods

Study design

Between January 1999 and December 2003, 1300 women with normal ovulatory cycles seeking infertility treatment were recruited in this prospective cohort study. The study was carried out in the Centre for Reproductive Medicine at the Dutch-speaking University Hospital of Brussels, which is a tertiary hospital. Inclusion criteria were: (i) infertility duration >12 months; (ii) regular menstrual cycles (21–35 days). Besides a full medical history and general clinical examination, the diagnostic work-up of these couples included the following: a complete endocrine investigation of the hypothalamo-hypophyseo-gonadal axis including ovulation confirmation; thyroid function and prolactin status; evaluation of semen characteristics according to the criteria of Kruger et al. (1986); minor pelvic ultrasound examination; hysterosalpingography; and when indicated, hysteroscopy and/or laparoscopy. Overall, 1206 women completed the diagnostic investigation and the cytogenetic analysis. In the case of a chromosome abnormality being found, genetic counselling was offered to the patient at the Centre for Medical Genetics. The study design was approved by the Ethics Committee of the Vrije Universiteit Brussel, and all patients gave informed consent for the study.

Cytogenetic analysis

Cytogenetic analysis was carried out on phytohaemagglutinin-stimulated and cultured peripheral lymphocytes using standard techniques (Rooney and Czepulkowski, 1992). Fifteen high-level G-banded metaphases (between 550 and 850 bands visible) were analysed per patient. In cases of structural abnormality, fluorescent in situ hybridization (FISH) analysis and high resolution banding (HRB) on a new blood sample were performed to elucidate the aberration. The pericentric inversion of the heterochromatic region of chromosome 9 [Inv(9)(p12q12)] and polymorphisms were considered normal variants and no additional work-up was carried out (Dutch Society of Obstetrics and Gynecology, 1996). In cases of suspected mosaicism (i.e. loss of the same chromosome in at least three cells or gain of the same chromosome in at least two cells in the first 15 metaphases), FISH was performed, the number of analysed metaphases was increased to a total of 115 and an additional analysis of 200 metaphases was performed on a second blood sample. If the aberrant cell line was confirmed, the patients’ peripheral blood karyotype was considered as mosaic. The term ‘low level sex chromosome mosaicism’ (LLM) is not well defined. However, it is considered to be clinically insignificant if <10% of the analysed cell lines are involved (Gardner and Sutherland, 1996). Moreover it has been shown that LLM in females has no effect on the course and outcome of assisted reproductive treatment (Sontag et al., 2001). The above definition of LLM, which is frequently encountered in the literature (Meshede et al., 1998; Scholtes et al., 1998; Gekas et al., 2001; Sontag et al., 2001), was also used in the current study and therefore these cases were not considered abnormal. For the purpose of this study we considered as secondary infertility only infertility after spontaneous conceptions.

Statistical analysis

Nielsen and Wohlert (1991) have reported a 0.79% frequency of CAs among 17,038 newborn girls. To detect an increase of 1%, deemed to be clinically important, the sample size required would be 1125 women, who have completed the infertility work-up and the karyotype analysis. The sample size was calculated according to the normal approximation of the binomial distribution, using a two-sided test with an α level of 0.05 and a β level of 0.02. A total of 1300 women were recruited for the study with an anticipated 10% drop-out.

Exact χ²-test was used to analyse nominal variables in the form of frequency tables. Normally distributed (Kolmogorov–Smirnov test with Lilliefors correction) metric variables were tested with the t-test for independent samples. For non-normally distributed variables, the Mann–Whitney test was used. All tests were two-tailed with a confidence level of 95% (P < 0.05). Values are expressed as mean ± SE.

Results

Cytogenetic analysis was performed in 1206 normovulatory subfertile women. The mean age of the women was 32.0 ± 0.1 years while the mean duration of infertility was 3.1 ± 0.1 years. Most of the women presented with primary infertility (secondary versus secondary infertility, 66.6 versus 33.3% respectively). In the majority of cases (39.6%) infertility was due to the presence of andrological factor. In 22.2% of the cases female factor was present; in 14.2%, infertility was due to a combination of male and female factor, while in 24% of the cases, no cause for infertility could be identified.

