Letters to the Editor

Use of hydrogen peroxide for vaginal contraception

Dear Sir,

We report a 37 year old Turkish patient using hydrogen peroxide (H₂O₂) as a vaginal contraceptive for 2 years after the birth of her first child. The woman was breast-feeding for only 3 months and she had regular sexual intercourse during the whole time she used the method. Immediately before vaginal intercourse she inserted a cotton pad soaked with 3% H₂O₂ solution (i.e. a commercial formulation available from pharmacies) deep into the vagina to the cervix os and removed it again after intercourse. Reported side-effects included local burning and vaginal discharge during the first few applications. The patient conceived again, 5 months after discontinuing H₂O₂ use, resulting in a normal pregnancy. The Papanicolaou and vaginal smears that were obtained during routine obstetric care did not show any evidence of cervical inflammation or lower genital tract complication. She had been told of the contraceptive method by a relative who, after the birth of her seventh child, had used this method with the same formulation, successfully for 20 years. Moreover, this method of family planning is used in certain regions of her native country Turkey by certain sectors of the population.

In in-vitro investigations, H₂O₂ has been shown to exert concentration-dependent (0.01–0.4 mM, Oehninger et al., 1995; 0.25–5.0 mM, de Lamirande and Gagnon, 1992) toxic effects on spermatozoa, ranging from immobilization to cell death. These deleterious effects are mainly attributable to the peroxidation of membrane lipids. Because of its spermicidal properties, H₂O₂ could well offer an effective and inexpensive method of contraception that is characterized by convenience of use and is associated with few side-effects. Moreover, the production of H₂O₂ by certain strains of Lactobacillus acidophilus plays an important role in maintaining or restoring the physiological microflora in the vagina (Klebanoff et al., 1991). However, the cytopathic properties of H₂O₂ may also be involved in the aetiology of cervical cancer (Fernandez et al., 1995). We therefore believe that patients practising this form of contraception, thus exposing the cervix to high concentrations of this toxic agent over many years, should be informed of the dangers involved and offered regular cervical cytology testing by Papanicolaou smear.

References


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Chromosomes of multipronuclear zygotes resulting from ICSI

Dear Sir,

Based on chromosome analyses of multipronuclear zygotes obtained after intracytoplasmic sperm injection (ICSI), Macas et al. (1996a) speculated on adverse effects of this technology on the distribution of paternal chromosomes prior to first cleavage division. Subsequently, we stressed the need for further investigation of chromosomal alterations induced by ICSI particularly because we have been able to confirm the observation of an obviously irregular oocyte chromosome segregation (Rosenbusch and Sterzik, 1996). Recently, Macas et al. (1996b) presented further data and provided an interesting discussion on possible genetic consequences of direct sperm insertion. However, some points in this report caught my attention and perhaps the authors on their part are willing to make a statement on the following remarks.

First, as indicated in the Introduction, Macas et al. (1996b) designed their study ‘to obtain insight into the regularity of sister chromatid exchange at the final stage of the second meiotic division’. However, sister chromatid exchange results from the interchange of homologous segments between two chromatids of one chromosome and is detectable only by special staining methods. As Macas et al. stained their slides homogeneously with Giemsa, something must have been confused here. What has been studied are, simply speaking, the results of anaphase of meiotic division II during which the centromeres divide and the sister chromatids move to opposite poles in preparation for the extrusion of the second polar body.

Secondly, Macas et al. noted that the rate of aneuploidy found among microinjected zygotes was 56.7%. In future discussions, this generalized finding will need careful interpretation because different phenomena are involved, i.e. gametes presenting with aneuploid chromosome complements before injection and an irregular, possibly ICSI-induced chromatid segregation in normal oocytes. For instance, a tripronuclear zygote with one polar body and a chromosome distribution of 23/24/22 would mean that the metaphase II oocyte had a correct chromosome number. Also, the distribution 23/19/4 would indicate a normal extrusion of the second polar body and a normal haploid oocyte chromosome set regardless of the formation of a supernumerary pronucleus. On the other
hand, a zygote with a 22,X,-D; 22,X,-D; 23,Y karyotype implicates the presence of an aneuploid oocyte but a correct segregation of the maternal chromosomes. As discussed by the authors, the situation can become more complicated as soon as an aneuploid oocyte experiences an irregular chromatid segregation. However, presenting the results accordingly might be helpful in distinguishing ICSI-induced from gamete-transmitted abnormalities. In this respect, the comparison of the total rates of aneuploidy detected after ICSI and conventional in-vitro fertilization (IVF) is another critical point. The sex chromosome constitution of IVF zygotes has not been provided so that the paternal or maternal origin of abnormal metaphases is not evident. (Incidentally, an unequivocal classification of pronuclei is only possible in a 23,X/23,Y/23,Y zygote). In short, it is conceivable that sperm-transmitted aberrations have been included which cannot be compared with ICSI-induced oocyte abnormalities.

