Letters to the Editor

Leukaemia inhibitory factor expression in human follicular fluid

Dear Sir,

We were interested to read the paper by Arici et al. (1997) who found leukaemia inhibitory factor (LIF) expression in human follicular fluid (hFF). We would like to confirm this fact and make some short comments.

Since we have postulated that LIF could play a role in the inflammatory-like processes involved in the periovulatory events, we have performed a study to assess its presence in hFF and evaluate this cytokine as a marker of oocyte quality. The preliminary results of this study were presented at the 13th Annual Meeting of the European Society for Human Reproduction and Embryology (ESHRE) (Jean et al., 1997).

Briefly, the hFF samples evaluated in our study (n = 96) were obtained from individual follicles at the time of oocyte retrieval for patients undergoing in-vitro fertilization (IVF). The aspirated follicles were immediately examined for the presence of oocytes and hFF were then classified into two groups based on the morphology of the oocyte. Group 1 (n = 70) included hFF corresponding to oocytes that could be inseminated, while group 2 (n = 26) was composed of hFF containing atretic oocytes. The LIF concentration of each hFF sample was determined in both groups by an enzyme-linked immunosorbent assay (ELISA) method (kit Medgenix, Fleurus, Belgium) while the patient serum served as a negative control for LIF detection. The sensitivity (20 pg/ml), the precision (intra-assay variation <5%), and the reproducibility (inter-assay variation <10%) of this ELISA have been reported previously (De Groote et al., 1994) while a good correlation (r = 0.96; P < 0.0001) between LIF measurements in several biological fluids using this ELISA and the corresponding DA-1a bioassay has been demonstrated previously (Gard et al., 1993; De Groote et al., 1994).

As reported by Arici et al., we confirm that LIF was expressed in hFF samples 35 h after administration of human chorionic gonadotrophin (HCG). However, despite the lack of available data in the literature, we were astonished by the very low mean concentration of LIF measured by these authors (13.0 ± 1.1 pg/ml) in comparison with the LIF concentrations reported in our study (group 1: 2833.37 ± 1270.08 pg/ml and group 2: 1602.33 ± 1159.01 pg/ml). In addition, the mean concentration of LIF in the pre-HCG hFF samples (0.8 ± 0.3 pg/ml) appears to be very low, especially with respect to the sensitivity for LIF (2 pg/ml) of the ELISA used by Arici et al. This level of LIF seems to us to be too low to be considered as a significant expression of LIF in hFF samples obtained from patients prior to the administration of HCG.

Nevertheless, we fully agree with these authors that LIF is probably one of the factors in hFF involved in the ovulatory process and embryo development. As they reported a significant correlation between embryo quality and corresponding hFF concentrations, we have demonstrated that the LIF level in hFF could be related to the oocyte quality. Thus, both the findings by Arici et al. and ourselves support the hypothesis that LIF expression in hFF is required in the early reproductive events.

Finally, although further studies have to be conducted to assess accurately the level of LIF in hFF, this cytokine appears to have a promising role in extending our knowledge of the processes leading to fertilization and embryo development.

References


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Dear Sir,

We read with interest the letter by Jean et al., and we appreciate their comments. We were pleased to see that their results confirm ours. However, we were surprised by the relatively high concentrations of leukaemia inhibitory factor (LIF) measured by these authors; their values are >200-fold higher than the ones we observed. The most likely possibility is the use of different enzyme-linked immunosorbent assay (ELISA) kits. Our kit came from R&D Systems, Rochester, MI, USA, while their ELISA was from Medgenix, Fleurus, Belgium. We are planning to purchase an ELISA from Medgenix, so that we can compare the two kits.

We thank Jean et al. for their interest.

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Letters to the Editor

Deception by chimerism

Dear Sir,

With great interest we read the debates and articles on the molecular diagnosis of Y chromosomal microdeletions (Pryor and Roberts, 1998; Simoni et al., 1998; Vogt, 1998). We recently had an interesting case adding an unexpected aspect to the question of quality control.

