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The benefits of low-dose aspirin therapy in women with impaired uterine perfusion during assisted conception

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The objective of this long-running study was to determine whether the addition of low-dose aspirin to a standard hormone replacement therapy (HRT) protocol improved uterine perfusion during assisted conception. A total of 99 women scheduled for frozen embryo replacement were studied. Endometrial preparation was with a standard buserelin/HRT protocol. Uterine perfusion was assessed by Doppler ultrasound and classified as impaired or normal. In their first attempts, those with impaired perfusion (group I, $n = 37$) received low doses of aspirin [150 mg ($n = 26$) or 300 mg daily ($n = 11$)], starting from day 13 of HRT. Women with normal perfusion (group II) did not receive aspirin. In subsequent attempts, those from group I were arbitrarily allocated to start aspirin on day 1 or day 13 of HRT, and 10 women from group II were arbitrarily selected to receive aspirin from day 1 of HRT. In group I, the cancellation (46 versus 36%) and pregnancy rates (15 versus 25%) in those who received 150 or 300 mg aspirin daily were similar. In those with cancelled first attempts, good perfusion was achieved in 82 versus 20% ($P < 0.02$) of subsequent attempts using aspirin from day 1 versus day 13 of HRT. Higher pregnancy rates (47 versus 17%) were achieved in those taking aspirin from day 1 of HRT. In group II, pregnancy rates were not statistically different in those who did or did not receive aspirin during their subsequent attempts (10 versus 35%). The addition of low-dose aspirin to a standard HRT protocol in women with impaired uterine perfusion is associated with improved blood flow and satisfactory pregnancy rates.

Key words: aspirin/buserelin/HRT protocol/frozen embryos/pregnancy/uterine perfusion

Introduction

Measurement of uterine blood flow by Doppler ultrasound is a relatively new technique applied to the management of assisted conception cycles (Goswamy *et al.*, 1988). Clinical studies have shown that embryos fail to implant in women with impaired uterine perfusion during in-vitro fertilization (IVF) (Battaglia *et al.*, 1990; Steer *et al.*, 1992). Impaired uterine blood flow has been suggested as a possible cause of infertility (Goswamy and Steptoe, 1988).

Despite awareness of the importance of good uterine blood flow for successful assisted conception, there are no clear guidelines for the management of those with impaired uterine perfusion. One suggested option is to abandon the treatment cycle and to administer cyclic oestrogen/progesterone therapy in the 3 months preceding further attempts (Goswamy *et al.*, 1988). However, prolonged hormone therapy can be inconvenient and is not of benefit for some women. Changes in uterine perfusion occur quickly in response to oestrogen therapy, but no further improvement is achieved with plasma oestradiol rising above 65 pg/ml (De Ziegler *et al.*, 1992). This observation suggests that hormone therapy alone would not be of universal benefit in women with impaired uterine perfusion.

A major factor controlling tissue perfusion is the equilibrium between thromboxane and prostacyclin (Bussolino *et al.*, 1980). Thromboxane predominantly has vasoconstrictory and platelet aggregatory properties, whilst prostacyclin is vasodilatory. In low daily amounts, aspirin shifts the balance towards prostacyclin production (Thorp *et al.*, 1988). Low-dose aspirin is currently safely employed to improve utero-placental perfusion in women at risk of developing pregnancy-induced hypertension (McParland *et al.*, 1990; Viinikka *et al.*, 1993). However, little information is available regarding the possible benefits of low-dose aspirin therapy in infertile women with impaired uterine perfusion.

The aims of the present study were first to determine whether the addition of low-dose aspirin to a standard hormone replacement therapy protocol improved uterine perfusion, and second to analyse the clinical pregnancy rates in frozen embryo replacement cycles involving the use of aspirin.

