Aneuploidy is the term used for numerical abnormality of chromosomes. The frequency of aneuploidy in human oocytes ranges between 12.0 and 37.3% (Szczygiet and Kurpisz, 2001). The mean number of spermatozoa with aneuploidy has been reported as 2.5% for all larger studies performed on sperm chromosomes (Szczygiet and Kurpisz, 2001). An oocyte or spermatozoon with an extra or missing chromosome involved in conception leads to an aneuploid embryo. Depending on the chromosome involved, these genetic abnormalities can prevent the implantation of the embryo, lead to early spontaneous abortion or, very rarely, result in liveborn children with specific physical and/or mental abnormalities. Trisomy 21, Down syndrome, is the most common form of aneuploidy. The other common aneuploidies are trisomies 13 and 18, Kleinfelter and Turner syndromes. Aneuploidies of these chromosomes rarely go to term and result in liveborn children. Trisomies of chromosomes 15, 16 and 22 result in early spontaneous abortions. Monosomies, except X and 21, will be discarded before implantation.

Aneuploidy is the most frequent abnormality in normally developing embryos (Munné et al., 1995). The reported rate of aneuploidy for preimplantation embryos at cleavage stage is 60% (Gianaroli et al., 1999; Kuliev and Verlinsky, 2002; Munné, 2002). Advanced maternal age increases the risk of having an aneuploid pregnancy (Munné et al., 1995). The chance of delivering an affected child is 0.26% at age 30, 0.52% at age 35 and 1.5% at age 40 years (Hook, 1981; Hook et al., 1983). Preimplantation genetic diagnosis for aneuploidy screening (PGD-AS), performed by polar body or blastomere analysis, is used in infertile patients treated with assisted reproduction technologies, especially in those with a poor prognosis, e.g. repeated IVF failure, advanced maternal age, or recurrent spontaneous abortion. The aim of this paper is to clarify the impact of PGD-AS in repeated implantation failure. In this review, the data collected so far regarding PGD-AS in this patient group will be discussed in depth.
described in this paper, to increase implantation rates by choosing chromosomally normal embryos. PGD using fluorescence in-situ hybridization (FISH) might improve IVF outcome in patients over 35 years of age and in those with recurrent spontaneous abortions (ESHRE PGD Consortium Data Collection, 2002). In this review, PGD-AS in repeated implantation failure (RIF) will be discussed in depth.

**PGD-AS in RIF using FISH**

RIF is usually defined as three or more failed IVF attempts, or repeated transfer of more than 10 morphologically good embryos to a normal uterus without achieving successful implantation and pregnancy. After excluding hormonal, uterine and immunological factors, chromosomal abnormalities are thought to be most commonly responsible for implantation failure. It was first stated by Munné et al. (1993) that embryos with chromosomal abnormalities have lower implantation potential. Genetic testing of preimplantation embryos for chromosomal aneuploidy allows selection of chromosomally normal embryos to be transferred into the uterus, which will increase the chance of conceiving, especially in patients with a poor prognosis, such as RIF, advanced maternal age, and recurrent spontaneous abortion (Munné et al., 1999, 2003; Kahraman et al., 2000; Kuliev and Verlinsky, 2003a).

PGD-AS is usually performed in patients with advanced maternal age (over 35 years) on day 3 preimplantation embryos. Evaluation of the chromosomal status of the embryo increases the chance of conceiving in this group (Gianaroli et al., 1999). In the study by Munné et al. (2003), 138 patients undergoing PGD-AS because of advanced maternal age (mean age 39 years) had a significantly higher implantation rate (fetal heartbeat per embryo replaced) when compared with patients receiving standard IVF (mean age 39 years) (Table 1). In another study (Gianaroli et al., 1999), the increase in implantation rate with PGD-AS was significantly higher in patients ≥38 years of age and the implantation rate of patients ≥38 years of age was reported to be comparable with the 36–37 years age group when PGS-AS was performed (Gianaroli et al., 1999). The decrease in assisted reproduction success in patients with advanced maternal age is associated with endometrial and oocyte related factors. Altered hormonal status, decreased uterine blood flow and insufficient endometrial proliferation reduce endometrial receptivity, causing implantation failure. In patients with advanced maternal age, ageing of the oocytes and increased chromosomal abnormalities are other contributing factors to low success rate in assisted reproduction. In this group of patients, the positive clinical impact of PGD-AS, doubling the implantation rate in IVF patients aged 40 years and over, is obvious (Munné et al., 2003). These results confirm that in advanced maternal age, the functional and structural decline of the oocyte (Navot et al., 1991) is more profound than endometrial receptivity in age-related decrease in fertility. In older women with donor oocytes, the higher implantation rate (Sauer, 1996; Abdalla et al., 1997) also implies that the age-related decline in female fertility is mainly due to oocyte related factors.