Finally, Table I in Macas et al. (1996b) reveals an astonishingly high incidence of diploid or near-diploid chromosome complements in groups B (two individual metaphases) and C (one single metaphase) of the microinjected oocytes. Also, 30% of the tripronuclear IVF zygotes were in the diploid range. It has been reported that cytoplasmic vacuoles might be confused with true pronuclei and that this error can largely be avoided by inspection of the cells under the phase contrast microscope because vacuoles do not contain nucleoli (Van Blerkom and Henry, 1987). From my own experience I know that the assessment of polar bodies and pronuclei in IVF oocytes can be impaired by large numbers of attached spermatozoa and remaining cumulus cells. However, this should not apply to ICSI zygotes. Macas et al. mentioned that the zygotes were re-examined using phase or interference optics but unfortunately, I found no explanation for the frequently missing concordance of chromosome count and number of pronuclei.

Interpretation bias

The term ‘sister chromatid exchange’ is used to describe the mutual exchange of sister chromatid segments that occurs during early oogenesis. However, once meiosis becomes arrested at metaphase of the second meiotic division (metaphase II), no further structural rearrangement on chromosomes occurs. Since our study was conducted on oocytes in the metaphase II, it is obvious and understood that the effect of intracytoplasmic sperm injection (ICSI) on the regularity of sister chromatid exchange between two female-derived pronuclei, and not chromosome, was evaluated. We feel the confusion may have arisen due to the wrong choice of words, i.e. ‘chromatid exchange’ to describe the movement of the chromatids to the opposite poles of the oocyte. However, this should not apply to ICSI zygotes. Macas et al. mentioned that the zygotes were re-examined using phase or interference optics but unfortunately, I found no explanation for the frequently missing concordance of chromosome count and number of pronuclei.

Conceptual problem

The main goal of this study was to evaluate whether ICSI causes oocyte cytoskeletal damage and our experiments were designed to specifically confirm this hypothesis. Moreover, our discussion is carefully written to highlight our findings and not to be too speculative and go beyond the framework of the study. We did not distinguish between ICSI-induced and gamete-transmitted abnormalities as this was not the goal of the study. It could be that other cytogenetic models, for example, the chromosome analysis of unfertilized oocytes after ICSI and human spermatozoa inserted into hamster eggs might offer a better opportunity to resolve the problem in which the extent of gamete abnormalities might contribute to the total rate of aneuploidy after ICSI.

Additional comments

In some IVF studies it has been shown that one pronucleus in trippronucleate zygotes may escape syngamy, so that an embryo at the first cleavage division would contain two haploid metaphases (Rudak et al., 1985; Angell et al., 1986; Macas et al., 1988; Pieters et al., 1992). There is no direct information on these events after ICSI; however, our observation of the second polar body chromatin suggests that a similar mechanism might be involved in the production of most diploid or near diploid zygotes in this study.

References


Bernd Rosenbusch
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Dear Sir,

We appreciate the interest Dr Rosenbusch has expressed in our recent article. In response to his comments we would like to make the following points:

References


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‘Curing’ empty follicle syndrome

Dear Sir,

We read with interest the report by Ndukwe et al. (1997) on ‘curing empty follicle syndrome’. We share with the authors the same emphasis on the strong relationship between human chorionic gonadotrophin (HCG) administration and the aetiology of this syndrome (Asch et al., 1992; Zegers-Hochschild et al., 1996), from our experience with a similar case of empty follicle syndrome that was created by inappropriate timing of HCG administration. A 35 year old woman with a 7 year history of secondary subfertility due to severe oligozoospermia underwent oocyte retrieval following controlled ovarian stimulation. No oocytes were found in the aspirate from 14 mature (size\(\geq 16\) mm) preovulatory follicles from one ovary 36 h after presumed HCG administration. On direct questioning it transpired that the patient had received her HCG injection (10 000 i.u.) only 12 h earlier. Further aspiration from the other ovary was deferred for 24 h, when 7 oocytes were obtained from the aspiration of nine follicles from that ovary of which fertilized normally following micro-injection with husband’s spermatozoa and resulted in the transfer of three 4-cell embryos. A viable intrauterine twin gestation was confirmed on ultrasound scanning. We are concerned however, that Ndukwe et al. consider empty follicle syndrome wholly as a drug-related syndrome, as this fails to explain recurrence of the syndrome (Coulam et al., 1986 and our own experience). A 28 year old woman with a 7 year history of unexplained primary subfertility had two retrieval cycles (9 months apart). Her response to standard ovulation stimulation was judged to be normal by both hormonal and ultrasound monitoring. Despite confirming the appropriateness of timing and administration of the HCG and the precaution of administering different batches each time, aspiration and flushing of 28 and eight follicles in the first and second cycle respectively failed to yield any oocytes. Thus empty follicle syndrome may be a manifestation of clinical dysfunction as well.

