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Corresponding Author
Anu Hirachan,
International Fertility center, Greenpark, Delhi, India
dr.anuhirachan@gmail.com

Author Designation
[1]Fellowship in Reproductive Medicine
[2]Senior IVF consultant

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Enhancing Embryo Implantation Success: The Role of Alpha Thymosin in Modulating Immune Response in Recurrent Implantation Failure

[1]Anu Hirachan and [2]Rita Bakshi
[1-2]International Fertility Center, Greenpark, Delhi, India

ABSTRACT
Recurrent implantation failure (RIF) is a significant challenge in reproductive medicine, affecting approximately 10-15% of couples undergoing assisted reproductive techniques. Despite various factors being implicated, the exact cause of RIF remains elusive. This study investigates the potential immunomodulatory role of Alpha thymosin, a small protein secreted by the thymus gland, in improving implantation success rates. The study involved 14 participants at the International Fertility Centre, New Delhi, who had a history of RIF. Participants received 3.2 mg of Alpha thymosin divided over two doses at the beginning of their menstrual cycle, with an additional dose prior to embryo transfer. Data collection and analysis were performed using SPSS version 26, focusing on associations between treatment and pregnancy outcomes. Of the 14 participants, 64.3% tested positive for serum beta HCG post-treatment, with 88.9% of these showing cardiac activity in ultrasounds. Statistical analysis revealed no significant correlation between Alpha thymosin administration and serum progesterone levels or patient demographic variables (age and BMI). Alpha thymosin may enhance the uterine environment's receptivity to embryo implantation through immunomodulation. However, given the small sample size and exploratory nature of this study, further research, including randomized controlled trials, is necessary to conclusively determine its efficacy.

INTRODUCTION

Implantation failure is used to describe both patients who have never shown quantifiable signs of implantation such as increased levels of hCG and those who have increased hCG production without later ultrasound evidence of a gestational sac[1]. Recurrent implantation failure (RIF) is a challenging condition in reproductive medicine and is only applicable to patients undergoing ART[2]. Although there is no accepted formal definition for recurrent implantation failure, Orvieto et al suggest that it is after three failed in vitro fertilization-embryo transfer (IVF-ET) cycles with good quality embryos transferred[3].

The prevalence of recurrent implantation failure varies depending on the population studied and the criteria used for diagnosis, but it is estimated to occur in approximately 10-15% of couples undergoing assisted reproductive techniques such as in vitro fertilization[4]. The exact cause of recurrent implantation failure is not fully understood, as it can be influenced by various factors including maternal age, embryo quality, uterine abnormalities and immune system dysregulation.

Successful implantation is a process of maternal-fetal immune tolerance involving various molecules. Trophoblast invasion can activate the maternal immune response to fetal antigens. Local immune cells at the implantation site in the endometrium, which are activated by the embryos, mediate maternal-fetal immune tolerance and promote placental development. They involve in regulating the differentiation of decidual cell, remodeling uterine vascular, promoting epithelial attachment and regulating immune activation. In this stage, immune cells, including innate lymphocytes, T cells, decidual dendritic cells and macrophages, are activated and they are also associated with adverse pregnancy outcomes such as RIF[5].

Alpha thymosin is a small protein secreted by the thymus gland and plays a crucial role in immune system regulation. The exact mechanism of action of alpha thymosin in recurrent implantation failure is not yet fully understood, but it is believed to have immunomodulatory effects that may modulate immune responses in the uterine environment, promoting a more favorable environment for successful embryo implantation. Several studies have looked at the role of immunomodulation in the implantation success in patients with recurrent implantation failure. One study found that immunomodulation in women with recurrent implantation failure significantly improved pregnancy rates compared to a control group[6].

However, there is dearth of evidence on the use of alpha thymosin in recurrent implantation failure in the Indian setting. The present study aims to study the role of alpha thymosin in recurrent implantation failure.