Implantation failure might also be due to an excess of chromosomally abnormal embryos in patients with RIF. The detected rates of aneuploidy in RIF patients from various studies are given in Table 1. Munné et al. (2003) found that RIF (≥2 IVF cycle) patients have similar rates of abnormal embryos when compared with patients having had one or no previous cycles (31 and 33% respectively), and suggested that in this group of patients, the poor implantation rate was not due to an excess of chromosomally abnormal embryos. In a recent study, chromosomal analysis of embryos from 276 couples with different indications for PGD-AS showed no significant difference for chromosomal abnormalities observed in patients of advanced maternal age, those with RIF, and those with repeated spontaneous abortion (Kahraman et al., 2004). Gianaroli et al. (2002) found that the most frequent chromosomal defects in 66 cycles of RIF patients with ≥3 IVF failures were mosaicism, haploidy and polyploidy. In contrast, others reported that RIF patients had a 1.9-fold higher rate of chromosomal anomalies, mostly aneuploidy, when compared with patients who underwent PGD for sex-linked diseases (Pehlivan et al., 2003). Moreover, it was found that the number of chromosomal anomalies detected increases with the number of failed IVF cycles (Gianaroli et al., 1997a). The rates are 40 and 50% respectively with two and three failed IVF cycles, and 67% with more than five failed IVF cycles (Gianaroli et al., 1997a).

The optimum method of management of implantation failure has not yet been clarified. PGD is a treatment option in the management of RIF. The study of Munné et al. (2003), and two other studies (Gianaroli et al., 1999; Kahraman et al., 2000) conducted in RIF patients using FISH for PGD-AS, all reported similar implantation rates when compared with a control group (Gianaroli et al., 1999) or a group of patients with advanced maternal age (Kahraman et al., 2000) (Table 1). Another study (Pehlivan et al., 2003), performed in RIF patients (mean age 36 years), reported an implantation rate of 19%, compared with 24% in patients who underwent PGD for sex-linked diseases (mean age of 31 years). A recent study reported similar aneuploidy and pregnancy rates among advanced maternal age, RIF and repeated spontaneous abortion groups (Kahraman et al., 2004). Day 4 replacement of the embryos after PGD showed that patients (mean age 40 years) with two or more previously failed IVF cycles have similar implantation rates when compared with controls (mean age 39 years) (14.3 and 11.5% respectively) (Munné et al., 2003). Data from European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium reported that using FISH for PGD-AS the pregnancy rate per transfer was 7% in RIF patients (ESHRE PGD Consortium Data Collection, 2002). Although there is no direct evidence that these patients benefit from PGD-AS, the use of PGD-AS for RIF could be useful to discover the reason for IVF failure, which in many cases is the lack of chromosomally normal embryos.

