Immunological Treatment of Implantation Failure

G. DE PLACIDO, F. ZULLO, N. COLACURCI, D. PERRONE, A. NAZZARO, F. PAOLILLO, AND U. MONTEMAGNO

Department of Obstetrics and Gynecology
II School of Medicine
Naples, Italy

INTRODUCTION

The non-rejection of the implanting blastocyst is one of the most fascinating topics in reproductive immunology. The interest in it has enormously increased in the last years since, with the development of the *in vitro* fertilization-embryo transfer (IVF-ET) technique, the great number of very early abortions (biochemical pregnancies) has become apparent;[1,2] thus, the implantation rate must be considered as the real limiting factor in all the assisted reproductive techniques. It seems likely that several immunological mechanisms such as the modification of the mother immune response by hormones and by non-hormonal substances, fetal and maternal suppressor cells, and maternal blocking antibodies are involved in the maintenance of pregnancy.[3-5] An immunological recognition of the blastocyst has been claimed to be a necessary prerequisite for a successful pregnancy. It has been further postulated that failure in this immunological recognition could lead to repeated unexplained abortions.[6] So far immunization treatment of women with recurrent abortion has been introduced, using different protocols: third party leukocytes,[7] husband's leukocytes,[6] or trophoblast membrane preparation.[8] Recently, a passive immunization treatment by using Ig from a donor pool (IVIG) has been proposed as well, which should provide immunological protection of pregnancy in the same way as the previous techniques.[9]

Considering the very high reliability of this technique, which has no side effects, a pilot study was undertaken to examine the possibility to extend this treatment to two other groups: 1) women with two or more previous very early pregnancy failures (including biochemical pregnancies) following assisted reproductive techniques; 2) women with repeated ($n \geqslant 3$) unsuccessful embryo transfers either uterine or tubal. So far we have treated 19 women (12 group A and 7 group B) obtaining 5 ongoing pregnancies out of a total of nine positive β-HCG.

MATERIALS AND METHODS

Inclusion criteria for the passive immunization protocol were: 1) women with two or more previous very early pregnancy failures (including biochemical pregnancies)

TABLE 1. Results Obtained Using the Intravenous Immunoglobulin Protocol

Patients	Cause of Infertility	Previous Attempts	Current Attempts	Results
1	Tubal	3 UET[a] 2 BP	IVF	No pregnancy
2	Male Factor	4 IUI 3 DIPI 2 UET 1 TET 1 BP	ZIFT	No pregnancy
3	Unexplained	4 IUI 3 UET	ZIFT	Ongoing pregnancy (14 week)
4	Unexplained	4 DIPI 1 UET 2 TET	ZIFT	No pregnancy
5	Tubal	3 UET 1 EA	IVF	Ongoing pregnancy (16 week)
6	Unexplained	5 IUI 3 DIPI 2 GIFT 1 BP 1 UET 1 EA	ZIFT	Ongoing pregnancy (8 week)
7	Tubal + male factor	3 UET	IVF	No pregnancy
8	Unexplained	3 DIPI 3 GIFT 1 BP 1 UET 1 EA	ZIFT	Early abortion (7 week)
9	Unexplained	4 IUI 3 DIPI 2 UET 1 TET 2 BP	ZIFT	No pregnancy
10	Unexplained	4 DIPI 1 UET 2 TET	ZIFT	Ongoing pregnancy (20 week)
11	Tubal	4 UET 1 EA 1 BP	IVF	No pregnancy
12	Unexplained	4 DIPI 3 UET	ZIFT	No pregnancy
13	Unexplained	3 IUI 2 DIPI 1 GIFT 1 UET 2 EA	ZIFT	No pregnancy
14	Unexplained	4 IUI 3 DIPI 2 UET 1 TET 1 BP	ZIFT	Ongoing pregnancy (15 week)
15	Tubal + male factor	3 UET 1 BP	IVF	No pregnancy
16	Unexplained	3 IUI 3 DIPI 1 GIFT 1 EA 1 UET 1 BP	ZIFT	Biochemical pregnancy
17	Unexplained	2 IUI 4 DIPI 1 GIFT 1 EA 1 UET 1 BP	ZIFT	Biochemical pregnancy
18	Unexplained	4 IUI 3 DIPI 2 UET 1 TET 1 BP	ZIFT	No pregnancy
19	Tubal	3 UET 1 BP	IVF	Early abortion (7 week)

[a] *Abbreviations:* IUI = IntraUterine Insemination; DIPI = Direct IntraPeritoneal Insemination; UET = Uterine Embryo Transfer; TET = Tubal Embryo Transfer; EA = Early Abortion; BP = Biochemical Pregnancy.