Materials and methods

A total of 99 women receiving hormone replacement therapy (HRT) for frozen embryo replacement were studied in 176 cycles from 1991 to 1993. The frozen embryos were derived from IVF cycles in which follicle stimulating hormone (FSH, Metrodin; Serono Laboratories, Welwyn Garden City, UK) and/or human menopausal gonadotrophin (Pergonal; Serono Laboratories) combined with luteinizing hormone-releasing hormone (LHRH) agonist were administered for ovarian stimulation (Macnamee and Brinsden, 1992).

Embryo cryopreservation

Embryos were cryopreserved at the bi-pronucleate or early cleavage stages using propanediol as the cryoprotectant (Lassalle

et al., 1985; Wada *et al.*, 1994). Bi-pronucleate embryos were thawed the day before transfer and cultured overnight. Only those that cleaved were transferred to the uterus. Embryos cryopreserved at an early cleavage stage were thawed and transferred on the same day (Sathanandan *et al.*, 1991). A maximum of three embryos were transferred per attempt.

Buserelin/HRT protocol

Buserelin, 500 μg s.c. daily, was started on day 22 of menses to achieve pituitary desensitization. Endometrial development was promoted with a standard HRT protocol (Sathanandan *et al.*, 1991): oestradiol valerate (Progynova; Schering, UK), 2 mg orally daily from days 1–5, 2 mg twice daily from days 6–9 and 2 mg three times daily from day 10 onwards. Injections of progesterone (Gestone; Payne and Burn Ltd, UK), 50 mg i.m daily for 2 days and 100 mg daily thereafter, were started only when adequate uterine perfusion had been achieved, between days 15 and 21. The endometrial thickness was normal (≥8 mm in diameter) before starting gestone injections in all the women studied. Those becoming pregnant continued the HRT until the 12th week of gestation.

Assessment of uterine perfusion

The initial assessment of uterine perfusion in each treatment cycle was on day 13 of HRT, using a Phillips/Vingmed CFM 700 Doppler ultrasound system with a vaginal probe. The blood flow velocity waveform of the anterior branch of the right and left uterine arteries was measured and classified according to the following criteria (Goswamy *et al.*, 1988): type O, when there was no diastolic forward flow; type A, the diastolic flow was present but not continuous with the preceding systolic waveform; type B, the diastolic flow was continuous with the preceding systolic waveform but not extending to the next cardiac cycle; and type C, when the diastolic and systolic waveforms were continuous from one cycle to the next.

The pulsatility index (PI) was defined as the peak-to-peak excursion of the waveforms divided by the mean height (Thaler *et al.*, 1990). The results were classified as PI < 3 or PI > 3 (Steer *et al.*, 1992). Uterine perfusion was considered to be impaired when there was bilateral blood flow velocity waveform

Table I. Characteristics of the women in groups I and II

Parameter	Group I	Group II
No.of women	37	62
Age (years)[a]	31.5 (26–40)	32.5 (20–42)
Cause of infertility		
Tubal	23 (62%)	35 (57%)
Unexplained	8 (22%)	12 (19%)
Male factor	5 (13%)	5 (8%)
Endometriosis	1 (3%)	10 (16%)

[a]Values are median (range in parentheses).

Table II. Results of the first attempts in groups I and II[a]

Aspirin regimen (daily dose)	Group I			Group II
	Day 13 (150 mg)	Day 13 (300 mg)	Total	No aspirin
No. of women	26	11	37	62
No. cancelled due to:				
Poor uterine perfusion	12 (46%)	4 (36%)	16 (43%)	—
Failed thaw	—	—	—	2 (3%)
No. (%) reaching embryo transfer	14 (54%)	7 (64%)	21 (57%)	60 (97%)
Mean (±SD) embryos transferred	2.6 (0.6)	2.7 (0.5)	2.6 (0.7)	2.8 (0.6)
No. pregnant (% per transfer)	2 (15%)	2 (25%)	4 (19%)	17 (28%)

[a]See Table I.