A chromosomal abnormality was observed in seven individuals, 0.58% (95% CI: 0.28–1.19) of the women analysed. This was not significantly different (P = 0.43) from the CAs reported in newborn girls (0.79%; 95% CI: 0.68–0.94) by Nielsen and Wohlert (1991) (Table I). The age of the patients and the duration of infertility were not different (P > 0.05) between patients with normal and abnormal karyotype (Table II). The cause of infertility was not associated with the prevalence of CAs in the patients analysed. However, a significantly higher (P = 0.04) prevalence of CAs was observed in women with secondary infertility (1.25%) compared to those with primary infertility (0.25%) (Table II).

In five patients (0.4%) a balanced reciprocal translocation was present; in one patient (0.08%), a paracentric inversion of chromosome X was observed, while in one case (0.08%),
Prevalence of chromosomal abnormalities in the study population

Patients characteristics according to the result of the karyotype

Table I. Prevalence of chromosomal abnormalities in the study population and historical newborn girls

<table>
<thead>
<tr>
<th>Chromosomal abnormalities (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population (n = 1206)</td>
<td>0.58 ± 0.28</td>
</tr>
<tr>
<td>Patients with primary infertility (n = 805)</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>Patients with secondary infertility (n = 401)</td>
<td>1.25 ± 0.40</td>
</tr>
<tr>
<td>Newborn girls (n = 17 038)</td>
<td>0.79 ± 0.68</td>
</tr>
<tr>
<td>Adult females* (n = 17 038)</td>
<td>0.49 ± 0.37</td>
</tr>
</tbody>
</table>

*Anticipated prevalence of chromosomal abnormalities at adult age female general population [estimated from newborn girls (Nielsen and Wohlert, 1991) after exclusion of subjects having trisomy 21; trisomy 18; trisomy 13; trisomy 8; +mar; +ring; deletions; duplications; and fragile X, who carry a high risk of dying or being mentally retarded and therefore not be represented in the normal fertile adult population].

Table II. Patients characteristics according to the result of the karyotype analysis and proportions of patients with abnormal karyotype according to type and cause of infertility

<table>
<thead>
<tr>
<th>Normal karyotype</th>
<th>Abnormal karyotype</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>32.0 ± 0.1</td>
<td>33.3 ± 1.0</td>
</tr>
<tr>
<td>Duration of infertility (years)*</td>
<td>3.1 ± 0.08</td>
<td>4.6 ± 1.6</td>
</tr>
<tr>
<td>Type of infertility, % (n)</td>
<td>99.75 (803)</td>
<td>0.25 (2)</td>
</tr>
<tr>
<td>Primary</td>
<td>98.75 (396)</td>
<td>1.25 (5)</td>
</tr>
<tr>
<td>Secondary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cause of infertility, % (n)</td>
<td>99.4 (474)</td>
<td>0.6 (3)</td>
</tr>
<tr>
<td>Male</td>
<td>99.6 (267)</td>
<td>0.4 (1)</td>
</tr>
<tr>
<td>Female</td>
<td>99.4 (170)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>Combined</td>
<td>99.3 (288)</td>
<td>0.7 (2)</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

NS = not significant.

gonosomal mosaicism was detected (Table III). Low-level mosaicism (n = 5, 0.4%) and inversion of chromosome 9 (n = 14, 1.2%) were not considered as chromosomal abnormalities (Table IV). In Tables III and IV the infertility history and treatment after the chromosomal analysis was carried out are shown in patients with abnormal karyotype and LLM respectively.

