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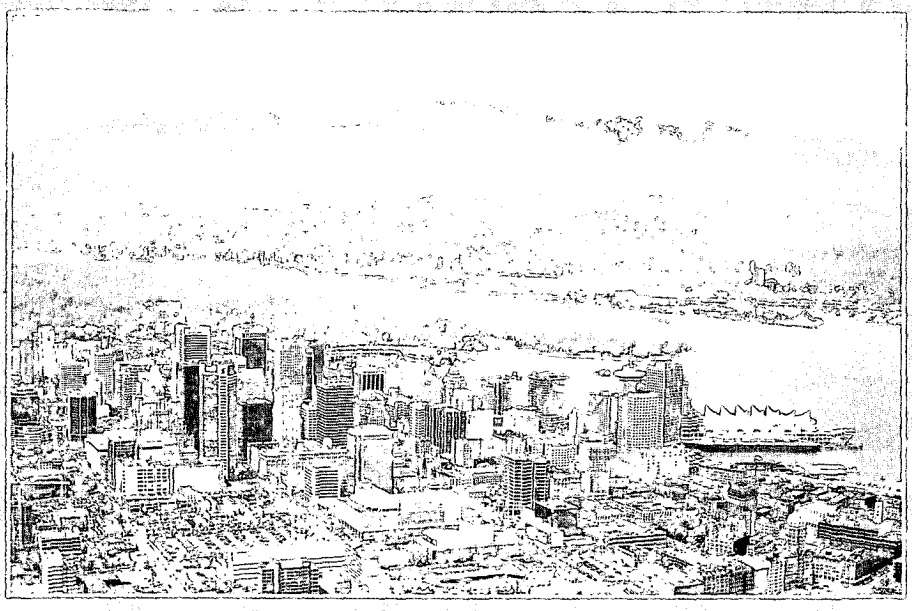

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Lymphocyte immunotherapy before in vitro fertilization may improve pregnancy rates (PRs) in patients with previous failed cycles

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SUMMARY

Women who failed to conceive despite at least two embryo transfer (ET) cycles were immunized with paternal leukocytes. The pregnancy outcomes of the next ET were compared to those of age matched controls who had ETs on the same day, had at least one previous ET failure but who were not immunized. The biochemical, clinical, and delivered PRs in 45 immunized patients were 28.9%, 24.2% and 22.2%, respectively compared to 21.9%, 17.0% and 13.4% for 82 controls. The delivery rate in 36 subsequent cycles showed the same trend (33.3% vs 16.7%). There were insufficient numbers to show statistical significance. These data show a trend toward improved PRs with ET following LI. Hopefully, these data may stimulate interest in a larger worldwide collaborative study of LI for treatment of failed implantation following in vitro fertilization cycles-ET.

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INTRODUCTION

Two independent statistical analyses from the worldwide collaborative observational study and meta-analysis on allogeneic leukocyte immunotherapy (LI) concluded that LI was effective for decreasing the risk of another spontaneous abortion (SAB) in women with a history of recurrent SAB (Coulam et al, 1994); however, the treatment benefit was small, i.e., it helped only 8-10% of affected couples (Coulam et al, 1994).

Explanations for why the treatment was not more effective include the possibility that only a small percentage of the patients with recurrent SAB have a problem that would respond to LI. Another possibility is that LI by itself is not maximally effective. One controlled study, for example, found that LI was more effective when the patients and controls were treated in the luteal phase with progesterone (P) (Check et al, 1995). Patients on LI and P therapy not only have a lower SAB rate (26.0%) than the controls given P only (57.1%), but the conception rate after four cycles was also higher (65.7% vs 45.1%). This led to a delivery rate that was more than twice as high in the group receiving the additional LI (48.5% vs 19.3%).

The study presented herein evaluated whether LI could improve pregnancy rates (PRs) following in vitro fertilization-embryo transfer (IVF-ET) in women failing to conceive despite previous ETs.

MATERIALS AND METHODS

Leukocyte immunization was offered to women prior to having another ET if they had failed to establish a viable pregnancy after at least two previous attempts. The number of previous failures ranged from 2 to 12. This wide range was due to previous treatment at other IVF centers and the use of other clinical options. They were informed of the theoretical benefits of LI, the slight risks of therapy, and told that there were no studies as yet proving that this therapy improves PRs following IVF-ET. Forty-five women elected to participate in the LI program.