Table III. Results of the subsequent attempts in group I[a] based on the outcome of the first attempts

Subsequent aspirin regimen (daily dose)	First attempt cancelled		First attempt completed		Total	
	Day 1 (150 mg)	Day 13 (150 mg)	Day 1 (150 mg)	Day 13 (150 mg)	Day 1 (150 mg)	Day 13 (150 mg)
No. of women	11	5	12	5	23	10
No. cancelled due to:						
Poor perfusion	1 (9%)	4 (80%)	2 (17%)	—	4 (17%)	4 (40%)
Failed thaw	1 (9%)	—	—	—	—	—
No. reaching embryo transfer*	9 (82%)[b]	1 (20%)[c]	10 (83%)	5 (100%)	19 (83%)	6 (60%)
Mean (±SD) embryos transferred	2.7 (0.6)	3	2.8 (0.5)	2.7 (0.6)	2.7 (0.6)	2.8 (0.5)
No. pregnant (% per transfer)	4 (44%)	—	5 (50%)	1 (20%)	9 (47%)	1 (17%)

[a]See Table I.
*b versus c: $P < 0.02$.

type O or A and PI > 3, or adequate when there was unilateral or bilateral blood flow velocity waveform type B or C and PI < 3. In those with impaired perfusion, the Doppler examinations were repeated at 2 day intervals until uterine blood flow improved, otherwise the cycle was abandoned on day 21 of HRT if poor perfusion persisted.

Aspirin therapy regimens

Initial attempts

Those with impaired perfusion (group I, $n = 37$) were arbitrarily allocated to receive 150 mg ($n = 26$) or 300 mg ($n = 11$) aspirin daily, starting from day 13 of HRT. Women with normal perfusion (group II, $n = 62$) did not receive aspirin.

Subsequent attempts

In their subsequent attempts, women from group I (i.e. those who had poor perfusion on day 13 of previous attempts) were arbitrarily allocated to start aspirin (150 mg/day) from day 1 ($n = 23$) or day 13 ($n = 10$) of HRT. Ten women from group II were selected arbitrarily to start aspirin on day 1 of HRT.

Women receiving aspirin in their initial or subsequent attempts continued treatment until the demonstration of fetal heart activity if they achieved a pregnancy.

Statistical analysis

Proportions were compared using Fisher's exact probability tests. Other parameters were compared using Mann–Whitney U tests. Differences were considered significant if $P < 0.05$.

Results

There were no significant differences between women in groups I and II in age and cause of infertility (principally tubal disease; Table I).

A total of 21/37 (57%) group I women achieved improved uterine perfusion during aspirin therapy, reaching embryo transfer in their first attempts (Table II). The median duration of aspirin therapy leading to improved uterine perfusion was 3.9 days (range 2–8 days). There were no significant differences between those who received 150 or 300 mg of aspirin daily in terms of the cancellation rates per attempt (46 versus 36%), the number of embryos transferred per cycle (mean 2.6 versus 2.7) and the clinical pregnancy rates per embryo transfer (15 versus 25%).

Group II showed a pregnancy rate of 28% despite cancellation of two first attempts due to failure of the embryos to survive the freeze/thaw process (Table II).

In subsequent attempts undertaken by group I women (Table III), a significantly higher proportion of those whose first attempts were cancelled (82 versus 20%; $P < 0.02$) achieved good uterine perfusion and reached embryo transfer using low-dose aspirin from day 1 compared with day 13 of HRT. For those group I women whose first attempts were not cancelled, no difference was found in cancellation rates comparing with those who received aspirin from day 1 versus day 13 of subsequent HRT (Table III). The clinical pregnancy rate per embryo transfer appeared higher for all group I women who had received aspirin from day 1 compared with day 13 of HRT (47 versus 17%; Table III), although this trend was not significant.

In subsequent attempts undertaken by group II women, no significant difference was found in clinical pregnancy rates when comparing those who did with those who did not receive aspirin from day 1 of HRT (10 versus 35%; Table IV).

The miscarriage rate of all pregnancies achieved by group I women with poor uterine perfusion (first and subsequent cycles combined) who had received aspirin from day 1 of HRT was similar to that of women with normal perfusion in group II (11 versus 17%; Table V). The miscarriage rate was significantly higher in those group I women who received aspirin from day 13 of HRT compared with women with normal perfusion (60 versus 11%; $P < 0.05$).

