Article

Influence of spermatogenic profile and meiotic abnormalities on reproductive outcome of infertile patients

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Abstract

Genetic aspects of male infertility and the possible risks of new assisted reproduction and their influence on the development of zygotes and children born after intracytoplasmic sperm injection (ICSI) need further research. These patients have an increased risk of diploidy, and disomies are frequent in their spermatozoa. Meiotic disorders are more common in testicular biopsies of patients with severe oligoasthenozoospermia. For these reasons, a detailed andrological study is absolutely mandatory before accepting a couple with these characteristics into an IVF–ICSI programme. When an andrological patient has plasma FSH values >10 IU/l and/or very low total motile sperm count $<1 \times 10^6$, despite a normal karyotype, they clearly need a testicular biopsy and a meiotic study in order to rule out meiotic arrest or synaptic anomalies. Another important aspect to be considered is the possible benefit of applying preimplantation genetic diagnosis in these cases because they normally have a high percentage of chromosomally abnormal embryos, although in the present study this was not evident. All studies agree on the necessity of conducting follow-up studies in the population of children born after IVF–ICSI. In this way, it will be possible to find out if these infertile patients and their offspring have a higher risk of suffering epigenetic errors and imprinting disorders.

Keywords: *congenital malformations, genetic risks, imprinting disorders, male infertility, meiotic studies, preimplantation genetic diagnosis*

Introduction

Classically, it has been male infertility that has produced the worst results from the application of conventional IVF techniques. Not until the development of sperm microinjection (ICSI) and the first pregnancies resulting from it did the prognosis for cases of male infertility increase significantly (Palermo *et al*., 1992). This technique was much more effective than partial zone dissection or subzonal insemination (Palermo *et al*. 1993). As of that moment, IVF laboratories became the best therapeutic tool to solve the problems of severe male factor infertility. The results were good and, more importantly, they could be repeated easily in all IVF laboratories throughout the world, thus complying with the first premise that any new technology must meet: it must be reproducible in different scenarios.

The high level of efficacy of ICSI techniques had to be accompanied by acceptable levels of safety, and for that reason, a comparative study between the rates of congenital malformations observed in children born by IVF and those who were born following application of ICSI was performed. The results were reassuring in that they did not show any greater incidence of malformations in the group of children born after ICSI (Van Golde *et al*., 1999).

From a strategic point of view, the position was very clear from the start. The incorporation and the efficacy of ICSI should not represent any lesser need for andrological studies, but on the contrary, it seems indispensable that before an infertile couple with male factor are included in an IVF–ICSI programme, they must undergo a detailed andrological study

to rule out additional genetic risk factors. For this reason, a number of andrological screening studies have been carried out which have made it possible to learn about fundamental aspects of andrological physiopathology such as the sperm chromosome component, analysed by means of fluorescence in-situ hybridization (FISH) techniques (Aran *et al*., 1999), and studies of meiosis in tissue material obtained by testicular biopsy (Egozcue *et al*., 2000a,b). With this knowledge, it has been possible to establish diagnostic protocols that should be applied to all patients who suffer severe male factor infertility that requires IVF–ICSI.

Materials and methods

In a first study, 103 andrological patients were included who presented severe oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6$ spermatozoa/ml) presumably of idiopathic origin, with normal karyotype, which had to be treated with ICSI. These patients were studied through two spermograms, testicular volume, concentration of basal plasmatic FSH and testicular biopsy for histological and meiotic study (Vendrell *et al*., 1999).

Next, the influence of the different spermatogenic patterns was studied on the early development of embryos obtained in the later IVF–ICSI cycle that these same patients underwent (Vendrell *et al*., 2003). In addition, the final result of the cycle in relation to the pregnancy rates obtained was considered (Aran *et al*., 2003).

Finally, the possible benefit that could be offered by preimplantation genetic diagnosis (PGD) for those andrological patients with greater genetic risk when they underwent an IVF–ICSI cycle was considered (Aran *et al*., 2004).