Discussion

The prevalence of chromosomal anomalies is known to be higher in women with amenorrhea or dysovulation compared with normal individuals (Hens et al., 1989). The current study has assessed the prevalence of chromosomal abnormalities in normovulatory women seeking infertility treatment. It was shown that this is not expected to be >1.19%, with the best estimate being 0.58%. This is the first report on the prevalence of chromosomal abnormalities in infertile normovulatory women, who represent the majority of infertile patients (Jones and Toner, 1993). Previous studies in the literature have focused on assessing the prevalence of karyotype disorders in groups of women according to the assisted reproduction treatment proposed (Gekas et al., 2001). However, this assumes the presence of uniformly accepted indications for treatment between centres, which is not always the case (Goverde et al., 2000; Van Rumste et al., 2003). Moreover, this approach cannot justify, nor can it rule out, the need to routinely karyotype normovulatory women prior to assisted reproduction treatment.

The observed prevalence of CAs in normovulatory women was not different from that reported in newborn girls (0.79%; 95% CI: 0.68–0.94). Our findings suggest that performing karyotype analysis in normovulatory subfertile women will not result in the detection of a higher prevalence of CAs than the prevalence reported in the female newborn population (Nielsen and Wohlert, 1991). Routine karyotype in these women does not appear therefore to be justified and thus a substantial increase in the cost of treatment can be avoided.

It should be noted, however, that the most appropriate control group for comparing the prevalence of CAs in normovulatory women would have been a group of fertile women attending, during the study period, a maternity clinic after spontaneous conception. Such a group could not be obtained since routine karyotype is not justified in the case of pregnant women. As a result and since the prevalence of chromosomal abnormalities in this population has not so far been reported in the literature, historical control data from a screened neonatal population has been utilized as the best estimate (Nielsen and Wohlert, 1991). These values, however, might be an overestimation of the frequency of CAs at adult age since a number of the children with chromosomal anomalies will die or be mentally retarded and not be represented in the normal fertile adult population. Even in this scenario the prevalence of karyotype abnormalities in normovulatory women remains not significantly different from the anticipated prevalence in the adult female population (Table I).

A novel finding of this study was that a significantly higher prevalence of CAs exists in women with secondary, in comparison to those with primary, infertility. In previous reports the estimation of the frequency of CAs was not performed according to the type of infertility present. For the first time, we have evaluated prospectively the prevalence of CAs in relation to the history of previous (non-)conception. The significantly higher prevalence of CAs observed in women with secondary infertility (four cases of translocation and one inversion) might not be associated with inability to conceive but rather with failure of pregnancy to progress. Indeed, a significantly high (6%) frequency of balanced translocations has been observed in patients with recurrent miscarriage (Stern et al., 1999). It is possible that the presence of an unbalanced translocation in some gametes of translocation carriers may result in failure of the developing embryo to implant or in early pregnancy loss. The recent development of preimplantation genetic diagnosis (PGD) represents a valuable diagnostic method for genetic counselling and infertility treatment of these patients. Our study was not powered to address differences in the prevalence of chromosomal abnormalities between women with secondary infertility and those in the general population. The significantly higher incidence of chromosome aberrations in women with secondary infertility as opposed to those with primary infertility suggests, however, that this subgroup of patients might benefit from a routine karyotype analysis. In line with our observation, a recent retrospective study found that in women...
with a history of gestation (G) \( \geq 1 \) parity (P) 0 the prevalence of CAs (3.25%) was 4-fold greater compared to the general population (Nielsen and Wohlert, 1991), but similar in the case of the G0P0 group (0.87%) (Clementini et al., 2005). The above findings suggest that further research needs to be carried out with regard to the impact of chromosomal aberrations in secondary infertility.

In conclusion, the current study has shown that the incidence of chromosomal abnormalities in normovulatory subfertile women is not different from that known to be present in the general female population and therefore routine cytogenetic analysis cannot be advocated in this category of patients. Nevertheless, the higher prevalence of CAs in women with secondary infertility requires further investigation.

### References


Karyotype testing in infertile women


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