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COMMENTARY

Aspirin and heparin to improve live birth rate in IVF for unexplained implantation failure?

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Abstract The data concerning use of aspirin and/or heparin in IVF failure patients is reviewed. A number of methodological and biological problems are identified. A strategy to achieve reliable conclusions is explained.

KEYWORDS: aspirin, controlled clinical trials, heparin, immunology, IVF failure, pregnancy

The paper by Akhtar et al. (2013) in this issue of *Reproductive Biomedicine Online* describes a retrospective cohort-controlled trail of the effect of aspirin, heparin and their combination on the outcome of IVF in women with one prior IVF failure. The live birth rate was not improved by any of the treatments. In fact, they lowered success in all the treatment groups by 8.7–15.7%, and 12.6% overall, which could suggest that intervention may actually reduce the success rate! What are the actual statistical implications?

None of the differences between treated and untreated groups were statistically significant, and the upper limit of the confidence interval of the risk ratio was >1 in all groups, so a small benefit of treatment cannot be excluded. Indeed, in some groups, 20% (aspirin alone), 50% (heparin alone) and 6% (combination) improvements in the relative risk of a live birth could occur in repeat trials of this size notwithstanding the point estimate of <1. A reduction in live birth rate to achieve significance in a repeat study with an 80% chance of not missing a real difference (i.e. achieving $P < 0.05$) would require approximately 187 treated and 187 control patients. Akhtar et al. had 103 in each group, so a database of sufficient size should be achievable. Adequately powered observational cohort-controlled studies are much easier to execute than prospective randomized control trials. With the present data set, it is not possible to conclude that some patients did not benefit and, given the cost of IVF, it is problematic to argue that relatively inexpensive and well-tolerated treatments such as low-dose aspirin and/or heparin should not be given. There are some important issues that should be kept in mind in interpreting the present data, and these would be pertinent to a further study to clarify the effect of treatment.

For IVF patients, there is a published randomized controlled trial (RCT) showing a significant benefit of aspirin (Ruopp et al., 2008), but other analyses of aspirin use in unselected IVF patients have not confirmed (nor disproved) benefit irrespective of when aspirin was started with respect to embryo transfer (Gelbaya et al., 2007; Groeneveld et al., 2011; Khairy et al., 2007; Siristatidis et al., 2012). In women suffering recurrent unexplained miscarriages who had a positive test for anticardiolipin (ACL) antibody, outcome was improved with aspirin + heparin in women with ACL (Kutteh, 1996; Rai et al., 1997), but in at least one study, women negative for ACL antibody also appeared to have better outcome when treated with combined aspirin + heparin (Coulam and Acacio, 2012). Indeed, Rai et al. (2000) reported a slightly higher live birth rate (risk ratio 1.08) in

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miscarriage patients (at least three <13 weeks or one >13 weeks) with low-dose aspirin alone, and in the subgroup of women with one late miscarriage, the risk ratio of 1.31 achieved statistical significance.

ACL antibodies were not tested in the IVF study by Akhtar et al. (2013) because testing was regarded as 'not cost effective'. Indeed, Hornstein et al. (2000) have argued against ACL antibody testing in IVF patients as success rates were similar in antibody-positive and antibody-negative patients, and Steinvil et al. (2012) have similarly argued against screening for thrombophilia in IVF patients because the success rate in test-positive patients was not inferior to that in test-negative patients. However, patients with condition A that is treatable with aspirin and/or heparin may have the same adverse outcome without treatment as those with conditions X, Y and Z that do not respond to treatment. Since the outcome is improvement with treatment, the endpoint used by these two studies is irrelevant. Cardiolipin, for example, is not expressed on the cell surface as are other phospholipid antigens. Antiphospholipid antibody assay results (including ACL) in different laboratories differ (Kutteh and Franklin, 2004). Hence for ACL/APL antibody testing, one should only use the laboratory where a positive predicts a response to treatment, and for APL, more than just ACL antibody may need to be tested in order not to overlook other significant APL antibodies (Coulam and Acacio, 2012). Additionally, it has been reported that a more extensive thrombophilia screen may be a better diagnostic test in infertility where IVF may be used than testing done in previous studies (Coulam and Jeyendran, 2009).

