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A Randomized Study Comparing the Efficacy of Reducing the Spontaneous Abortion Rate following Lymphocyte Immunotherapy and Progesterone Treatment versus Progesterone Alone in Primary Habitual Aborters

Key Words

Leukocyte immunization
Luteal phase defect

Abstract

Presented herein is a randomized prospective study performed to evaluate the efficacy of the addition of lymphocyte immunotherapy (LI) to progesterone (P) therapy (LI/P) for the prevention of spontaneous abortion (SAB) in primary aborters with a history of three SABs. The incidence of intrauterine pregnancies in four cycles was 23 of 35 (65.7%) patients for LI/P vs. 14 of 31 (45.1%) patients treated with progesterone alone. SABs occurred in 6 of 23 (26.0%) LI/P-treated patients compared to 8 of 14 (57.1%) given progesterone alone. The mean number of previous abortions in both groups was 3.9. The mean age of the LI/P group was 34.1 vs. 33.6 years for the group treated with progesterone alone. These data could be interpreted to show that progesterone therapy and LI independently inhibit SAB or that LI/P acts synergistically to inhibit immune destruction. LI/P therapy was found to be more effective than progesterone therapy alone.

Introduction

The use of lymphocyte immunotherapy (LI) for recurrent spontaneous abortion (RSA) is considered by some researchers as very effective in preventing subsequent spontaneous abortions (SABs), but is considered by other groups to be no better than placebo. The latter groups justify their conclusions based on various studies demonstrating benefits of the following placebo therapies for RSA: cerclage [1]; infusion of trophoblast membrane vesi-

cles without eliciting an immune response [2]; saline [3], or psychotherapy [4].

Though there have been several studies demonstrating a benefit to LI [5-12], only the study by Mowbray [5] had randomized controls. However, subsequently, three other controlled studies concluded that LI was not effective in preventing SABs [3, 13, 14]. In fact, a recent meta-analysis concluded that 'we do not believe that the current evidence supports any benefit of immunotherapy' and that 'fair evidence exists against its use' [15].

The meta-analysis by Fraser et al. [15] found only four randomized controlled immunotherapy treatments for RSA, but included as one of them an infusion of trophoblast membrane (which has been considered as placebo therapy) [2].

The maintenance of pregnancy during the first trimester is dependent on progesterone secretion by the corpus luteum, as evidenced by SAB if the corpus luteum of pregnancy is removed in the early first trimester [16] or if pregnancies achieved by donor oocytes are not supported by progesterone [17]. Some data suggest that if the serum progesterone drops below 15 ng/ml, SAB is almost inevitable [18]. Other data have demonstrated that with aggressive progesterone therapy, the abortion rate can be kept to only 30% when progesterone was <15 ng/ml [19] or 40% even when progesterone was <8 ng/ml [20]. Some females seem to have luteal phase defects (LPDs) repetitively and others only intermittently [21–23]. Recently, some data suggest that not only will the lack of progesterone lead to structural endometrial abnormalities but also may lead to an increase in embryotoxic factors [24].

One of the reasons a woman might abort despite LI could be the coincidental development of a LPD on the cycle of conception. Furthermore, this patient could also have an excess of embryotoxic factors. Possibly, the addition of progesterone therapy to LI (LI/P) might reduce the SAB rate to an even greater degree.

Presented herein are our data on a randomized comparative study for primary aborters with 3 or more SABs treated with luteal phase and first trimester progesterone therapy vs. LI/P therapy combined.

Materials and Methods

Patient Selection

The study evaluated a group of 66 consecutive primary aborters with ≥ 3 SABs. Each patient was randomly assigned to treatment with progesterone therapy in the luteal phase and during the first trimester versus LI/P therapy. Patients were randomized according to the last digit of their social security number. All patients were required to demonstrate the following: absence of submucous leiomyomata or uterine septi by hysterosalpingography or hysteroscopy; normal chromosome analysis in the male and female partner; negative cervical cultures for *Mycoplasma*, *Ureaplasma*, and *Chlamydia* in the female partner; the absence of leukocytospermia ($> 1 \times 10^6$ /ml leukocytes/ml semen) in the male partner, and the female negative for anticardiolipin antibodies and lupus anticoagulant. Furthermore, each woman had to demonstrate the presence of a mature follicle (18–24 mm average diameter by pelvic sonography associated with a serum estradiol (of ≥ 200 pg/ml approximately 2 weeks before expected menses). Only patients conceiving within four cycles of therapy were included in the study.