In young patients with a good prognosis, the rate of chromosomally abnormal embryos detected is 28% and the aneuploidy rate is 5.4% (Munné et al., 1995). Very few studies have evaluated the value of PGD-AS in young patients with RIF. It was suggested that patients with RIF at a young age might benefit from PGS-AS (Kahraman et al., 2000). In the study by Kahraman et al. (2000), the pregnancy rate in patients with RIF and a mean age of 30 years was reported as 30% with PGS-AS studied in five chromosomes. The higher fertilization
Table 1. Some of the studies performed with day 3 cleavage stage embryo biopsy of one or two blastomeres for aneuploidy screening in poor prognosis assisted reproduction patients. AK = altered karyotype, AMA = advanced maternal age, CGH = comparative genomic hybridization, FISH = fluorescence in-situ hybridization, RIF = repeated implantation failure, RM = repeated miscarriages, TC = previous trisomic conceptions, ZH = zona hatching.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control group</th>
<th>Method</th>
<th>Analysed chromosomes</th>
<th>Mean age (years)</th>
<th>Analysed embryos (n)</th>
<th>Mean no. embryos transferred</th>
<th>Day of transfer</th>
<th>Abnormal PGD result (%)</th>
<th>Aneuopeiody rate (%)</th>
<th>Pregnancy rate (%)</th>
<th>Implantation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>(n)</td>
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</tr>
<tr>
<td>Kahraman et al. (2004)</td>
<td>–</td>
<td>FISH</td>
<td>X, Y, 13, 16, 18, 21, 22</td>
<td>35.1</td>
<td>1147</td>
<td>2.7</td>
<td>4</td>
<td>40.9</td>
<td>55.4</td>
<td>31.6</td>
<td>16.2</td>
</tr>
<tr>
<td>Balaban et al. (2004)</td>
<td>–</td>
<td>FISH</td>
<td>X, Y, 13, 16, 18, 21</td>
<td>Not mentioned</td>
<td>240</td>
<td>2.0a</td>
<td>5</td>
<td>69.2</td>
<td>67.5</td>
<td>22.1</td>
<td>17.6</td>
</tr>
<tr>
<td>Munné et al. (2003)</td>
<td>–</td>
<td>FISH</td>
<td>X, Y, 13, 15, 16, 18, 21, 22</td>
<td>39.8a</td>
<td>1071a</td>
<td>2.7</td>
<td>5</td>
<td>70.3a</td>
<td>39.8a</td>
<td>34.7a</td>
<td>17.6a</td>
</tr>
<tr>
<td>Pehlivan et al. (2003)</td>
<td>–</td>
<td>–</td>
<td>X, Y, 13, 15, 16, 18, 21</td>
<td>36.2a</td>
<td>430a</td>
<td>1.9a</td>
<td>5</td>
<td>67.4a</td>
<td>50.2a</td>
<td>34.0a</td>
<td>19.8a</td>
</tr>
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<td>Wilton et al. (2003)</td>
<td>–</td>
<td>–</td>
<td>X, Y, 13, 15, 16, 18, 21</td>
<td>31.6b</td>
<td>80b</td>
<td>2.4b</td>
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<td>36.3b</td>
<td>26.3b</td>
<td>33.3b</td>
<td>24.1b</td>
</tr>
<tr>
<td>Kahraman et al. (2000)</td>
<td>–</td>
<td>–</td>
<td>X, Y, 13, 15, 16, 18, 21</td>
<td>19.6</td>
<td>126</td>
<td>1.8e</td>
<td>3</td>
<td>37.6</td>
<td>33.1</td>
<td>26.3</td>
<td>10.6b</td>
</tr>
<tr>
<td>Gianaroli et al. (1999)</td>
<td>–</td>
<td>–</td>
<td>X, Y, 13, 15, 16, 18, 21</td>
<td>34.0</td>
<td>329</td>
<td>2.3a</td>
<td>3</td>
<td>37.9</td>
<td>37.4</td>
<td>24.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Gianaroli et al. (1997)</td>
<td>–</td>
<td>–</td>
<td>X, Y, 13, 15, 16, 18, 21</td>
<td>30.3</td>
<td>695a</td>
<td>3.2b</td>
<td>3</td>
<td>60.0a</td>
<td>43.2</td>
<td>37.0a</td>
<td>24.2b</td>
</tr>
</tbody>
</table>

aResults of study group.
bResults of control group.
cTotal number of biopsied embryos with satisfactory results.
dMean number of embryos transferred per cycle.
eNumber of embryos transferred per patient.
fPregnancies with gestational sac with fetal heartbeat detection.
gSome embryos are screened for extra chromosomes.

Studies performed to evaluate the value of PGD-AS in cases of RIF have different designs. The number of probes used and the pairs of chromosomes evaluated differs between studies performed by FISH. The study groups, stimulation protocols and day of embryo transfer also differ among studies performed so far. Some of the studies performed with day 3 cleavage stage embryo biopsy for aneuploidy screening in poor prognosis assisted reproduction patients are shown in Table 1. From these results, there is no clear indication that RIF patients benefit from PGD using FISH, as mentioned previously by Munné (2003).

PGD using FISH gives information about limited numbers of chromosomes, enabling a maximum of nine chromosomes per cell to be analysed. Analysis of more than one blastomere,
when available, is an opportunity to detect further chromosome abnormalities. It is particularly important to exclude the high frequency of chromosomal mosaicism in human preimplantation embryos, as this usually causes misdiagnosis of PGD performed with FISH (Munné, 2002). PGD-AS by FISH does not always give a result, and some embryos are not sufficiently well developed for biopsy on day 3. The other dilemma is the lack of embryos for transfer in some cycles.