When there is a small subset of patients with condition A diluted in a larger group, it becomes exceedingly difficult with the number of patients usually available to detect a treatment effect, and if a benefit is discovered, many patients who do not have condition A will receive ineffective and potentially problematic treatment (Clark, 2012). Increasing the sample size where patients who benefit are countered by those who are harmed will never lead to a conclusive result and will fail to detect subgroups where aspirin ± heparin is indicated and where treatment is contraindicated. The solution is to test all patients and then to analyse the relationship between treatment and successful live birth and positive or negative test results. Better diagnosis leads to better treatment results (Clark, 2011a,b)! One cannot expect a patient to benefit from treatment for condition A unless they actually have that condition and the treatment is effective in countering the abnormality (Clark, 2012). An autoantibody or thrombophilic abnormality seems *a priori* more likely to be clinically significant in women who have manifested a clinical problem, e.g. have had recurrent pregnancy failures. In unexplained recurrent miscarriage patients randomized to aspirin and/or heparin or placebo, in a much larger study reported by Kaandorp et al. (2010), the risk of a live birth improved to 1.26 in the subgroup with thrombophilia and was unchanged in patients without thrombophilia, but due to the small size of the thrombophilia group there was insufficient power to achieve $P < 0.05$. However, in IVF failure patients, Urman et al. (2009) noted a higher live birth rate in non-thrombophilia women treated with luteal phase heparin during a subsequent IVF–intracytoplasmic sperm injection cycle, but the 1.3 risk of a live birth did not achieve statistical significance due to a small sample size and no information was provided concerning the outcome if thrombophilia(s) had been present. The current study by Akhtar et al. may also have missed detecting a subgroup where treatment may be beneficial and may also have missed defining a subgroup where treatment decreases success rates.

In any observational study, one must ask about selection bias. Were the treatment and control groups comparable? It is believed that randomization protects against imbalances but that is not necessarily correct, and elements in the randomization process can have unintended consequences (Clark, 2010, 2012). What were treated versus untreated patients told? That could affect outcome. But telling a patient there is uncertainty and hence randomization (which is justifiable in a RCT) may also affect outcome. Cohort-controlled observational studies can provide more useful information than RCT, but need due care for biological rigor and control for all of the variables described above is required in both observational and RCT-type studies. One way to approach the analysis is by logistic regression analysis or the entire group (intention-to-treat analysis) and separate analysis of the success and failure groups to determine what factors may identify the former.

There are some additional methodological issues that arise in the Akhtar et al. (2013) paper. With better diagnosis of immunological problems in IVF failure patients, it is clear that aspirin and heparin are not as effective as when combined with intravenous immunoglobulin (IVIG) or IVIG + anti-tumour necrosis factor α (TNF-α) drugs (Clark, 2011a,b; Winger et al., 2011a,b). In the latter studies, a live birth rate of approximately 50% per embryo transferred has been reported. In Akhtar et al. (2013), live birth rate has not been corrected per embryo transferred. Assuming two embryos were transferred on average, a live birth rate per embryo of 23.8% was obtained in the control group. In the IVIG and/or anti-TNF-α studies, the live birth rate per embryo in the heparin + aspirin group was 13% in those with immune test abnormalities and 48% in those without (Winger et al., 2011b). Winger et al. (2011a,b) reported only patients with 'good' ovulation responses. Those with inferior responses may have 'sicker' oocytes. Indeed, the percentage of surviving IVF embryos after in-vitro incubation may be used to determine a die-off ratio (Winger et al., 2012), and those with high death rates may have problems uncorrectable with aspirin and/or heparin, thus dooming an improvement in success rate. Therefore, to assess the effect of aspirin and/or heparin, one needs to parse the outcome based on ovulation response and die-off ratio since only a select subgroup may benefit.

Oocyte and fertilized egg (zygote) analysis of polar bodies 1 and 2 have shown a significant percentage of chromosomal abnormalities, and one would *a priori* expect the various treatments discussed above to benefit only normal embryos. FISH analysis of five chromosomes has shown an increasing incidence with maternal age (Kuliev et al., 2011), but even young women in the <30-year age group may have abnormalities. Comparative genomic hybridization, which more efficiently tests for abnormalities using all of the chromosomes, has yielded estimated abnormality rates between 5.6% in ovum donor women (corrected for the need to test both polar bodies) and 65% in infertile women (Fragouli, 2009; Sher et al., 2007). In Sher et al. (2007), developmental arrest eliminated many of the