Lymphocyte Preparation and Immunization

Four hundred milliliters of whole blood was obtained from the male partner and the plasma and erythrocytes were removed. The remainder was resuspended with an equal volume by weight of sterile saline. The cells were then layered over sodium metrizoate/Ficoll and centrifuged. The mononuclear cell layer was then aspirated and washed three to five times with sterile saline with a final slow spin to remove platelets and then resuspended with 5 ml of sterile saline. The lymphocyte suspension was then aspirated into five syringes (numbers 1–4 with 0.5 ml and No. 5 with 3.0 ml). The patient then received two intramuscular and two subcutaneous injections with the 0.5-ml suspensions and the 3-ml suspension was given by intravenous saline drip. The therapy was repeated within 2 weeks of the diagnosis of pregnancy. The mean number of lymphocytes received by each patient per inoculum was $214.4 \pm 99.7 \times 10^6$.

Progesterone Therapy

Progesterone therapy was initiated as 50-mg oral micronized capsules 4×/day beginning 3 days after attaining a mature follicle, as long as the serum progesterone level was > 2 ng/ml. A late-luteal phase endometrial biopsy was performed and the dosage was increased for the next cycle if the biopsy dated more than 2 days out-of-phase. A repeat biopsy in the subsequent cycle was performed only if the dosage of progesterone was increased from the previous cycle. The patient was only allowed to try to conceive once the biopsy was in-phase. Once a pregnancy was established, the dosage of progesterone was increased to 400 mg/day, unless the patient was already taking that level, in which case it was increased to 600 mg. Serum progesterone levels were measured once a week during the first trimester, drawn 4–6 h from the previous progesterone capsule; a level of ≥ 35 mg/ml was maintained by adjustment of dosage.

Lymphocytotoxic Antibody Assay

Each patient had a lymphocyte antibody assay measured prior to LI. The patient's serum was serially diluted using 30% McCoys with fetal calf serum. Leukocytes from the partner were isolated, washed and suspended in Hanks' buffered saline solution. The serum was then pipetted (0.002 ml) beneath 0.005 ml of heavy mineral oil that had been added to 24 wells of a tray; 0.001 ml of the cell suspension was then added and incubated for 1 h at room temperature. Next, 0.005 ml of ABC (rabbit) complement was added to each well and incubated for 2 h at room temperature. Finally, 0.008 ml of eosin/formaldehyde solution was added, the trays sealed, and read under an inverted phase microscope.

Evaluation of Data

All pregnant patients were required to have a final sonogram 12 weeks from conception (one having already been performed at approximately 6 weeks from conception). Spontaneous abortion rates were compared using χ^2 analysis.

Any woman attaining a β -human chorionic gonadotropin (β -hCG) subunit of > 100 mIU/ml was considered pregnant.

The pregnancies were further divided into 5 groups: group 1, β -hCG > 100 but no sac demonstrated by ultrasonography; group 2, a sac demonstrated but no fetal pole; group 3, a sac demonstrated with fetal pole but no viability; group 4, sac with viable fetus at 6 weeks from conception but nonviable or pregnancy loss by 12 weeks from conception, and group 5, viable pregnancy 12 weeks from conception.

Results

Intrauterine pregnancies in four treatment cycles occurred in 23 of 35 (65.7%) patients given LI/P (there was 1 ectopic pregnancy also) vs. 14 of 31 (45.1%) treated with progesterone alone ($p = 0.09$, χ^2 analysis). SABs occurred in 6 of 22 (26.0%) intrauterine pregnancies treated with LI/P vs. 8 of 14 (57.1%) given progesterone alone ($p = 0.073$, χ^2 analysis).

The pregnancies divided according to group found, 0 group 1, 2 group 2, 0 group 3, 3 group 4 and 17 group 5, in those treated with LI/P vs. 1, 1, 1, 5, and 6 for the 5 groups, respectively, in those treated with progesterone only. The mean number of previous SABs was 3.9 ± 1.1 in both groups.

The mean age of the LI/P group was 34.1 ± 3.8 and 33.6 ± 5.4 for the group treated with progesterone only. Unfortunately, karyotyping was available for only 2 of the abortions, both in the progesterone only group, and found 46,XX in one and 69,XXX triploid in the other.

Discussion

In randomized studies we have previously found that progesterone therapy will reduce SABs in patients treated with follicle-maturing drugs [25, 26] and in an uncontrolled study of women with previous histories of SABs. Anecdotally we had not been impressed by a significant reduction in abortions in primary aborters with three or more losses treated with progesterone. Thus, the patients given progesterone therapy were only made aware of our previous work with progesterone to reduce SABs, but no personal data about LI therapy was given to them since there was none. Thus, we believe the group treated with progesterone only should serve as a placebo control. Any information concerning the benefit provided by progesterone for reducing SABs given to the group treated with progesterone would have also been provided to the LI/P group.

One study compared the pregnancy rates in women with habitual abortions, who were given antenatal counseling and psychological support, to the rates in women given no specific counseling. The group given the counseling had a significantly higher rate of successful pregnancies (86%) than did the control group (33%) [4]. These 2 groups, however, in the present study, were given equal antenatal counseling and were evaluated in exactly the same way during the first trimester.