Infertility affects ~7% of the male population. There is increasing evidence for a genetic cause for at least a subset of fertility disorders and DNA analysis is being introduced more and more into the routine infertility work-up. A set of spermatogenesis-specific genes are known to be involved in fully normal germ cell proliferation and recently microdeletions of the Y chromosome have been detected in 5–10% of men with idiopathic azoospermia and oligozoospermia. According to Vogt et al. (1996), the microdeletions can be subgrouped into the AZF (azoospermia factor) a to c region. Candidate genes, e.g. RBM or DAZ, can be allocated to these regions.

A 53 year old patient presented with the questions of his chances of fertility prior to his second marriage. He had an adult daughter from a previous relationship. Semen analysis revealed azoospermia. He had been treated for chronic myeloic leukaemia (Philadelphia chromosome positive) 3 years ago. Total body irradiation and chemotherapy associated with autologous bone marrow transplantation in most cases lead to complete and irreversible spermatogenic failure, through sporadic recovery of the reproductive axis has been reported.

Factors that determine the fate of spermatogenesis after oncological therapy are not well understood (Chatterje et al., 1996). To further elucidate the pathogenesis of azoospermia and provide better prognostic information to the patient, we performed Y chromosomal microdeletion analysis, which is normally included into the diagnostic work up of men presenting with severe impairment of spermatogenesis in our infertility clinic. We routinely use nine different Y chromosomal markers and one autosomal marker to ensure proper polymerase chain reaction (PCR) conditions.

In our patient, amplification of Y chromosomal markers for the different AZF regions failed completely, however, the autosomal marker could be amplified by PCR from genomic DNA obtained from leukocytes. In subsequent steps we tried to amplify SRY, a crucial marker for the testis formation, which was also absent in this patient. The lack of any specific male marker in the genomic DNA from the blood led us to speculate on the sex of the bone marrow donor, which indeed turned out to be female. Analysis of DNA isolated from buccal cells of the patient then showed the normal presence of all Y chromosomal markers.

Molecular diagnostics on genomic DNA isolated from lymphocytes is becoming an important tool in many different clinical conditions. In infertile males, this diagnostic procedure is now being performed world-wide using sequenced-tagged site (STS) markers covering the AZF areas (Simoni et al., 1997). Physicians and laboratories should be aware of the fact that chimerism is created by transplantation centres, which in our case lead to a laboratory deception in the analysis on Y chromosome deletions. We suggest that SRY, as a male marker, should be included into DNA diagnostic of infertile men to assure sex identity.

References


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Putting new drugs to good use

Most infertility specialists have encountered the problem of having to abandon a treatment cycle because the man was unable to produce a semen sample on demand. The need to ejaculate at a determined time in the clinic can be so stressful for some otherwise normal men, that they may not be able to achieve an erection at the desired time. A number of methods to tackle this problem have been described. These include: freezing a semen sample in advance; using an electrically operated mechanical vibrator; and even using electroejaculation to retrieve spermatozoa. We recently had a patient, who normally had no problems masturbating or having sex, but who could not achieve an erection in order to produce the semen sample which was needed for intrauterine insemination. He had failed to achieve an erection even after 2 h of stimulation with the vibrator in the ‘masturbatorium’. Before abandoning the cycle, we offered him a trial with Viagra (sildenafil citrate) to help him have an erection. Within 25 min of taking 50 mg of Viagra, and resuming vibratory stimulation, he was able to ejaculate successfully. We feel infertility clinics should keep an emergency stock of Viagra on hand, so that this can be used to help infertile men who need the additional help to ejaculate in the clinic.