PGD-AS and embryo quality, blastocyst culture in RIF

In many assisted reproduction centres, embryos to be transferred into the uterus are chosen based on their morphological characteristics. Pronuclear scoring, commonly used as a single selection criterion for transfer, is also related to chromosomal abnormalities (Coskun et al., 2003; Balaban et al., 2004). Embryos with poor pronuclear morphology have a high risk of chromosomal abnormalities (Kahraman et al., 2002). Over 40% of morphologically normal embryos have chromosomal abnormalities (Márquez et al., 2000), and morphological analysis of the embryo is not satisfactory (Gianaroli et al., 1997b; Magli et al., 1998). The rate of aneuploidy has been reported as 26% with a normal pronucleus pattern, whereas aneuploidy was observed in 83% of embryos with ≥2PN anomalies (Balaban et al., 2004). It was reported that success rates are improved with PGS-AS in good quality embryos, resulting in higher implantation and pregnancy rates (Pehlivan et al., 2003). The significant increase in implantation rate was most obvious in patients with eight or more 2PN zygotes (Munné et al., 2003).

Arrested development of the embryo is more frequent in aneuploidy (Jones and Trounson, 1999). However, chromosomal abnormality of the embryo does not always affect its development. Chromosomally abnormal embryos also reach the blastocyst stage (Magli et al., 2000; Sandalinas et al., 2001). Development up to the blastocyst stage has been observed in 33% of chromosomally abnormal (Rubio et al., 2000) and in 19% of aneuploid embryos (Sandalinas et al., 2001). The pronuclear pattern is closely related to blastocyst formation; embryos with normal pronuclear pattern show a higher incidence of blastocyst formation (Balaban et al., 2001; Scott et al., 2000). The percentage of aneuploid embryos detected by FISH analysis, with normal pronuclear morphology, reaching blastocyst stage by day 5 of in-vitro culture has been reported as 29% (Balaban et al., 2004). Blastocyst transfer and day 2 transfer resulted in similar implantation rates in patients using their own oocytes, reported as 11.9 and 10.7% respectively (Simon et al., 1999). The data in the literature also show that implantation and pregnancy rates dramatically decrease in repeated cycles of IVF with blastocyst transfer (Shapiro et al., 2001). Although co-culture of the embryos is another alternative in the management of RIF, these results suggest that methods other than blastocyst culture should be used in the management of RIF patients.

In a recent randomized controlled study, the outcome after blastocyst transfer combined with PGD-AS using FISH for chromosomes X, Y, 13, 16, 18, 21 and 22 was compared with a control group without PGD-AS in advanced maternal age couples (mean maternal age 39 years in control and 40 years in study groups) (Staessen et al., 2004). In this study, FISH analysis revealed a normal result in 36.8% of patients. The implantation rates of the two groups were similar (11.5 and 17.1% in control and study groups respectively), but a significantly higher number of embryos was replaced in the control group (Staessen et al., 2004). In PGD-AS cycles (n = 148) in 11 genetically normal embryos, no morula or blastocyst formation occurred. Moreover, the authors suggested that in patients from whom expanded blastocysts are obtained, the chance of selecting a chromosomally normal embryo increases from 41.3% on day 3 to 65% on day 5 (Staessen et al., 2004). In the study by Pehlivan et al. (2003), who performed PGD-AS by FISH for chromosomes X, Y, 13, 16, 18, 21 and 22, all pregnancies in the RIF group occurred after the transfer of at least one chromosomally normal blastocyst on day 5, and no pregnancy was achieved after replacement of slower developing embryos without cavitation (morula transfer) (Pehlivan et al., 2003). It was suggested that the use of PGD-AS along with blastocyst transfer could improve IVF outcome (Pehlivan et al., 2003). Identifying abnormalities in morphologically good quality embryos by PGD-AS increases the chances of patients to conceive by preventing the transfer of chromosomally abnormal embryos that will fail to implant or abort.