Table IV. Results of the subsequent attempts in group II[a]

	Aspirin regimen	
	Day 1	No aspirin
No. of women	10	34
No. cancelled due to failed thaw	—	—
No. reaching embryo transfer	10	34
Mean (±SD) embryos transferred	2.5 (0.6)	2.8 (0.5)
No. pregnant (% per transfer)	1 (10%)	12 (35%)

[a]See Table I.

Discussion

The observation that human embryos fail to implant in women with impaired uterine perfusion (Battaglia *et al.*, 1990; Steer *et al.*, 1992) prompted us to avoid embryo transfer in patients with persistently poor uterine blood flow. In this study, the high proportion of women (37%) found to have impaired perfusion indicates that this problem may be fairly common in HRT cycles

Table V. Miscarriage rates in groups I and II[a] (first and second cycles combined)

	Group I aspirin regimen		Group II aspirin regimen	
	Day 1	Day 13	Day 1	No aspirin
No. pregnant	9	5	1	29
No. miscarried*	1 (11%)	3 (60%)[b]	—	5 (17%)[c]
No. (%) ongoing/delivered	8 (89%)	2 (40%)	1 (100%)	24 (83%)

[a]See Table I.
*b versus c: $P < 0.05$.

for frozen embryo replacement and further suggests that hormone therapy alone does not invariably lead to good uterine perfusion.

The absence of clear guidelines for management of women with impaired uterine perfusion during assisted conception is because the aetiology of the disorder is not fully understood in individual cases. For instance, this study did not find differences in age and causes of infertility when comparing women with poor or normal perfusion, which is in agreement with the findings of Goswamy *et al.* (1988). A more detailed investigation of all factors regulating local tissue perfusion is beyond the scope of this study.

To our knowledge, the present study is the first to use low-dose aspirin in combination with HRT for frozen embryo replacements. The rationale for administering aspirin is that any shift in thromboxane/prostacyclin equilibrium towards prostacyclin production would lead to peripheral vasodilatation and an enhanced uterine blood flow.

This study has shown that both low-dose aspirin regimens (the short and long regimens in which the aspirin therapy started on day 13 and day 1 of HRT respectively) led to improved uterine blood flow in several cases. However, the trend observed was that a larger proportion of women with poor uterine perfusion who used the long aspirin regimen benefited from treatment (i.e. achieved improved perfusion), compared with those treated with the short aspirin regimen. The advantage of long- versus short-term aspirin regimens is possibly due to more time being available during the former regimen to correct any imbalance in the thromboxane/prostacyclin equilibrium.

The dose of aspirin selected for the short regimen (150 versus 300 mg/day) did not significantly affect treatment outcome. Other workers employed even smaller aspirin doses (50 mg/day) and observed improved utero-placental perfusion, thus preventing pregnancy-induced hypertension (Viinikka *et al.*, 1993). This study has shown that low daily amounts of aspirin are sufficient to effect observable changes in uterine blood flow in the non-pregnant uterus.

The role of aspirin therapy in human embryo implantation is of interest, since Testart and Gauthier (1991) have linked the administration of aspirin with an increased embryo implantation rate in one animal study. The high pregnancy rate (47% per embryo transfer) and low miscarriage rate (11%) observed in women with poor perfusion following treatment with the long aspirin regimen suggests that low-dose aspirin may benefit implantation in humans. The benefits of low-dose aspirin on embryo implantation may be secondary to improved uterine perfusion, since this study did not observe any advantage in using aspirin in women with normal uterine perfusion. The value of aspirin for human embryo implantation requires continued evaluation since the number of pregnancies in this study was small.

Finally, some of the women with poor uterine perfusion in this study failed to achieve improved perfusion, even when treated with the long aspirin regimen. The reasons for the treatment failure in these cases are uncertain, but may be related to individual variations in response to low-dose aspirin or to differences in the underlying cause of impaired perfusion. Management of this subgroup of patients merits further evaluation.