The results of the study presented herein could be interpreted as either (1) LI therapy does reduce the rate of SAB vs. placebo in a group of primary aborters (minimum three previous losses), or (2) that combination LI/P acts synergistically in inhibiting SAB compared to controls (progesterone alone).

Recently, there has been evidence that recurrent SABs may, in some cases, be related to embryo and/or trophoblast-toxic factors (possibly cytokines) which are produced in response to stimulation by sperm or trophoblast antigens [27]. A large majority of women with LPDs and RSAs were found to produce these embryotoxic factors versus lower levels in women with anatomic etiologies for SABs [27]. In fact, many women with LPDs have an increased concentration of T lymphocytes in the endometrium that are capable of cytokine secretion, as compared to endometrial biopsy specimens from women without LPDs [28]. Possibly, the combination of LI/P somehow suppresses these embryotoxic factors better than progesterone alone and possibly better than LI alone.

If, in fact, LI does help prevent SABs, the mechanism is still poorly understood. Future prospective randomized studies might compare LI/P to LI alone and progesterone alone to help answer these questions. This future study would be aided by the measurement of the embryotoxic and trophoblastic cytokines.

A very good review and commentary on the problems with conclusions as to whether LI can reduce the risk of another SAB in patients with RSAs has been provided by Clark and Daya [29]. The study by Cauchi et al. [3], which did not support the positive data of Mowbray et al. [30], failed to use an adequate number of lymphocytes [31] nor did they give two thirds of the dose of lymphocytes intravenously; similarly, the study by Ho et al. [13] failed to use two thirds of the dose intravenously and did not specify cell concentration as elaborated by Clark and Daya [29]. Furthermore, in the study of Ho et al. [13], there was a reduction in SABs (38 vs. 21%) in those given LI vs. those receiving maternal lymphocytes. Yet, these were two of the four studies used for the meta-analysis by Fraser et al. [15].

There are many pitfalls in using the meta-analysis statistical approach [32]. Once the trials were selected, Fraser et al. [15] used the appropriate statistical methodology to combine and analyze the data. They correctly used a random effect model to measure the treatment effect. However, as Thompson and Pocock [33] point out, 'the random effects model is no panacea for heterogeneity'. These issues have also been addressed by Thacker [32] who advises that in reviewing a meta-analysis one should

consider whether the authors have assessed the comparability of the cases, controls, and circumstances in the studies selected; he also considered an alternative explanation in the discussion and analyzed the relationship of study characteristics to problems under review. The failure to analyze other possible interpretation for the heterogeneity of the studies limits the validity of the conclusion drawn by the authors. But, as in all statistical analyses, the best methodology applied to an improperly selected sample does not validate the analysis.

The small preliminary study presented herein certainly should not be considered the final word on the benefits of LI; however, the marked reduction in SABs in a very recalcitrant group following LI/P therapy expresses the need for continued studies of LI and not to cease its investigation as a therapeutic tool based on the conclusions of the meta-analysis of Fraser et al. [15], since none of their studies evaluated the combination of LI and progesterone.

As previously mentioned, there is evidence that progesterone may have immunosuppressive effects [24, 34]. Eckler et al. [24] gave luteal phase progesterone therapy to 325 women with RSA in whom embryotoxic factors had been found, and remeasurement of the embryotoxic factor was available on repeat testing in 141 pregnancies in 117 women [24]. They found that 40 of 56 women (71%) still positive for embryotoxic factors aborted vs. only 11 of 85 women (13%) now found negative for those factors

on repeat testing after progesterone therapy [24]. The selection criteria for the study by Ecker et al. [24] was any woman registering with SAB problems with two or more losses. This was similar to our selection criteria for our previous study reaching similar conclusions about the possible benefit of progesterone therapy [35]. However, progesterone therapy alone was not nearly as effective in the group of primary aborters with a history of three or more SABs used in the present study. Perhaps embryotoxic factors may still be operational in this more difficult group, but progesterone therapy alone is insufficient to suppress them adequately. Possibly future studies will find that the combination of LI and progesterone therapy is more efficient in suppressing these toxic cytokines.

It is hoped that these data will stimulate interest in a large prospective cooperative study among various groups interested in reproductive immunology with uniform selection criteria (i.e., primary aborters with three or more previous SABs) and uniform treatment (number of cells, percent given intravenously, booster injections, repeating LI if no pregnancy by a certain time period). Though our experimental design does not allow a conclusion as to whether the combination of LI/P therapy is better than LI alone, since the SAB rate in this small study was one of the lowest reported to date in primary aborters with RSA, we hope that a large cooperative study would include evaluation of LI and progesterone together as one of the therapies for evaluation.

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