PGD-AS and assisted hatching in RIF

Another factor to consider in implantation failure is the inability of the blastocyst to escape from its zona pellucida (Cohen, 1993; De Vas and Van Steirteghem, 2000). The procedure of assisted hatching, which helps the blastocyst to break its zona pellucida, is another option in the management of RIF. The different techniques of assisted hatching (e.g. chemical, mechanical, enzymatic, laser) performed to improve the implantation of the embryo have all yielded similar implantation and pregnancy rates (Balaban et al., 2002). A recent randomized prospective study to assess the use of assisted hatching using a diode laser showed that in fresh embryos after RIF, the implantation and pregnancy rates were both improved, although not significantly (Primi et al., 2004). A study comparing assisted hatching with PGD showed that implantation rates were similar in these procedures (Gianaroli et al., 1999). PGD-AS in patients aged ≥36 years resulted in a lower number of embryos replaced with increased implantation rates, but clinical pregnancy rate was similar to that in controls who underwent assisted zona hatching (Gianaroli et al., 1999) (Table 1).

PGD-AS using comparative genomic hybridization in RIF

Comparative genomic hybridization (CGH) enables complete karyotypic diagnosis (Wilton et al., 2001). CGH, which seems to be more effective than FISH, is expected to improve IVF outcome in poor prognosis patients, increasing pregnancy and implantation rates. The transfer of euploid embryos with complete karyotypic diagnosis is expected to provide higher implantation rates. The application of CGH for PGD-AS using single blastomeres biopsied from 141 cleavage stage embryos from 20 patients (mean age 34 years) with RIF showed that 60% of the biopsied embryos had chromosome abnormalities.
(Voullaire et al., 2002). Aneuploidy for one or two chromosomes was found in 25% of the embryos (Voullaire et al., 2002).

In the study conducted by Wilton et al. (2003), which included 20 patients (mean age 34 years) with RIF who had at least 10 embryos transferred previously without pregnancy, the results of analysis of 198 embryos by CGH ($n = 141$) or FISH ($n = 57$) were compared. The implantation and pregnancy rates were observed to be higher in this study, with a small population of patients (Table 1). Wilton et al. (2003) suggested that RIF patients would benefit from PGD-AS when performed with CGH. This study reported that the proportion of abnormal blastomeres incorrectly diagnosed as normal by FISH is 60% for five chromosomes and 40% for nine chromosomes (Wilton et al., 2003).

Analysis of embryos for PGD-AS by CGH from RIF patients (mean age 34 years) showed that chromosomes 4, 7, 9, and 20 were involved in embryos with single aneuploidy, and 1, 3, 4, 6, 8, 9, 10, 11 and 12 were involved in embryos with double aneuploidy (Voullaire et al., 2002). In RIF patients, the involvement of chromosomes other than those included in the FISH probes would explain the minor improvement in outcome with PGD-AS by FISH. Moreover, it was reported that complex abnormality more often occurs in embryos from women with RIF. Outcome by PGD-AS in RIF patients using CGH necessitates further research.

CGH involves freezing of the embryos, as the whole procedure takes 5 days after blastomere biopsy to determine the karyotype of the embryo, which prevents the transfer of the analysed embryo in the same cycle. In addition, a frozen cycle, the outcome of the thawing procedure is poor for biopsied embryos (Joris et al., 1999; Magli et al., 1999). After the thawing procedure, 50% of the embryos will be destroyed (Wilton et al., 2003). These disadvantages make CGH impractical for routine use (Kuliev and Verlinsky, 2003b).

**PGD-AS by polar body biopsy in RIF**

Most PGD-AS procedures, including the studies mentioned above, are performed on cleavage stage embryos on day 3 from a single blastomere. Alternatively, evaluation of the genetic material of the oocyte for aneuploidy by polar body biopsy also provides useful information. The polar body diagnosis of common aneuploidies by FISH was suggested to be useful for detection of oocytes with common chromosomal trisomies in IVF patients of advanced maternal age (Verlinsky et al., 1996). Errors in meiosis I of the oocyte contribute to 80% of the aneuploidies seen in embryos (Dailey et al., 1996). PGD by first and second polar body FISH analysis avoids common aneuploidies associated with advanced maternal age in IVF patients (Verlinsky et al., 1998). The most common anomaly in polar body (PB) 1 was a missing chromatid, resulting in trisomy in the remaining embryo (51%) and in PB2, missing and extra signals were of similar rates (39% and 44% respectively) in the oocytes of patients with a mean age of 38 years who underwent FISH analysis for five chromosomes (Verlinsky et al., 2001).