Aniruddha Malpani
Malpani Infertility Clinic, Bombay, India

Selective cost of delayed childbearing

Dear Sir,

In recent years the tendency to postpone marriage and childbearing has spread throughout the developed countries,
proportion of aged (Central Italy the average maternal age at delivery and the negligible price in perinatal deaths. Age at delivery started to increase, women have paid a not mortality, since the beginning of the eighties when maternal to the fact that in spite of the very low risk of perinatal (Bowman and Saunders, 1995; Gosden and Rutherford, 1995; Lansac, 1995; Breart, 1997). Here we wish to draw attention (Fretts et al. 1995) among the EU countries. From 1980 to 1994, year of the latest available data from the Italian vital statistics bureau (ISTAT), in Northern and Central Italy the average maternal age at delivery and the proportion of aged (≥35 years) mothers increased steadily, and linearly from 1985 onwards: the average age increased from 27 to 30 years, and the proportion of aged mothers by about 70%. During the same period the overall stillbirth rate decreased drastically, but among the aged mothers, in spite of a 60% reduction, it remained higher than that among mothers <35 years, and in 1994 had not yet reached the stillbirth rate suffered by the young mothers ten years earlier (Table I).

We calculated in terms of stillbirths the selective cost of late childbearing among the women aged ≥35 years in excess of a 9% baseline proportion of mothers ≥35: this 9% low, reached in 1980 thanks to the reduction in the number of children per family, was assumed to be the minimum unavoidable proportion of mothers who would have a late pregnancy. In the 10 years from 1985 to 1994, the excess quota accounted for a total of >100,000 mothers (50,000 in the last 4 years of the study), 700 of whom lost their child at birth and an almost identical number lost their child within the first month of extra-uterine life (Table I). The emotional price of the losses is even greater in northern and central Italy where women have attained the lowest fertility (1.05 children per family, was assumed to be the minimum unavoidable of childbearing women will be aged ≥35 years by 2015, that is in less than one generation.

The decision to start a family late may allow greater parental awareness in pregnancy management and child raising, but might be a disadvantageous gamble from a personal and social point of view. Only personal consciousness of the risk and a far-seeing policy of incentives for young couples to start a family may counterbalance the tendency and limit personal and social costs to those instances of subfertility and perinatal pathology which are unavoidable.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total no. births</th>
<th>Average age of mothers (years)</th>
<th>Mothers aged ≥35 years</th>
<th>Stillbirth rate (%) among mothers</th>
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<tbody>
<tr>
<td></td>
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<td>All</td>
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<tr>
<td>1980</td>
<td>343,677</td>
<td>26.99</td>
<td>8.95</td>
<td>12.75</td>
</tr>
<tr>
<td>1985</td>
<td>305,891</td>
<td>27.75</td>
<td>10.46</td>
<td>5.64</td>
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<tr>
<td>1994</td>
<td>296,412</td>
<td>29.61</td>
<td>15.14</td>
<td>3.96</td>
</tr>
</tbody>
</table>

*Perinatal mortality (no. per thousand), i.e. stillbirths + deaths within 1 month of delivery was 7.61 (total); 7.05 (mothers aged <35 years) and 10.76 (mothers aged ≥35 years).*