Results

Motile sperm concentration ranged between 0 and $\leq 1.5 \times 10^6$ /ml, being $< 1 \times 10^6$/ml in 88 patients (85.4%). Testicular volume was <15 ml (Tanner, 1978) in 73 patients (70.9%) and increased concentrations of basal FSH (>10 IU/l) were observed in 34.8% of patients. Quantitative analysis of testicular biopsy, based on Silber's criteria (Silber and Rodriguez Rigau, 1981), showed a mean number of mature spermatids per tubule that ranged between 0.15 and 21.5. Histological diagnosis was incomplete maturation arrest in 91.3% of cases. Despite the normal karyotypes, meiotic studies were normal in only 62.1% of patients and severe arrest and synaptic anomalies were found in 20.4 and 17.5% of patients respectively.

In patients with sperm concentration $< 1 \times 10^6$/ml ($P < 0.001$), motile sperm concentration $< 0.5 \times 10^6$/ml ($P = 0.001$) and serum FSH concentration >10 IU/l ($P < 0.05$), meiotic abnormalities were significantly more frequent (**Table 1**). After multivariate analysis, sperm concentration and serum FSH concentration were the only independent predictive factors of abnormal meiotic pattern (**Table 2**).

When these patients completed an IVF–ICSI cycle, it was possible to study fertilization and cleavage as well as 4-cell stage embryo division rate on day 2, and to compare the results with those obtained in a group of normozoospermic patients. The first interesting observation was that the fertilization rates were normal regardless of the spermatogenic pattern, except in cases with sperm concentration $\leq 1 \times 10^6$/ ml. However, the percentage of 4-cell embryos on day 2 was significantly lower in the group of patients with oligoasthenozoospermia ($P < 0.01$, **Table 3**). The same thing happened in the group of patients with meiotic anomalies and with sperm counts lower than 1×10^6/ml, who also presented a significant reduction in the number of 4-cell embryos on day 2. Once again, multivariate analysis showed that the the two findings, meiotic anomalies and low sperm count, were independent risk variables for a low rate of embryo division (**Table 4**).

A retrospective study analysed the outcome of IVF–ICSI cycles in terms of pregnancy in 44 andrological patients with meiotic anomalies who underwent 66 cycles of treatment (51 meiotic arrest, 15 synaptic anomalies). The control group included 93 andrological patients with normal meiotic studies who underwent 158 cycles during the same period of time. No statistical differences were found in pregnancy, implantation and miscarriage rates among the three groups (normal meiosis, meiotic arrest or synaptic anomalies) (**Table 5**).

Finally, this study investigated whether it was possible to improve the results by applying PGD techniques in the 27 IVF–ICSI cycles undergone by 25 couples infertile for male factor in which the male partner presented meiotic anomalies. On comparing the results with those obtained in 66 IVF–ICSI cycles without PGD performed in 44 couples also infertile for male factor whose meiotic studies were also abnormal, it was shown that 42.5% of the embryos in the altered meiosis group in which PGD was performed were abnormal. There were no significant differences in the rates of fertilization, pregnancy, implantation or miscarriage between the two groups (**Table 6**), although this was probably due to the small size of the sample included so far in this preliminary evaluation.

Discussion

It is obvious that new assisted reproduction techniques have given possibility of becoming fathers to men whose only alternative would have been to resort to artificial insemination with donated semen. Moreover, clinical observations derived from the records of congenital malformations are important in casting a shadow of caution and of safety over the unquestionable efficacy of these techniques. Initial studies showed that the children born after IVF or ICSI did not present a higher rate of congenital malformations than that observed in the general population (Van Golde *et al*., 1999). However, these were preliminary studies and wider series were necessary to confirm these initial data; soon some worrying studies appeared on rates of congenital malformations that were significantly increased with the use of either IVF or ICSI techniques (Hansen *et al*. 2002; Koivurova *et al*. 2002). Nevertheless, at the same time more reassuring opinions were published, which made it possible to continue applying these techniques without fear (Steinkampf and Grifo, 2002). More wide-ranging studies with a longer follow-up period were necessary in order to be able to state that in general, children conceived after ICSI did not have a higher rate of malformations than children conceived naturally (Van Steirteghem *et al*., 2002). In addition, studies are now