Letters to the Editor

Cytogenetic analysis is essential before ICSI

Dear Sir,

There appears to be little relationship between specific physical and spermiogram abnormalities and male chromosomal defects (Pandiyan and Jequier, 1996). However, it is estimated that 12% of men with azoospermia and 7% of men with oligozoospermia have an abnormal karyotype (De Braekeele and Dao, 1991). In severe oligozoospermia this abnormality is often a translocation. With the advent of intracytoplasmic sperm injection (ICSI) to treat males with severe oligozoospermia and azoospermia such a situation needs consideration before therapy. When chromosome anomalies are present pregnancy rates may be jeopardised and inherited cytogenetic defects passed on (Testart et al., 1996).

Since July 1996, 138 male patients attending the Rotunda Hospital, Dublin, for treatment with ICSI have had cytogenetic analysis performed. Overall six patients had an abnormal karyotype (4.3%). In the oligozoospermic group (<20x10^6 spermatozoa/ml), five out of 102 patients (5%) with severe oligozoospermia (<1x10^6 spermatozoa/ml) were found to have balanced translocations. Of these, four were Robertsonian translocations, three of chromosomes 13/14 and one of chromosomes 14/21. The fifth abnormality was a reciprocal translocation of chromosomes 4/21. No sex chromosomes aneuploidy mosaic was discovered. Of 36 patients with azoospermia, however, only one abnormality, a sex chromosome aneuploidy (XXY), was seen.

These data suggest, as found elsewhere (Testart et al., 1996), that chromosome analysis is necessary for patients with severe oligozoospermia. The abnormality we found most frequently was a Robertsonian translocation which can result in chaotic embryonic cleavage division (Delhanty et al., 1996). The incidence of chromosomal abnormality is inversely proportional to the sperm count (Kjessler, 1974) and, furthermore, infertile males with a normal sperm count have an increased incidence (2.2%) of abnormal karyotype (Yoshida et al., 1995).

Therefore, despite the undoubted cost (Pandiyan and Jequier, 1996), we recommend following a full explanation of the reasons, that mandatory screening of all infertile male patients independent of sperm count prior to treatment with ICSI is carried out to reduce any risk of propagation of genetic defects. Appropriate counselling and follow-up must also be provided by a clinical geneticist when results of concern are obtained.

Dear Sir,

Kobayashi et al. (1996) reported a case of an intrauterine pregnancy that was spontaneously conceived during a symptomatic missed abortion of an ectopic gestation resulting from an ovulation in the previous cycle. It has long been a matter of debate whether natural superfetation (conception during pregnancy) is at all possible in humans. While it may be concluded from the report of Kobayashi et al. (1996) that ovulation can occur before complete clearance of human chorionic gonadotrophin (HCG) from a tubal abortion, there is recent evidence in the literature suggesting that even an intrauterine pregnancy may not preclude conception resulting from ovulation stimulation in connection with assisted reproduction techniques (ART). In 1995, the Department of Pathology of our University and a private local ART centre published a case of a spontaneous intrauterine abortion at 15 gestational weeks consisting of two fetuses with a developmental age of ~98 days, a fetal remnant of approximately the same age and two embryos at ~41 days of age after gamete intra-Fallopian transfer (GIFT) (Krenn et al., 1995). The different developmental stages of the embryos and fetuses could not be attributed to intrauterine growth retardation because the development of the embryos was adequate compared with their placenta. Early embryonic death and retention of a pair of twins out of a quintuplet after GIFT was another possible explanation but was considered unlikely because the embryonic tissues showed no autolytic changes. Indeed, it was felt that the large difference in developmental age (>50 days) could only be plausibly interpreted by assuming that the fetuses and embryos were conceived from subsequent ovulations, although it was not possible to prove this assumption from the results of the morphological and histological examination of the abortus...
without the knowledge of endocrine profiles during early pregnancy. Nevertheless, the report of Krenn et al. (1995) highlighted the possibility that intruterine superfetation may occur after ovulation stimulation and ART techniques.

References

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Dear Sir,
Steck and Bussen (1997) have reported an earlier case which raised the possibility of superfetation after a gamete intrallopian transfer (GIFT)-induced pregnancy (Krenn et al., 1995). In their report, the patient underwent ovulation induction by human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG), and nine oocytes were recovered from 11 follicles at the time of GIFT. After the establishment of pregnancy, the patient was diagnosed as suffering from hyperstimulation syndrome, which usually includes multiple enlarged follicles. In addition, during early pregnancy, the HCG concentration in the sera of the pregnant woman is very high. As the authors mentioned in the discussion of their paper, it is plausible that the high concentration of HCG produced by the three placentae of the first multiple (triplet) pregnancies stimulated the follicles, and triggered the second ovulation under such unusual conditions (hyperstimulation). The interval between the two fetations was ~7 weeks, indicating that the gestational age of the first pregnancy was 9 weeks at the time of the second ovulation. The maternal serum HCG concentration reaches a peak at ~9 weeks of pregnancy (Cunningham et al., 1997); thus, the second ovulation and fertilation seem to be closely related to the hyperstimulation due to ovulation induction by HMG–HCG for GIFT.