FRAXA premutations are not a cause of familial premature ovarian failure

Dear Sir,

Conway et al. (1998) report that the transmission of premature ovarian failure (POF) (MIM 311360) was associated with fragile X (FRAXA) premutation in three out of 23 pedigrees. POF is defined as secondary amenorrhoea with high gonadotrophin values occurring before the age of 40. From a population of 71 women affected by POF and attending the Reproductive Endocrinology Service of the Departments of Obstetrics and Gynaecology in Milan and Varese (Vegetti et al., 1998), we selected a sample of 29 families showing the transmission of the condition. Pedigree analysis suggested a
dominant pattern of inheritance through maternal or paternal relatives and, in five families, through both. The analysis of 29 probands indicated that the median age at POF onset was 38 years (range 20–40 years), and that menarche occurred at 12 years (range 10–18 years). Within each family the proband and all of the relatives affected by POF were phenotypically normal; women with a family history of mental retardation were excluded from the study. Furthermore, proband chromosome analysis based on high resolution banding, failed to reveal any structural anomalies. To test the possible association between POF and the fragile X premutation, peripheral blood DNA from the probands was digested with EcoRI or EcoRI/EagI, and hybirdized with the StB12.3 probe, as described by Rousseu et al. (1991). Southern blot analysis revealed a FRAXA premutation in only one out of the 29 affected females (see Figure 1). However, the hybridization intensity of the fragment corresponding to the FRAXA premutation (5.6 kb) in the proband (II-4) was less than that observed in the premutated control (PC). This result suggests the occurrence of a post-zygote event leading to a mosaic condition in which case the FRAXA premutation can be considered a sporadic event. Moreover, the mother (I-8) showed a normal number of CCG repeats. Our data agree with the results of Kenneson et al. (1998) (who found no evidence of FRAXA premutation in a sample of 17 cases of familial POF and 108 sporadic cases, but one premutated allele in one of the control women) but differ from those reported by Conway et al. (1998) which indicate a higher than expected (Sherman, 1995) prevalence of FRAXA carriers among women with POF. The discrepancy between the two studies may lie in the different criteria used for the sampling, including family history and median age at menopause. Since it has been reported that the onset of menopause is earlier in FRAXA carriers (Partington et al., 1996), pedigrees showing the transmission of fragile X syndrome (as reported by Conway et al., 1998) should probably be avoided when investigating the possible association between FRAXA premutation and POF. Furthermore, Conway et al. (1998) reported a median age at menopause onset of 26, which differs from the 38 years found in our study. This difference in median age may reflect the occurrence of subpopulations of women affected by POF, who may be characterised by a different aetiopathogenesis. In conclusion, any assessment as to whether FRAXA carriers are at greater risk of developing early menopause requires studies with more controlled parameters.

References

Figure 1. Pedigree and Southern Blot analysis of the identified fragile X (FRAXA) carrier and her mother. I-5 and II-4 respectively ceased menstruation at 29 and 40 years. II-3 was not available for analysis, but had regular menses at the age of 48. PC = premutated FRAXA control.

To whom correspondence should be addressed

Dear Sir,
In their letter Dr Marozzi et al. present the third series of women with premature ovarian failure in whom screening for Fragile X (FRAXA) premutations has been undertaken. Neither Marozzi et al. nor Kenneson et al. (1997) detected an excess of FRAXA premutations in their studies, as was the case in our own series (Conway et al., 1998). The reason for these discrepancies is simply related to the difference in the age of onset of ovarian failure in each study population. The median age of menopause in the Marozzi series was 38 while in the study of Kenneson et al. (1997) only a minority of women in fact had premature menopause at all as 85% of women in their series experienced the menopause between the ages of 40 and 47. In our own series the median age of onset of amenorrhoea was 26 years (range 11–39) and in the seven women with premature ovarian failure (POF) and FRAXA permutations the median age of onset was 24 years (11–35).
Quite obviously, very few women in this age group were included in either the Marrozzi or the Kenneson study.

While we did not select our patients on the basis of age, our clinic is a tertiary referral centre based in a Reproductive Endocrine unit rather than a routine menopause clinic and as such it tends to attract a younger age group. I would suggest though, that if one is studying the pathogenesis of a premature menopause it would be unhelpful to focus on the 35–40 year old age group, most of whom will form the young end of the physiological menopausal process being only 2SD below the mean age menopause at 50.8 years.

One method of verifying our observation that women with POF have an excess of FRAXA premutations is to perform the reverse experiment – screening for early ovarian failure in women known to transmit Fragile X syndrome. This in fact has been undertaken by an international consortium which found that women with FRAXA premutations but not, intriguingly, those with a full mutation, experienced an early menopause (Allingham-Hawkins, 1998). Clearly there is more to this association than can be explained by the FMRI protein alone.

References


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Erratum

Enhancement of motility and acrosome activity reaction in human spermatozoa: differential activation by type-specific phosphodiesterase inhibitors
by J.D. Fisch, B. Behr and M. Conti


The figure legends for Figures 1 and 2 on page 1250 were unfortunately transposed.