abnormal oocytes/zygotes, so 89% of post-fertilized euploid oocytes developed into blastocysts in contrast to 20% of aneuploid post-fertilization oocytes. Therefore, transfer of blastocysts after 5 days of in-vitro culture might be expected to bias results towards a higher success rate. In Akhtar et al. (2013), transfer was performed at 2, 3 or 5 days after IVF, but we are not told if the outcomes with treatment differ in these groups. Embryo biopsy is not necessarily helpful since a high percentage of embryos are a mosaic of normal and abnormal blastomeres. Polar bodies can (in theory) be removed without deleting blastomeres; deleting a blastomere can reduce the subsequent success rate *in vivo* (Collins, 2007). Additional laboratory testing for NK cell numbers and TNF-α overproduction with respect to interleukin 10 successfully identifies those who benefit from IVIG and/or anti-TNF-α, where success rates can be doubled compared with Akhtar et al. (2013), and interestingly, this subgroup has the highest die-off ratio (Winger et al., 2011a,b, 2012). It is unclear if the benefit of IVIG and/or anti-TNF-α would occur if aspirin + heparin were omitted. Interestingly, anti-TNF-α treatment was most effective when given between 60 and 120 days before oocyte collection, during the period of folliculogenesis (Winger et al., 2011a), so again, treatment before embryo transfer may be important and may generate better-quality embryos that will contribute to success even when there are uncorrectable factors causing a high die-off. Perhaps aspirin + heparin also need to be given much earlier than in Akhtar et al. (2013).

A cohort-controlled observational study can provide useful information as can a RCT, but both types of study need due care for biological rigor and control for all of the variables described above. Cohort-controlled studies are particularly useful in identifying variables associated with treatment outcomes and are needed before proceeding to a RCT (Clark, 2010, 2012). The strategy is to compare those succeeding and failing in an observational study of treatment A before comparing outcome in suitable patients given A versus control treatment. A follow-up of the cohort-controlled study of Akhtar et al. (2013) with attention to the issues set out above could be quite helpful in clarifying possible benefits and harms of adjuvant treatments in IVF patients. Due to the need for a large number of well-defined and thoroughly tested patients, a multicentre registry might provide a feasible approach to answering some important questions. Performing all of the testing would be expensive, but in the long run, given the cost of IVF, ignorance would be even more costly, and would be arguably immoral.

References

Akhtar, M.A., Eljabu, H., Hopkisson, J., Raine-Fenning, N., Quenby, S., Jayaprakasan, K., 2013. Aspirin and heparin as adjuvants during IVF do not improve live birth rates in unexplained implantation failure. Reprod. Biomed. online 26, 586–594.

Clark, D.A., 2010. Anti-TNF-α therapy in immune-mediated subfertility: state of the art. J. Reprod. Immunol. 85, 15–24.

Clark, D.A., 2011a. The power of observation. Am. J. Reprod. Immunol. 66, 71–75 [erratum Am. J. Reprod. Immunol. 66, 162].

Clark, D.A., 2011b. Intravenous immunoglobulin and idiopathic secondary recurrent miscarriage: methodological problems. Hum. Reprod. 26, 2586–2587.

Clark, D.A., 2012. The end of evidence-based medicine. Inflammopharmacology 20, 187–193.

Collins, J.A., 2007. Preimplantation genetic screening in older mothers. N. Engl. J. Med. 357, 61–63.

Coulam, C.B., Jeyendran, R.S., 2009. Thrombophilic gene polymorphisms are risk factors for unexplained infertility. Fertil. Steril. 91, 1516–1517.

Coulam, C.B., Acacio, B., 2012. Does immunotherapy for treatment of reproductive failure enhance live births? Am. J. Reprod. Immunol. 67, 296–303.

Fragouli, E., 2009. Comparative genomic hybridization of oocytes and first polar bodies from young donors. Reprod. Biomed. Online 19, 228–237.

Gelbaya, T.A., Kyrgiou, M., Li, T.C., Stern, C., Nardo, L.H., 2007. Low-dose aspirin for in vitro fertilization: a systematic review and meta-analysis. Hum. Reprod. Update 13, 357–364.

Groeneveld, E., Broeze, K.A., Lambers, M.J., Haapsamo, M., Dirclx, K., Schoot, B.C., Salle, B., Duvan, C.I., Schats, R., Mol, B.W., Hompes, P.G., IPD MARIA study group. 2011. Is aspirin effective in women undergoing in vitro fertilization (IVF)? Results from an individual patient data meta-analysis (IPD MA). Hum. Reprod. Update 17, 501-509.