Magli et al. (2004) reported the results of PGD-AS by FISH for chromosomes X, Y, 13, 15, 16, 18, 21 and 22 in poor prognosis IVF patients (mean age 38.4; advanced maternal age, repeated IVF failure) in 113 cycles. In some cycles ($n = 19$), polar body biopsy of both polar bodies was carried out simultaneously, and in others ($n = 62$), blastomere biopsy only was performed. In 32 cases of incomplete diagnosis in polar body analysis, blastomere biopsy was performed in regularly developing embryos. No statistically significant difference was reported in the implantation rates of the three-biopsy groups (15% in polar body, 26% in polar body and blastomere and 25% in blastomere group). The mean age of the all the biopsy groups was 38 years, and no detrimental effect on embryo viability was observed after removal of a blastomere subsequent to polar body biopsy.

A recent study demonstrated the reliability of CGH as an alternative to PGD using FISH on PB1 and metaphase II (MII) oocytes ($n = 30$) donated by 21 women with normal karyotype (mean age 33 years) and three translocation carriers (aged 21, 35 and 35 years) (Gutierrez-Mateo et al., 2004a). The detected aneuploidy rate was reported as 48%, and it was mentioned that if FISH with nine chromosomes had been used, 33% of the PB1-MII oocyte duplicates diagnosed as aneuploid by CGH would have been misdiagnosed as normal (Gutierrez-Mateo et al., 2004a). In another study of the same authors (Gutierrez-Mateo et al., 2004b), PB1 was analysed by CGH and the corresponding MII was analysed by FISH for at least nine chromosomes (1, 13, 15, 16, 17, 18, 21 and X). CGH and FISH analyses showed 88% reciprocal results obtained from 42 PB1-MII pairs from 33 patients (mean age 35.8), and the aneuploidy rate was reported as 57.1%, with chromosomes 1, 4, 22 and 16 more frequently involved (Gutierrez-Mateo et al., 2004b). In this study, analysis using CGH led to diagnosis of 16 (38.1%) out of 42 aneuploidies which would have been missed by FISH, as chromosomes 3, 4, 6, 7, 8, 9, 10, 14 and 19 were involved. In addition, Wells et al. (2002) applied CGH to first polar bodies removed from 12 oocytes of a 40-year-old woman, suffering from secondary infertility with a previous history of six failed IVF cycles. Depending on the results of CGH analysis, one embryo containing a normal number of chromosomes was transferred on day 4, yielding no pregnancy. In this patient, CGH to first polar body revealed that four of the five embryos reported as euploid by FISH analysis of single blastomeres on day 3 for nine chromosomes (X, Y, 13, 15, 16, 17, 18, 21, 22) were at risk of aneuploidy for chromosomes that are not usually tested by FISH. The results of the two studies mentioned above are challenging, as they support the idea that involvement of chromosomes in aneuploidy that do not cause trisomic live birth or spontaneous abortion might be common at conception. This might be the reason for implantation failure in some cases, and analysis of all chromosomes might improve the outcome in RIF.

There are several limitations of polar body biopsy for aneuploidy screening, as reported by Munné (2001, 2002). The most important handicap is that only maternal genetic contribution can be evaluated by polar body biopsy. Neither paternal inherited aneuploidies nor chromosomal abnormalities such as polyploidy and haploidy can be identified (Munné, 2002). In addition, for a more accurate diagnosis, after the analysis of PB1, the testing of PB2 is also required (Munné, 2002). Both polar bodies can be removed simultaneously for...
aneuploidy screening (Gianaroli et al., 2001). However, the defects originating either after fertilization or the first embryonic divisions cannot be diagnosed (Gianaroli et al., 2001).

**Paternal contribution to aneuploidy in RIF**

The proper alignment of the chromosomes in the meiotic spindle is regulated by metaphase I–anaphase I checkpoint, which is more strict in spermatozoa than in female meiosis (Hunt et al., 1995; LeMarie-Adkins et al., 1997). If there is an error in this alignment, meiosis is arrested in spermatozoa, although it continues in female meiosis, resulting in aneuploid oocytes, the origin of most of embryo aneuploidies. However, aneuploidy can be paternally derived and 8–12% of abortions with trisomy 13, 18 and 21 are of paternal origin (Nicolaides and Petersen, 1998).