Because PRs following IVF-ET may vary according to the physician doing the transfer, possibly the amount of endotoxin in the embryo media or other potential variables, the control group for each patient treated with LI were all the women having ETs the same day as long as they were within 2 years of age and had a history of at least 1 failed ET. Cases using intracytoplasmic sperm injection were not included. All transfers were of 3 day old embryos. Since the PRs at Cooper Center for IVF are equal following fresh or frozen ET, all transfers were combined for analysis. 82 controls were used. The PRs were compared by group using chi-square analysis. A p value of .05 was used.

The methodology of leukocyte preparation and immunization was the same as previously described (Check et al, 1995). The source of leukocytes was the male partner of the woman receiving immunization; a second immunization occurred during the first trimester.

RESULTS AND CONCLUSIONS

The mean $\pm$ SD number of previous transfers without a viable pregnancy was 5.2 ± 3.0 in the immunized group compared to 2.1 ± 2.0 in the

controls ($p<.05$, t-test). There were 8/45 (17.8%) immunized women who previously had a failed clinical pregnancy with ET at our center compared to 8/82 (9.8%) controls. The number of fresh transfers in the immunized group was 21 (46.7%) compared to 31 (37.8%) of the controls (p=NS, chi-square).

A comparison of clinical and delivered PRs are seen in tab. 1. There were no statistical differences though the delivered PR for the immunized group was about 65% higher. Power analysis found that a sample size of 300 patients per group would be required to detect a 10% difference in PRs between the two groups with 80% power at the .05 level of significance.

Table 1 Comparison of pregnancy outcomes following embryo transfers in first cycle following leukocyte immunization versus controls

	No. of patients	Chem. preg.	Clin. preg.	Delivered
LI	45	13 (28.9%)	11 (24.2%)	10 (22.2%)
Controls	82	18 (21.9%)	14 (17.0%)	11 (13.4%)

The immunized group had 12 more transfer cycles and 4 delivered (rate of 33.3%) and the control group had 24 more transfers with 4 deliveries (rate of 16.7%). Combining all IVF cycles there were 14 deliveries in 57 transfers (24.6%) for those immunized vs 15 of 106 cycles (14.1%) in the controls.

A previous uncontrolled study of patients given LI prior to IVF reached similar conclusions to our study (Carp et al, 1994), i.e., that LI may improve the chances of a successful pregnancy following IVF-ET in patients with a history of failed ETs. Though the results in the study presented herein did not reach statistical significance, the 65% improved delivery rate in the immunized group was more impressive if one considers that the group receiving LI had a mean of 5.2 transfers which failed to result in a live pregnancy compared to only 2.1 previous transfers in the controls.

The mechanism of how LI can improve PRs following ET is not apparent, but we have formed at least one hypothesis. The possibility exists that LI may work, at least in part, by augmenting the production of a 34 kD immunomodulatory protein known as progesterone induced blocking factor (PIBF) (Szekeres-Bartho et al, 1985; Szekeres-Bartho et al,1989a; Szekeres-Bartho et al, 1989b). Progesterone induced blocking factor may be one of the important factors causing a shift in the decidua favoring the production of TH2 cytokines over TH1 cytokines by TH cells and may also allow for inhibition of CD56+, CD16+ natural killer (NK) cells (Szekeres-Bartho et al, 1996); furthermore, PIBF neutralizes cytolytic action of NK cells (Szekeres-Bartho et al, 1985).

There is evidence that PIBF induction occurs soon after implantation (Check et al, 1996). There are some data suggesting that LI augments a PIBF response to P through an allogeneic stimulus (Check et al, 1997). Theoretically, the embryo/fetus in some women would not be sufficiently allogenic to stimulate the response but the leukocytes provide a more potent stimulus.

Before LI can be recommended universally as a treatment for patients with a history of failed ETs, it is necessary to complete a larger study in which a statistical difference in PRs between the immunized patients and control is

established. Since these preliminary data are encouraging, we are currently conducting a randomized study in which women who fail to attain a viable pregnancy after two ET cycles are randomized to either LI therapy or the control group. Progesterone induced blocking factor levels are also being measured pre and post-LI. Enrollment in the study has been slow due to the need to find women who are undergoing a minimum of 3 ETs.

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