Hornstein, M.D., David, O.K., Massey, J.B., Paulson, R.J., Collins, J.A., 2000. Antiphospholipid antibodies and in vitro fertilization success: a meta-analysis. Fertil. Steril. 73, 330–333.

Kaandorp, S.P., Goddijn, M., van der Post, J.A.M., Hutten, B.A., Verhoeve, H.R., Hamulyák, K., Mol, B.W., Folkeringa, N., Nahuis, M., Papatsonis, D.N.M., Büller, H.R., van der Veen, F., Middeldorp, S., 2010. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N. Engl. J. Med. 362, 1586–1596.

Khairy, M., Banerjee, K., El-Toukhy, T., Coomarasamy, A., Khalaf, Y., 2007. Aspirin in women undergoing in vitro fertilization treatment: a systematic review and meta-analysis. Fertil. Steril. 88, 822–831.

Kutteh, W.H., 1996. Antiphospholipid antibody-associated recurrent pregnancy loss treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. Am. J. Obstet. Gynecol. 174, 1584–1589.

Kutteh, W.H., Franklin, R.D., 2004. Assessing the variation in antiphospholipid (APA) assays: comparison of results from 10 centers. Am. J. Obstet. Gynecol. 191, 440–448.

Kuliev, A., Zlatopolsky, Z., Kirillova, I., Spivakova, J., Janzen, J.C., 2011. Meiosis errors in over 20,000 oocytes studied in the practice of preimplantation aneuploidy testing. Reprod. Biomed. Online 22, 2–8.

Rai, R., Cohen, H., Dave, M., Regan, L., 1997. Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ 314, 253–257.

Rai, R., Backos, M., Baxter, N., Chilcott, I., Regan, L., 2000. Recurrent miscarriage — an aspirin a day? Hum. Reprod. 15, 2220–2223.

Ruopp, M.D., Collins, T.C., Whitcomb, B.W., Schisterman, E.F., 2008. Evidence of absence or absence of evidence? A reanalysis of the effects of low-dose aspirin in in vitro fertilization. Fertil. Steril. 90, 71–76.

Sher, G., Keskintepe, L., Kestintepe, M., Ginsburgh, M., Maassarani, G., Yakut, T., Baltachi, V., Kotze, D., Unsal, E., 2007. Oocyte karyotyping by comparative genomic hybridization provides a highly reliable method for selecting 'competent' embryos, markedly improving in vitro fertilization outcome: a multiphase study. Fertil. Steril. 87, 1033–1040.

Siristatidis, C.S., Dodd, S.R., Drakeley, A.J., 2012. Aspirin is not recommended for women undergoing IVF. Hum. Reprod. Update 18, 233.

Steinvil, A., Raz, R., Berliner, S., Steinberg, D.M., Zeltser, D., Levran, D., Shimron, O., Sella, T., Chodick, G., Shalev, V., Salomon, O., 2012. Association of common thrombophilias and antiphospholipid antibodies with success rates of in vitro fertilization. Thromb. Haemost. 108, 1192–1197.

Urman, B., Ata, B., Yakin, K., Alatas, C., Aksoy, S., Mercan, R., Balaban, B., 2009. Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labelled pilot trial. Hum. Reprod. 24, 1640–1647.

Winger, E.E., Reed, J.L., Ahoush, S., Ahuja, S., El-Toukhy, T., Taranissi, M., 2011a. Degree of TNF-α/IL-10 cytokine elevation correlates with IVF success rate in women undergoing treatment with Adalimumab (Humira) and IVIG. Am. J. Reprod. Immunol. 65, 610–618.

Winger, E.E., Reed, J.L., Ashoush, S., El-Toukhy, T., Ahuja, S., Taranissi, M., 2011b. Elevated preconception $CD56^{+}16^{+}$ and/or Th1/Th2 levels predict benefit from IVIG therapy in subfertile women undergoing IVF. Am. J. Reprod. Immunol. 66, 394–403.

Winger, E.E., Reed, J.L., Ashoush, S., El-Toukhy, T., Taranissi, M., 2012. Die-off ratio correlates with increased TNF-α:IL-10 ratio and decreased IVF success rates correctable with Humira. Am. J. Reprod. Immunol. 68, 428–437.

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