TABLE 2. Differences in One Way MLC in Women with Habitual Abortion/Failure of Implantation

Responder Lymphocytes[a]	Stimulator Lymphocytes[b]	cpm in AB Serum[c]	Autologous Serum[c] (% of Stimulation in AB Serum)	Responder Lymphocytes[a]	Stimulator Lymphocytes[b]	cpm in AB Serum[c]	Autologous Serum[c] (% of Stimulation in AB Serum)
Women				Women			
1	–	400	115	11	–	314	91
	Own husband	25623	42		Own husband	41107	55
	Pool	41428	65		Pool	42818	18
2	–	1635	132	12	–	667	105
	Own husband	73777	38		Own husband	73777	65
	Pool	41653	71		Pool	41654	41
3	–	4200	96	13	–	1647	87
	Own husband	82200	111		Own husband	60553	161
	Pool	92428	103		Pool	52709	95
4	–	425	128	14	–	1564	94
	Own husband	17959	76		Own husband	44775	123
	Pool	34427	43		Pool	250774	117
5	–	1259	155	15	–	1791	116
	Own husband	62505	74		Own husband	83155	36
	Pool	42169	59		Pool	76459	21
6	–	679	108	16	–	3659	133
	Own husband	34258	144		Own husband	78924	39
	Pool	48519	112		Pool	91932	46
7	–	1259	89	17	–	473	152
	Own husband	72917	122		Own husband	29942	18
	Pool	84215	139		Pool	84421	80
8	–	318	141	18	–	987	85
	Own husband	54156	24		Own husband	141180	143
	Pool	92818	57		Pool	105492	109
9	–	1250	88	19	–	893	126
	Own husband	116790	46		Own husband	59004	23
	Pool	201123	32		Pool	201135	19
10	–	1647	157				
	Own husband	95150	129				
	Pool	68617	86				

[a] 2×10^5 cell/well. [b] Cells treated with mitomycin C, 2×10^5/well. [c] Mean of sextuplet.

following assisted reproductive techniques; 2) women with repeated ($n \geq 3$) unsuccessful embryo transfers either uterine or tubal. Informed consent was obtained from 19 women: 12 in group A and 7 in group B. The Intra-Venous Immunoglobulin (IVIG) protocol we have adopted has been: 20g of human Ig from a pool of 100 donors (Endobulin, Immuno) in slow infusion at the beginning of ovarian hyperstimulation by exogenous gonadotropins for IVF, followed by a second dose of 15g at the moment of positive β-HCG and then every 3 weeks up to week 20. The blocking capacity of sera was investigated in one way mixed lymphocytes culture (MLC): lymphocytes were separated by Ficoll gradient centrifugation and then transferred in RPMI supplemented with 10% serum; all sera from patients were heat inactivated; the inhibition exerted by each single serum was determined as a percentage of the response in AB serum (100 × cpm in own serum/cpm in AB serum).

RESULTS AND DISCUSSION

By using the IVIG technique, as reported in TABLE 1, we have obtained 9 pregnancies out of a total of 19 treated women. Five of these have an ongoing pregnancy (between 8 and 20 weeks), 2 have had a biochemically detectable pregnancy and 2 a very early abortion (within 8 weeks). Four out of the 5 pregnant women had an abnormal one way MLC suggesting a deficient blocking activity in the serum. These very preliminary results seem to show a positive effect of IVIG treatment in these indications of 55.5% (5 out of 9) in a population with a well documented inability to obtain a successful implantation. Considering the MLC results (TABLE 2) we can observe a significantly higher success rate of IVIG treatment in those patients with a presumptive lower serum blocking activity. Furthermore, the IVIG technique has some important advantages over the other immunization techniques, such as simplicity of use and the absence of major side effects,[10] particularly long-term ones, both on the mother and on the fetus.

REFERENCES

1. MILLER, J. F., E. WILLIAMSON, Y. G. GORDON, J. G. GRUDZINSKAS & A. SYKES. 1980. Fetal loss after implantation: A prospective study. Lancet **1:** 554.
2. EDMONDS, D. K., K. S. LINDSAY, J. F. MILLER, E. WILLIAMSON & P. J. WOOD. 1982. Early embryonic mortality in women. Fertil. Steril. **38:** 447.
3. BEER, A. E. 1983. Immunopathologic factors contributing to recurrent spontaneous abortions in humans. Am. J. Reprod. Immunol. **4:** 182.
4. THOMAS, M. L., J. M. HARGER, D. K. WAGENER, B. S. RABIN & T. J. GILL. 1985. HLA sharing and spontaneous abortions in humans. Am. J. Obstet. Gynecol. **151:** 1053.
5. FAULK, W. P. & J. A. MCINTYRE. 1987. Role of anti-TLX antibody in human pregnancy. *In* Reproductive Immunology. D. A. Clark & B. A. Croy, Eds.: 106. Amsterdam, Elsevier Science Publications.
6. BEER, A. E. 1988. Sem. Reprod. Endocrinol. **6:** 162. Thieme Medical Publishers.
7. UNANDER, A. M. & A. LINDHOLM. 1986. Transfusions of leukocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortions. Am. J. Obstet. Gynecol. **154:** 516.
8. JOHNSON, P. M., K. V. CHIA, C. A. HART, M. B. GRIFFITH & W. J. A. FRANCIS. 1988. Trophoblast membrane infusion for unexplained recurrent miscarriage. Br. J. Obstet. Gynecol. **95:** 342.
9. MUELLER-ECKHARDT, G., O. HEINE, J. NEPPERT, W. KUNZEL & C. MUELLER-ECKHARDT. 1989. Treatment of recurrent spontaneous abortion (RSA) by intravenous immunoglobulin (IVIG):

A pilot study. J. Reprod. Immunol. (Suppl. July) 4th International Congress of Reproductive Immunology; Abstract book, p. 113.

10. VARGA, P. J., Z. SZEREDAY, A. ARTNER & J. SZEKERES-BARTHO. 1989. Early pregnancy loss, premature and low birth weight delivery, and increased maternal lymphocyte cytotoxicity. Am. J. Reprod. Immunol. **19**: 136.