In conclusion, the addition of low-dose aspirin to a standard HRT protocol in women with impaired uterine perfusion has been shown to improve blood flow and is associated with satisfactory pregnancy rates.

References

Battaglia,C., Larocca,E., Lanzani,A., Valentini,M. and Genazzani,A.R. (1990) Doppler ultrasound studies of the uterine arteries in spontaneous and IVF stimulated ovarian cycles. *Gynecol. Endocrinol.*, **4,** 245–250.

Bussolino,F., Benedetto,C., Massobrio,M. and Camussi,G. (1980) Maternal vascular prostacyclin activity in pre-eclampsia. *Lancet*, **ii,** 702.

De Ziegler,D., Bessis,R., Fanchin,R. and Frydman,R. (1992) Transvaginal pulsed and color Doppler in gynecology: 1—uterine blood flow. *Contracept. Fertil. Sexual.*, **20,** 1155–1165.

Goswamy,R.K. and Steptoe,P.C. (1988) Doppler ultrasound studies of the uterine artery in spontaneous ovarian cycles. *Hum. Reprod.*, **3,** 721–726.

Goswamy,R.K., Williams,G. and Steptoe,P.C. (1988) Decreased uterine perfusion—a cause of infertility. *Hum. Reprod.*, **3,** 955–959.

Lassalle,B., Testart,J. and Renard,J.P. (1985) Human embryo features which influence the success of cryopreservation with the use of 1,2-propanediol. *Fertil. Steril.*, **44,** 645–651.

Macnamee,M.C. and Brinsden,P.R. (1992) Superovulation strategies in assisted conception. In Brinsden,P.R. and Rainsbury,P.A. (eds), *A Textbook of In Vitro Fertilisation and Assisted Reproduction.* Parthenon, Carnforth, UK, pp. 111–126.

McParland,P., Pearce,J.M. and Chamberlain,G.V.P. (1990) Doppler ultrasound and aspirin in recognition and prevention of pregnancy-induced hypertension. *Lancet*, **335,** 1552–1554.

Sathanandan,M., Macnamee,M.C., Rainsbury,P., Wick,K. Brinsden,P. and Edwards,R.G. (1991) Replacement of frozen–thawed embryos in artificial and natural cycles: a prospective semi-randomized study. *Hum. Reprod.*, **6,** 685–687.

Steer,C.V., Mills,C., Campbell,S., Mason,B.A., Tan,S.L., Collins,W.P. and Crayford,T. (1992) The use of transvaginal color flow imaging after in vitro fertilization to identify optimum uterine conditions before embryo transfer. *Fertil. Steril.*, **57,** 372–376.

Testart,J. and Gauthier,A. (1991) The action of anti-inflammatory drugs on the fertility of female rats with intra-uterine contraceptive devices. *J. Reprod. Fertil.*, **63,** 257–261.

Thaler,I., Manor,D., Brandes,J., Rottem,S. and Itskovit,J. (1990) Basic principles and clinical applications of the transvaginal doppler duplex system in reproductive medicine. *J. In Vitro Fertil. Embryo Transfer*, **7,** 74–85.

Thorp,J., Walsh,S. and Brath,P. (1988) Low-dose aspirin inhibits thromboxane, but not prostacyclin production by human placental arteries. *Am. J. Obstet. Gynaecol.*, **159,** 1381–1384.

Viinikka,L., Hartikainen-Sorri,A.-L., Lumme,R., Hillesmaa,V. and Ylikorkalaa,O. (1993) Low dose aspirin in hypertensive women: effect on pregnancy outcome and prostacyclin-thromboxane balance in mother and newborn. *Br. J. Obstet. Gynaecol.*, **100,** 809–815.

Wada,I., Macnamee,M.C., Wick,K., Bradfield,J.M. and Brinsden,P.R. (1994) Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. *Hum. Reprod.*, **9,** 543–546.

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