Letters to the Editor

Antiphospholipid antibodies in women undergoing in-vitro fertilization

Dear Sir,

The association between recurrent miscarriage and antiphospholipid antibodies (aPL) is one of the most consistently reported features of the primary antiphospholipid syndrome (PAPS) (Rai et al., 1995a,b). We were therefore surprised to read the paper by Birdsall et al. (1996) which failed to demonstrate an association between the presence of aPL and pregnancy loss in women undergoing IVF. We suggest that this is due to the authors not applying strict laboratory criteria before diagnosing a woman to have PAPS, and by not performing assays for the lupus anticoagulant, which is the aPL most commonly found in women with recurrent miscarriage (Rai et al., 1995b).

Birdsall et al. (1996) only measured the levels of anticardiolipin (aCL) and antiphosphatidyl serine (aPS) antibodies on a single occasion. However, before a diagnosis of PAPS can be made, positive titres of aCL should have been obtained on at least two occasions >8 weeks apart (Harris, 1987). This is particularly important as an association between adverse pregnancy outcome and aCL only holds for those with persistently positive titres (Ishii et al., 1990). In our own prevalence survey of 500 women with recurrent miscarriage only 33% of those with an initial positive immunoglobulin (Ig)G aCL titre and 29% of those with a positive IgM aCL had a repeat positive titre on subsequent testing 8 weeks later (Rai et al., 1995b). Transiently positive aCL titres, which are a common occurrence and which may be due to viral or other infections (Vaarala et al., 1986), are not thought to be significant as they do not bind to β2 glycoprotein I (β2 GPI), a plasma protein which serves as a natural inhibitor of coagulation and which is found on the trophoblast surface of placentae (Hunt et al., 1992; La Rosa et al., 1994). Only aCL that bind β2 GPI are associated with pregnancy loss (Aoki et al., 1994). Additionally, the majority of aCL titres in this study were low, the clinical significance of which is questionable.

The prevalence of aPL, including lupus anticoagulant, in women with infertility and the prognostic significance of these antibodies in women undertaking both natural and assisted conception needs to be established in a large study using strict laboratory protocols. This study does not answer either of these points.

References


Raj Rai and Lesley Regan

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Dear Sir,

Like Dr Rai and Professor Regan, we were surprised to find no association between miscarriage and the detection of antiphospholipid antibodies (aPL) in women having in-vitro fertilization (IVF) treatment. Rai and Regan suggest that our failure to demonstrate this association was because we did not apply strict criteria to the diagnosis of primary antiphospholipid antibody syndrome (PAPS) and did not screen for lupus anticoagulant.

We did not claim that the women in our study had PAPS and therefore were not using the suggested criteria for the diagnosis of this syndrome. We agree the addition of lupus anticoagulant may have been valuable. In contrast to Rai et al. (1995), however, we have found lupus anticoagulant to be detected less frequently than anticardiolipin in women with recurrent miscarriage (Birdsall et al., 1992). Thus testing for lupus anticoagulant may not have significantly increased the prevalence of aPL in the study population.

Rai and Regan also suggested that the cut-off point used in our study was too low, further contributing to the lack of any association between pregnancy loss and aPL. The only woman with aPL who had a miscarriage had low titres of anticardiolipin (5 MPL) and thus increasing the cut-off point would not have altered our findings.

We did not repeat aPL testing because the addition of β2 glycoprotein I (β2 GPI) to the antiphospholipid enzyme-linked immunosorbent assay has been shown to inhibit the binding of cofactor independent antibodies (Matsuura et al., 1990). Our assays included bovine β2 GPI and thus it is unlikely that the aPL which were detected were cofactor-independent.

The IVF population studied had a very low incidence of miscarriage overall (5.4%), independent of whether the women had aPL or not. It has been our experience that women attending a recurrent miscarriage clinic tend to have favourable
outcomes, regardless of whether they received pharmacological intervention (Liddell et al., 1991). This suggests that factors associated with attending a clinic may be beneficial and attending a fertility programme may have a similarly positive effect.

The major findings from our paper are that antiphospholipid antibodies are common in women having IVF and that their presence at the start of a treatment cycle is associated with a growth retarded baby should a pregnancy result. We hope that our study will generate more research into the causes, effects and treatments for women with antiphospholipid antibodies.

References

Larry Chamley and Mary Birdsall
Fertility Associates Ltd., 131 Remuera Road, Remuera, Auckland, New Zealand

Safety during sperm banking
Dear Sir,
Viral contamination during the preservation of human material has been highlighted by the transmission of hepatitis B in a cryopreservation facility (Tedder et al., 1995). The demonstration of hepatitis C virus (HCV) RNA in semen from an HCV-infected sperm donor (McKee et al., 1996) indicated the potential for HCV transmission and the contamination of cryopreservation. Recent presentation of a 19 year old patient with malignancy who was found to be HCV infected prior to semen storage led to a review of procedures.