Usually, it is difficult to become pregnant when a large mass such as submucous myoma exists in the uterine cavity. In this case, there were three concepti of 9 weeks’ gestation at the time of the second nidation. Previously, this patient had not become pregnant for 3 years, although the cause of infertility and the indications for GIFT were not explained. Nevertheless, the patient became pregnant even under such unusual conditions.

On the other hand, the patient in our case had no medication, and the spontaneous ovulation occurred during an ectopic pregnancy (Kobayashi et al., 1996). We suggest that the fertilized egg could implant in the uterine cavity without any problems since the preceding pregnancy was a peritoneal pregnancy. Thus, our case suggests the possibility that superfetation can occur not only after artificial induction of multiple ovulations but also during the natural ovulatory cycle.

References
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The postulated lagged correlation between dizygotic twinning rates and sperm count
Dear Sir,
I (James, 1997) have interpreted evidence to suggest that: (i) the dizygotic twinning rates fell in the USA between 1930 and 1964 and was roughly stable thereafter; (ii) sperm counts apparently fell in many parts of the USA in the 1960s and 1970s and stabilized in the 1980s; (iii) the dizygotic twinning rates in many parts of Europe were falling in the 1960s and 1970s and stabilized in the 1980s; and (iv) the declines in sperm count were a cohort phenomenon and data from Scotland and France suggest that sperm counts have been progressively lower in men born after 1950 or thereabouts.

Accordingly I proposed that there may be a correlation between dizygotic twinning rates and sperm counts a generation later (variations in both having the same cause). The support of this evidence for this hypothesis is admittedly thin (although it will be supported if the sperm counts of European cohorts cease declining a generation after their corresponding dizygotic twinning rates ceased declining). Meanwhile I wish to note a further piece of evidence for this postulated lagged correlation.

The secular movements of the age-specific dizygotic twinning rates of Sweden and Finland have been different during this century. Those of Sweden declined from 1925 to 1967 and stabilized thereafter (James, 1986; Eriksson et al., 1995). In contrast, those of Finland increased until 1960 and then subsequently declined (Eriksson et al., 1995). Sperm counts in Sweden have fallen in recent years (Anonymous, 1995; Bendvold et al., 1991), whereas those of Finland have not (Suominen and Vierula, 1993). If there were a lagged correlation between dizygotic twinning rates and sperm count, the sperm counts of Finland’s youngest cohort of men should by now be lower than those of their older cohorts. This point could be tested by reanalysing the existing data.

References


Eriksson, A.W., et al. (1995) Secular Clomid experiences. Quite rightly it is stated that ‘even the correct HMG and 28.57% in the long GnRHa protocol). However, the two GnRHa protocols (Table III); this of course can explain


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**Letters to the Editor**

**Table I. Cancellation rate of three different stimulation protocols**

<table>
<thead>
<tr>
<th></th>
<th><strong>Clomid + HMG</strong></th>
<th><strong>Ultrashort GnRHa</strong></th>
<th><strong>Long GnRHa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPU cycles</strong></td>
<td>341</td>
<td>351</td>
<td>77</td>
</tr>
<tr>
<td><strong>Cancelled cycles</strong></td>
<td>59</td>
<td>71</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>400</td>
<td>422</td>
<td>83</td>
</tr>
</tbody>
</table>

HMG = human menopausal gonadotrophin; GnRH = gonadotrophin-releasing hormone; OPU = oocyte puncture procedure.

*P = 0.0448 calculated by contingency table analysis (total \( \chi^2 = 10.41 \)).

**Table II. Total pregnancy rates*, miscarriage rates* and normal ongoing pregnancies of three different stimulation protocols**

<table>
<thead>
<tr>
<th></th>
<th><strong>Clomid + HMG</strong></th>
<th><strong>Ultrashort GnRHa</strong></th>
<th><strong>Long GnRHa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Not pregnant</strong></td>
<td>214</td>
<td>241</td>
<td>55</td>
</tr>
<tr>
<td><strong>Pregnant</strong></td>
<td>127</td>
<td>110</td>
<td>22</td>
</tr>
<tr>
<td><strong>Miscarried</strong></td>
<td>27</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td><strong>Take home baby rate</strong></td>
<td>100</td>
<td>89</td>
<td>11</td>
</tr>
</tbody>
</table>

*Includes ectopic and chemical pregnancies.