References


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Dear Sir,

We thank Khalaf and Braude (1997) for their interest in our paper on ‘curing’ empty follicle syndrome (Ndukwe et al., 1997).

Like the case described by Khalaf and Braude, we had cases where no oocytes were retrieved due to inappropriate dose or timing of human chorionic gonadotrophin (HCG) administration. We did not regard these cases as true cases of empty follicle syndrome and they were therefore excluded from our data for both our publications on empty follicle syndrome. This, however, underscores the importance of careful investigation to rule out any problems with HCG administration, wrong timing and incorrect dose of HCG before the diagnosis of empty follicle syndrome is made.

Coulam et al. (1986) reported a case in which oocytes were not retrieved from the same patient on two occasions. On the first occasion there was sub-optimal response to human menopausal gonadotrophin (HMG) with only two follicles. On the second occasion, there was a better response with six follicles. There was no comment on confirmation of the appropriateness of the timing, dose and administration of the HCG. This in our view does not prove the case that empty follicle syndrome is recurrent.

The case described by Khalaf and Braude, however, is a very interesting one and merits further study. We would recommend that they check her serum HCG concentration 12 h after HCG administration and defer oocyte retrieval if it is very low.

In another publication of ours on predicting empty follicle syndrome (Ndukwe et al., 1996), we defined a cut-off level of 10 IU/ml as that below which all our cases of empty follicle syndrome occurred.

The work done independently and contemporaneously by Zegers-Hochschild et al. (1995) and ourselves (Ndukwe et al., 1996, 1997) provides strong evidence that, provided HCG is administered appropriately, the fundamental problem with empty follicle syndrome seems to lie in the quality of HCG administered. Desialylation of some ampoules of HCG rendering them subject to rapid metabolic clearance by the liver and leading to very low serum concentrations of HCG is one possible explanation. How this can occur, despite the quality control measures by the manufacturers, merits further study.

References


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Letters to the Editor

Anything to learn from exceptionally good in-vitro fertilization treatment cycles?

Dear Sir,

We read with interest the case report by Marcus et al. (1996) which described the generation of 11 embryos in an in-vitro fertilization (IVF) treatment cycle. The transfer of three embryos resulted in a quadruplet birth. The remaining frozen embryos were donated to two different recipients and each patient had three embryos transferred. The first resulted in a twin birth and the second resulted in a singleton birth. We agree with Marcus et al. that some patients on assisted conception programmes have high fertility potential. The latter is essentially due to good oocyte, hence good embryo, quality. This has become more evident since we started our oocyte and embryo donation programmes (Serhal and Craft, 1989) where the embryos have the same high potential to achieve a pregnancy in different recipients.

We have made the same observation in our embryo donation programme. A 32 year old patient presented with 3 years’ infertility due to a male factor. She gave a history of one termination of pregnancy 10 years earlier. The couple underwent IVF: using the long down-regulation protocol, 22 oocytes were aspirated transvaginally and 17 fertilized. Three embryos were transferred. A pregnancy test was positive 12 days after embryo transfer and, after a further 3 weeks, a vaginal ultrasound scan showed two gestational sacs each containing a fetus with evident fetal heart activity. She gave birth to two healthy infants, a 2.6 kg male and a 2.36 kg female.

The couple donated their remaining 14 embryos. The latter were cryopreserved in three straws containing five, five and four embryos. A set of five embryos was donated to Recipient A and three were transferred. She conceived with two fetuses and gave birth to two female infants. The second set of embryos was donated to Recipient B who conceived one fetus and this resulted in the birth of a female infant. We still have four frozen embryos which are assigned to Recipient C to be used in the near future.

There is a wide range of fertility potential in assisted conception programmes where some patients easily achieve a pregnancy. The same principle will apply to women trying for natural pregnancy. We think that it is worth reviewing those patients with such excellent fertility potential to ascertain, if possible, the factors that might have contributed to such an outstanding performance. This might help to improve the outcome for less fortunate patients.