Aneuploidy in spermatozoa can be analysed by FISH, and many studies have shown that infertile men have higher rates of sperm aneuploidy when compared with fertile men (Moosam et al., 1995; Lahdetie et al., 1997; Bernardini et al., 1998; Aran et al., 1999; Pang et al., 1999; Pfeffer et al., 1999; Ushijima et al., 2000; Vegetti et al., 2000). Moreover, chromosomally abnormal spermatozoa can fertilize an oocyte, leading to implantation failure (In’t Veld et al., 1997; Pang et al., 1999), and sperm aneuploidy was suggested to be associated with implantation failure and fetal losses (Rubio et al., 2001).

Sperm chromosomal abnormalities were evaluated by FISH analysis of spermatozoa for chromosomes 13, 18, 21, X and Y in an at-risk population including 19 patients with RIF after intracytoplasmic sperm injection (ICSI) (≥3 failures) with normal karyotype and mean age of 37.5 years (Rubio et al., 2001). The results of this study showed that in implantation failure there is an increased incidence of sex chromosome disomies, and abnormal FISH results were found in six out of 19 (31.6%) couples with RIF (Rubio et al., 2001).

Severe male infertility is associated with an increased risk of inherited and de-novo chromosomal abnormalities, with most of the aneuploidies involving sex chromosomes (Liebaers et al., 1995; Bonduelle et al., 1998). The rate of gonosomal aneuploidy increases proportionally with the severity of the male factor condition (Gianaroli et al., 2000). Moreover, PGD cycles of ICSI for non-obstructive azoospermia using testicular sperm extraction (TESE) showed that only 24% of embryos were chromosomally normal, whereas 16% aneuploidy and 36% mosaicism were found (Silber et al., 2003). A significant involvement of the male partner in the aetiology of chromosomally abnormal embryos was shown in the study of Gianaroli et al. (2000), which found that 72% of embryos from patients with epididymal or testicular sperm aspiration with ≥1 IVF failures were chromosomally abnormal (45% monosomy and trisomy, 8.6% gonosomal triploidy). The role of the male gamete in the aetiology of chromosomal abnormalities of preimplantation embryos is significant. However, paternal factors contributing to numerical chromosome abnormalities in embryos that might lead to RIF need to be evaluated by further studies.

**Karyotype anomalies in RIF**

Karyotyping is usually performed in infertile couples with repeated spontaneous abortions, since abnormal chromosomal analysis is linked with spontaneous abortions. In cases of RIF, to clarify a similar relationship with chromosomal abnormalities, some authors have investigated the frequency of chromosomal aberrations in implantation failure. In one study (Stern et al., 1999) in a group of patients (n = 514) who failed to conceive after cumulative transfer of at least 10 embryos, the rate of chromosomal abnormality (either maternal or paternal) was reported as 2.5% where chromosomal translocations constituted 1.4% of cases. The authors suggested that RIF could be due to balanced maternal or paternal translocations which necessitate genetic examination in all RIF patients.

In another study in 65 couples with high-order implantation failure (≥6 IVF trials and ≥15 transferred embryos), mean age 29 years, karyotyping for chromosomal abnormalities showed 15.4% abnormal karyotype (10 couples: translocations in six, mosaicism in two, deletion and inversion in two) (Raziel et al., 2001). Although a causal link between chromosomal aberrations and implantation failure was not established in this study, due to a higher percentage of chromosomal aberrations in their series, the authors concluded that karyotyping should be recommended when evaluating RIF cases, which is also of assistance in further patient counselling (Raziel et al., 2001).

**Conclusions**

In order to have greater success in poor prognosis patients in assisted reproduction, evaluation of preimplantation embryos depending on morphology is not enough. PGD for aneuploidy is a safe and reliable procedure in a selected group of patients. PGD for aneuploidy using FISH improves IVF outcome in patients over 35 years of age and those with recurrent spontaneous abortions. The use of CGH in clinical practice of assisted reproduction and the data collected on the use of this procedure in PGD are very limited. Future prospects in PGD for aneuploidy screening include a full karyotype analysis in a single cell in 2 or 3 days.

The data in the literature do not provide firm evidence that, with the methods used today, patients with RIF will benefit from preimplantation genetic screening for aneuploidy. However, the use of PGD-AS in RIF can be useful to clarify the reason for IVF failure, which in many cases is a lack of chromosomally normal embryos.

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