There are a number of approaches to reducing the infection hazards of cryopreservation including screening for infection prior to donation and dedicated facilities for the storage of material from infected donors. However, it remains very important to minimize cross-contamination between materials during handling and cryopreservation. Current practices in the UK include the use of either straws or vials for semen containment and storage in the liquid phase of liquid nitrogen. Straws are inexpensive and economical to maintain; however they tend to be fragile and often lose the polymeric sealing plug during freezing which brings semen into direct contact with the liquid nitrogen and other extruded semen, which gives the possibility of microbial and genetic contamination. Two approaches should be addressed to prevent this risk: the first is to consider gaseous phase storage, the second is to change the storage of samples to allow secondary containment. Concerns about loss of motility during storage have discouraged the adoption of gaseous phase storage. The use of vertical heat sinks in cryotanks could virtually abolish thermal gradients. However, any procedural changes will have to be evaluated for evidence of any sperm impairment and considered carefully in relation to the long-term maintenance of sperm banks and related medico-legal issues (Steele et al., 1995).

Many advantages accrue through the use of cryovials for preservation. They are sturdy, they can be labelled easily and are amenable to secondary containment which straws are not. The sealant ring in cryovials can distort at low temperature and allow ingress of liquid nitrogen. This can be prevented by the use of cryoflex. As a simple alternative to the somewhat cumbersome secondary sealing in cryoflex of vials, ‘Nescofilm’ which is commonly used to seal toxic chemical bottles was applied. A total of 50 inner and 50 outer thread vials, containing a variety of inert fluids, were sealed with ‘Nescofilm’ and placed in batches, with unsealed controls, at different locations in a 330 litre liquid nitrogen bank. A week later, no liquid nitrogen was found in the sealed vials whilst over half the inner and outer thread control vials had nitrogen inside them. ‘Nescofilm’ sealing method was used for the double containment of sperm samples for the cancer patient who had acquired hepatitis C iatrogenically.

Nescofilm (Merck Ltd., Merck House, Poole, Dorset, UK) is normally used as a barrier for glass and metal containers to minimize contamination from the outside. For the purpose of preventing liquid nitrogen ingress during semen cryopreservation, the polymeric Nescofilm is wrapped outside the vial and makes no direct contact with semen. Toxicity to semen is envisaged to be minimal. No toxicity data exist for the use of cryoflex or the polymeric ring in vials or straws and their polymeric plug used during semen cryopreservation. Similarly, there are no data on the durability of Nescofilm in liquid nitrogen, but in our preliminary experience Nescofilm integrity appears to be unaffected at low temperature in liquid nitrogen.

References

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Ovulation induction and IUI in older women

Dear Sir,

We wish to compliment Corson et al. (1996) for their careful observations and thoughtful comments regarding ovulation induction (OI) with sequential clomiphene citrate (CC) and human menopausal gonadotrophin (HMG) or HMG alone coupled with intrauterine insemination (IUI) in 402 completed cycles in women age ≥40 years.

We would like to add our experience to theirs. We reviewed 466 completed IUI cycles, which resulted in 29 clinical pregnancies and 13 births. Our analysis is consistent with their findings of low term pregnancy rates in this group of patients (Table I). Our treatment groups and results differ from theirs in several respects. Only CC was used in 40% of our cycles and no medication was used in 12% of cycles. Clinical pregnancies and term pregnancies were as high in cycles of CC only as in cycles of CC–HMG or HMG only. Unlike Corson et al. (1996), we did observe term pregnancies in patients aged 43–45 years, but at a rate only half as high as for ages 40–42. The abortion rate was much higher in our study than in theirs for ages 40–41 (57 versus 30%), but was the same as theirs for ages 42–43 (57%). This difference might be explained by our use of oral progesterone, rather than human chorionic gonadotrophin (HCG), to support the luteal phase.

We additionally reviewed all pregnancies recorded in our clinic, excluding IVF and egg or embryo donation, to determine if the abortion rates observed for OI–IUI were different from those of non-OI or non-IUI pregnancies in patients age ≥40 years. The spontaneous abortion rate for all 102 non-IUI pregnancies in patients aged 40–43 was 39%. It was lower in spontaneous pregnancies than in OI pregnancies (18 versus 50%) (P = 0.003). The abortion rate for eight non-IUI pregnancies at age 44–46 years was 75%, irrespective of treatment. The oldest patient with term pregnancy was aged 46 years, and the pregnancy occurred spontaneously.

Our results suggest that very low term pregnancy rates occur for ages ≥40, and that CC is as effective as HMG or CC + HMG combinations for OI at these ages.

Table I. Clinical pregnancy and delivery rate with intrauterine insemination as a function of female age and ovulation induction regimen

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<tr>
<th>Age years</th>
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<th>CC + HMG/FSH</th>
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<td>Delivered %</td>
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<td>Pregnant %</td>
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<td>56</td>
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</tbody>
</table>

Reference


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Table I. Clinical pregnancy and delivery rate with intrauterine insemination as a function of female age and ovulation induction regimen

CC = clomiphene citrate; HMG = human menopausal gonadotrophin; FSH = follicle stimulating hormone.