References


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Dear Sir,

We appreciate the interest Dr Gorgy and colleagues have expressed in our recent article (Marcus et al., 1996). We fully agree with them that there is a group of patients in in-vitro fertilization (IVF) programmes who have high fertility potential. Indeed, we have a similar case to the one described by Gorgy et al., a patient aged 24 years who underwent an IVF treatment because of tubal damage. A total of 22 oocytes were collected and all the 16 embryos which resulted were cryopreserved because of increased risk of developing ovarian hyperstimulation syndrome. She subsequently attended the Clinic for frozen embryo transfer on hormone replacement treatment. Three frozen embryos were thawed and two embryos were transferred to the uterus. She conceived with a twin pregnancy and gave birth to two healthy babies. Subsequently, the couple donated their 13 spare frozen embryos to infertile couples. Recipient A received two embryos and recipient B received three embryos. Both recipients conceived twin pregnancies which are now ongoing over 24 weeks gestation. Recipient C is awaiting embryo transfer and we may still be able to assign embryos to yet another recipient.

It is worth remembering, in the UK, that donated embryos should not normally be used for treatment once the number of children born from donated oocytes has reached 10. This number may be exceeded only in exceptional cases, for example where the recipient wishes to have a subsequent child from the same donor (Human Fertilisation and Embryology Act, 1990). In this case, as well as the case described by Gorgy et al., the donor was aged <32 years, which is probably the reason for the good quality embryos and hence the high potential to achieve pregnancies. This is in contrast to the case originally reported by us (Marcus et al., 1996) when the age of the donor was 36 at the time of egg collection. The astonishingly high implantation rate achieved from the oocytes of this patient provides evidence that oocyte quality does not necessarily decline with increasing maternal age. The lower pregnancy rates achieved in older age women after IVF/embryo transfer are not therefore entirely due to decreased oocyte quality, but also to a decline in endometrial receptivity (Templeton et al., 1996; Marcus and Brinsden, 1996). We are currently analysing the factors affecting the success of treatment after cryopreserved-thawed embryo transfer of donated embryos.

References


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Comparative costs of methotrexate and laparoscopic surgery

Dear Sir,

Yao et al. (1996a) recently reported on the direct medical costs of methotrexate and laparoscopic surgery for the treatment of ectopic pregnancy. The mean costs of treatment with methotrexate were Canadian Dollar (CD$) 880, almost CD$1000 less than the mean costs of laparoscopic surgery.

The prospective profile of the two groups of this non-randomized study is reported not to show any differences. The authors refer for further details to a previous study (Yao et al., 1996b). It is hard to imagine that patient characteristics have played no role in the allocation of treatment. Gestational age, initial serum human chorionic gonadotrophin (HCG) concentration and initial tubal diameter on tubal sonography are reported not to be different between the two groups. However, nothing is stated about abdominal pain, findings at physical examination, the circulatory situation of the patients or cardiac activity at sonography. Furthermore, eight patients (12%) who were treated surgically required conversion to salpingectomy or mini-laparotomy. This might be due to the presence of tubal rupture or active bleeding before treatment. We are afraid that the two groups may not be as comparable as the authors would like us to believe.

Secondly, the authors excluded the visits to the physicians’ private offices. In our opinion, this decision hampers the interpretation of the results of the study, especially since the surgeon’s fee has been included in the calculation of the costs of laparoscopy.

Although the authors state that ‘the completeness of cost and clinical data based on actual cases reported contributed enormously to the strength of the study’, we think that their conclusion that methotrexate reduces costs considerably is premature. Furthermore, the conclusion that the presumed cost-reduction generates savings of health care costs should be handled with care, since a cost reduction will only generate savings if the resources are not reallocated for other purposes.

References


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Dear Sir,

We would like to thank Mol et al. (1997) for their comments and interest in our study.

First, we would like to address the issues of fetal cardiac activity and exclusion of office visits. Interestingly, four out of 40 patients in the methotrexate group had fetal cardiac activity pre-treatment and all were treated successfully with methotrexate. However, due to the small numbers, we did not conclude on the lack of adverse effect of fetal cardiac activity on methotrexate success (Yao et al., 1996).

The exclusion of office visit costs did not affect our cost analysis because patients were treated and counselled primarily in the gynaecological ultrasound units (included in the ultrasonographer’s fee), and were instructed to go to the emergency centre for increasing symptoms. Physician time spent on telephone follow-up of human chorionic gonadotrophin (HCG) concentrations was not a reimbursed activity in the Province of Québec during the study period.