**Table III. Mean values (±SD) of some variables in in-vitro fertilization programmes, after three different stimulation protocols**

<table>
<thead>
<tr>
<th></th>
<th><strong>Clomid + HMG</strong></th>
<th><strong>Ultrashort GnRHa</strong></th>
<th><strong>Long GnRHa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>31.95 ± 4.81</td>
<td>34.7 ± 5.14</td>
<td>35.95 ± 5.13</td>
</tr>
<tr>
<td><strong>No. oocytes</strong></td>
<td>5.88 ± 3.22</td>
<td>7.73 ± 4.33</td>
<td>8.01 ± 4.8</td>
</tr>
<tr>
<td><strong>No. fertilizations</strong></td>
<td>3.88 ± 2.49</td>
<td>4.45 ± 2.83</td>
<td>4.51 ± 0.91</td>
</tr>
<tr>
<td><strong>No. for embryo transfer</strong></td>
<td>2.29 ± 0.48</td>
<td>2.49 ± 0.87</td>
<td>2.51 ± 0.91</td>
</tr>
<tr>
<td><strong>No. for freezing</strong></td>
<td>0.99 ± 1.88</td>
<td>1.18 ± 2.16</td>
<td>1.06 ± 2.2</td>
</tr>
<tr>
<td><strong>Embryo score</strong></td>
<td>41.28 ± 22.8</td>
<td>41.89 ± 22.7</td>
<td>41.23 ± 24.23</td>
</tr>
<tr>
<td><strong>Basal FSH</strong></td>
<td>9.35 ± 6.04</td>
<td>9.94 ± 5.7</td>
<td>7.43 ± 3.97</td>
</tr>
<tr>
<td><strong>Basal LH</strong></td>
<td>4.43 ± 2.68</td>
<td>4.13 ± 2.91</td>
<td>3.83 ± 2.19</td>
</tr>
<tr>
<td><strong>Basal oestradiol</strong></td>
<td>32.93 ± 16.86</td>
<td>29.75 ± 18.08</td>
<td>30.87 ± 16.72</td>
</tr>
</tbody>
</table>

HMG = human menopausal gonadotrophin; GnRH = gonadotrophin-releasing hormone; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant.

*P values calculated by Analysis of Variance (ANOVA factorial).

Continuing the debate on the obvious need for milder forms of ovarian stimulation

Dear Sir,
The letter by Edwards *et al.* (1997) confirmed my own experiences. Quite rightly it is stated that ‘even the correct use of clomiphene and human menopausal gonadotrophin (HMG) can lead to pregnancy rates equivalent to those gained using the agonists’. However, it is not true as it is maintained that ‘the problem here is that more monitoring is needed with the former treatment’. I have been using clomiphene for more than 10 years; always with the same stimulation protocol without hormone analyses (Kemeter and Feichtinger, 1989) and it is still my most successful stimulation protocol. We have demonstrated in a prospective randomized study (Obruca *et al.*, 1995) that there do not exist any advantages of gonadotrophin-releasing hormone agonist (GnRHa) protocols with HMG or pure follicle stimulating hormone (FSH) in relation to the clomiphene combination protocols.

During the last 2 years, retrospective analysis of our in-vitro fertilization (IVF) programme showed, in view of the stimulation, the following results: the cancellation rate was 14.75% in the clomiphene group and significantly lower (7.23%) in the long GnRHa protocol group (Table I). The number of oocytes, fertilized oocytes and embryos transferred was on average higher in the two GnRHa Protocols (Table III); however, the pregnancy rate was highest after clomiphene and HMG (37.3 compared with 31.4% in the ultrashort GnRHa and 28.57% in the long GnRHa protocol). However, the miscarriage rate was 50% in the latter, and only 19 and 21% respectively in the other two protocols, so that finally the normal ongoing pregnancy rate (= baby take-home rate) was close to 30% after clomiphene and HMG, 25% after the ultrashort GnRHa Protocol, and only 14.3% in the long GnRHa protocol (Table II). All our patients undergoing the long GnRH protocol received recombinant gonadotrophins. The average number of embryos for freezing was about the same in all three groups, as were also the other parameters, such as embryo score, basal FSH, luteinizing hormone (LH) and oestradiol (Table III) concentration. Patients who received clomiphene plus HMG were significantly younger than those receiving the two GnRHa protocols (Table III); this of course can explain the higher pregnancy and lower miscarriage rate. On the other hand it shows us that we should stimulate younger patients with such a mild form of stimulation and not with dangerous and expensive GnRHa protocols. In a previous review of our material (Niederberger *et al.*, 1995), ovarian hyperstimulation was significantly lower in patients stimulated with clomiphene and HMG, and twice as high after GnRHa stimulation.

Perhaps these results can also demonstrate ‘that modest forms of stimulation obtain better or at least equal results, so that clinics using them may end up at the top of the league’. I fully agree that a ‘laissez-faire’ attitude to hormone stimulation, with increasingly powerful agents becoming more freely.

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available, encourages bad practice and short cuts, and that this results in various risks to our patients.

I would like to stress that I fully agree with Edwards et al. (1997), that there is a need for greater care in deciding individual protocols for specific patients and for more research on new forms of treatment. Meanwhile Edwards’ demand for milder forms of stimulations can very well be realized by clomiphene and HMG and the ultrashort protocol. Correctly applied, as my experience proves, they lead to very good pregnancy rates, even better than some of those of the ‘long protocol and high dose FSH clinics’.

References


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Erratum

Molecular biology in the work-up of the infertile male: the time to recognise the need for andrologists

by P.Patrizio and G.S.Kopf

Hum. Reprod., 12, 879–883

On page 879, line 4, the number of births was printed incorrectly.

The line should have read:

. . . 150 000 births have occurred worldwide through the use of the various assisted reproduction techniques. . . .