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Immunological Treatment of Implantation Failure

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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 ($n \geq 3$) 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	Autologous Serum ^c		Responder Lymphocytes ^a	Stimulator Lymphocytes ^b	Autologous Serum ^c	
		cpm in AB Serum ^c	(% of Stimulation in AB Serum)			cpm in AB Serum ^c	(% of Stimulation in AB Serum)
Women							
1	— Own husband Pool	400 25623 41428	115 42 65	11	— Own husband Pool	314 41107 42818	91 55 18
2	— Own husband Pool	1635 73777 41653	132 38 71	12	— Own husband Pool	667 73777 41654	105 65 41
3	— Own husband Pool	4200 82200 92428	96 111 103	13	— Own husband Pool	1647 60553 52709	87 161 95
4	— Own husband Pool	425 17959 34427	128 76 43	14	— Own husband Pool	1564 44775 250774	94 123 117
5	— Own husband Pool	1259 62505 42169	155 74 59	15	— Own husband Pool	1791 83155 76459	116 36 21
6	— Own husband Pool	679 34258 48519	108 144 112	16	— Own husband Pool	3659 78924 91932	133 39 46
7	— Own husband Pool	1259 72917 84215	89 122 139	17	— Own husband Pool	473 29942 84421	152 18 80
8	— Own husband Pool	318 54156 92818	141 24 57	18	— Own husband Pool	987 141180 105492	85 143 109
9	— Own husband Pool	1250 116790 201123	88 46 32	19	— Own husband Pool	893 59004 201135	126 23 19
10	— Own husband Pool	1647 95150 68617	157 129 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 ($100 \times \text{cpm in own serum/cpm in AB serum}$).

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.

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Repeated Implantation failure (RIF) is the failure of the embryo to implant onto the side of the uterus wall following IVF treatment. Regularly, this happens at 6-7 days after conception and involves the embedding of the growing embryo into the mothers uterus and a connection being formed . A successful implantation can be determined by using an ultrasound to view the sac which the baby grows in, inside the uterus. Recurrent implantation failure (RIF) refers to cases in which women have had three failed in vitro fertilization (IVF) attempts with good quality embryos. The definition should also take advanced maternal age and embryo stage into consideration. The failure of embryo implantation can be a consequence of uterine, male, or embryo factors, or the specific type of IVF protocol.Â Immunological factors such as cytokine levels and presence of specific autoantibodies should be examined, as well as any infectious organisms in the uterus leading to chronic endometritis. Uterine pathologies such as polyps and myomas as well as congenital anatomical anomalies should be ruled out.