While we appreciated the limitations of a retrospective, non-randomized comparison of the two treatment modalities (methotrexate versus laparoscopic surgery), we demonstrated that the two groups were similarly based on objective and quantifiable parameters (weeks of amenorrhoea, initial serum HCG, and initial tubal diameter on transvaginal ultrasound) (Yao et al., 1996). None of the patients described in the study had signs of haemodynamic instability or presented with an acute abdomen as such patients were routinely treated with laparotomy during the study period. Fernandez et al. (1993) described a prognostic scoring system for methotrexate treatment based on the grading of six criteria: gestational age, serum HCG, serum progesterone, degree of abdominal pain, volume of haemoperitoneum, and tubal diameter on ultrasound. The degree of abdominal pain could not be accurately quantified in a retrospective manner; therefore, it could not be used in the comparison.

Indeed, in non-randomized treatment allocation, selection bias could occur. In this instance, the concern would be a tendency to treat ‘sicker’ patients by laparoscopic surgery and ‘less sick’ patients by methotrexate. A total of five patients (7.6%) required conversion to mini-laparotomy and there were eight cases of non-elective salpingectomy. The reasons for those procedures were described and reflected more on the technical difficulty of the surgery and were not necessarily due to the ‘severity’ of the ectopic pregnancy. In fact, in the laparoscopy group, the incidence of tubal rupture was very low (five out of 66 patients or 7.5%) as was the incidence of significant haemoperitoneum >500 ml (one out of 66 patients or 1.5%) (Yao et al., 1996).

A lack of agreement on prognostic indicators for methotrexate treatment further compounded the issue (Yao and Tulandi, 1997). Our methotrexate success rate (72.5%) was relatively low compared with the 86% (Glock et al., 1994) to 94% (Stovall and Ling, 1993) quoted in the literature. This discrepancy suggests that our methotrexate treatment criteria may be less stringent. If there existed a selection bias, it probably operated to overestimate, rather than underestimate, the cost of methotrexate treatment.

Ideally, an alternative treatment method that is chosen over the gold standard (laparoscopy) would have an efficacy that is greater than or at least equal to the gold standard and a cost that is less than or equal to the gold standard. Methotrexate is non-invasive and without risks of general anaesthesia and...
trocars; therefore, it is particularly appealing as an alternative treatment modality. Retrospective design notwithstanding, our finding of methotrexate’s lower direct medical cost and lower success rate indicates that a prospective, randomized trial is imperative to establish unified guidelines on methotrexate treatment criteria, direct medical costs, and indirect or societal costs. Operator–receiver curves can then be used to determine the optimal stringency of treatment criteria that will maximize the number of patients eligible for the methotrexate treatment while maintaining a high efficacy rate and low cost.

References


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In-situ hybridization chromosome analysis of XYY and XXY males’ spermatozoa

Dear Sir,

We studied two oligozoospermic, abnormal karyotype men to evaluate the safety of using their spermatozoa for intracytoplasmic sperm injection (ICSI). Their karyotypes were 47,XYY and 46,XY/47,XXY. Fluorescent in-situ hybridization (FISH) was employed to analyse the genetic make-up of spermatozoa. Spermatozoa from a control subject was also analysed. No significant differences between the spermatozoa from the abnormal genotype subjects’ spermatozoa and the control spermatozoa were found (Table I).

The purpose of this study was to demonstrate that even in cases of mosaic aberrations, genetically normal spermatozoa are available for, and safe for use with, ICSI. No spermatozoa with abnormal karyotype were found in our study. Although the mechanisms of spermatogenesis are still not clearly understood, we surmise that the process itself safeguards against the production of genetically abnormal spermatozoa (Cozzi et al., 1994; Han et al., 1994; Martin et al., 1996). This suggestion is supported by the fact that XYY cells usually fail to develop into spermatozoa. Hence the high rate of azoospermia in men with abnormal karyotypes. ICSI and other assisted reproductive technology (ART) procedures are clearly viable treatment options for men with abnormal somatic cell karyotypes. However, in these cases it would be prudent to diagnose the embryo karyotype after fertilization and, prior to implantation and, at the appropriate time, amniocentesis should be performed.

Table I. Karyotypes obtained by fluorescent in-situ hybridization. Figures in parentheses are percentages

<table>
<thead>
<tr>
<th>Patient karyotype</th>
<th>No. spermatozoa tested</th>
<th>Sperm karyotype</th>
</tr>
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<tbody>
<tr>
<td>47,XYY</td>
<td>60*</td>
<td>25 (41.7)*</td>
</tr>
<tr>
<td>46,XY/47,XXY</td>
<td>82</td>
<td>44 (53.7)</td>
</tr>
<tr>
<td>46,XY (control)</td>
<td>748</td>
<td>404 (54.0)</td>
</tr>
</tbody>
</table>

*One spermatozoon was unstainable, and so the karyotype could not be determined.

References


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