MATERIALS AND METHODS

The present study was conducted in International Fertility Centre, New Delhi. After taking prior consent, patients with recurrent implantation failure were recruited on the first day of their menstrual cycle. Upon enrolment, participants received alpha thymosin supplementation, with each dose consisting of 1.6 milligrams (mg) of alpha thymosin. Two doses, totalling 3.2 mg of alpha thymosin, were administered on the first day of the menstrual cycle. Subsequently, patients were scheduled for follow-up appointments every alternate day, starting from day one of the menstrual cycle. Follow-up appointments occurred on day 3, day 5 and onwards until the endometrial lining thickness reached or exceeded 7.5 mm. Additionally, 48 hours prior to the embryo transfer procedure, patients received a single dose of alpha thymosin, equivalent to 1.6 mg, to optimize the uterine environment for successful implantation. Data was collected and cleaned using MS excel spreadsheet. The data was analysed using SPSS version 26. The quantitative data was expressed in mean and standard deviation, whereas the qualitative variables were expressed in frequencies and proportions. To determine the association between the dependent and independent variables, chi square test was used. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

There were 14 study participants with an average age of 36 years and a standard deviation of 5.53 years, with 50% of the participants falling into the age group of 35 to 39 years. The average Body Mass Index (BMI) was 21.47 kg/m^2 with a standard deviation of 2.94. The majority fell into the normal BMI category (71.43%), with smaller proportions being underweight (14.29%) and overweight (14.29%) and none categorized as obese. Majority of the women (64.29%) had a past history of having three previous implant failures, followed by four failures (28.57%) and only a small proportion (7.14%) experienced five failures. The entire cohort belonged to the upper socioeconomic class. (Table 1).

Table 1: Demographic profile of the study participants (n = 14)

Variables	Frequency	Proportion
Age Group		
25-29	2	14.29%
30-34	2	14.29%
35-39	7	50.00%
40-44	3	21.43%
BMI Category		
Underweight	2	14.29%
Normal	10	71.43%
Overweight	2	14.29%
Past History of Implant Failures		
3 times	9	64.29%
4 times	4	28.57%
5 times	1	7.14%

Table 2: Association of the beta HCG levels with different demographic variables

	Beta HCG		
Parameters	Negative	Positive	p-value*
Age Group			
25-29	0 (0.0%)	2 (100.0%)	0.875
30-34	1 (50.0%)	1 (50.0%)	
35-39	3 (42.86%)	4 (57.14%)	
40-44	1 (33.33%)	2 (66.67%)	
BMI Category			
Normal	4 (40.0%)	6 (60.0%)	0.774
Overweight	1 (50.0%)	1 (50.0%)	
Underweight	0 (0.0%)	2 (100.0%)	
Implant Failures			
3	3 (33.33%)	6 (66.67%)	0.523
4	1 (25.0%)	3 (75.0%)	
5	1 (100.0%)	0 (0.0%)	

*Chi square test

Table 3: Association of the cardiac activity with different demographic variables

	Cardiac Activity		
Parameters	Absent	Present	p-value*
Age Group			
25-29	0 (0.0%)	2 (100.0%)	0.029
30-34	1 (100.0%)	0 (0.0%)	
35-39	0 (0.0%)	4 (100.0%)	
40-44	0 (0.0%)	2 (100.0%)	
BMI Category			
Normal	1 (16.67%)	5 (83.33%)	0.754
Overweight	0 (0.0%)	1 (100.0%)	
Underweight	0 (0.0%)	2 (100.0%)	
Implant Failures			
3	0 (0.0%)	6 (100.0%)	0.707
4	1 (33.33%)	2 (66.67%)	

*Chi square test

Table 4: Association of the beta HCG levels with serum progesterone levels

Serum P4 Category*	Beta	HCG Negative	Beta	HCG Positive	p-value**
<0.8	2	33.33%	4	66.67%	0.356
>=0.8	3	37.50%	5	62.50%	

*Cut off calculated on the basis of the median value of serum progesterone (= 0.8), **Chi square test

Table 5: Association of the cardiac activity with serum progesterone levels

	Cardiac Activity		
Serum P4 Category*	Absent	Present	p-value**
<0.8	1 (25%)	3 (75%)	0.905
>=0.8	0 (0%)	5 (100%)	

*Cut off calculated on the basis of the median value of serum progesterone (=0.8), **Chi square test

Pre-embryo transfer endometrial thickness averaged at 8.5 mm (SD = 0.67 mm). The mean progesterone levels before embryo transfer were 0.90 mcg (SD = 0.29). All individuals underwent the transfer of exactly two embryos, highlighting a uniform treatment approach.

In all the women, the quality of embryo at the time of transfer on day 5 was good. 2 embryos were transferred into each of the study participants. Out of 14 women, 9 (64.3%) women tested positive for serum beta HCG. Among these 9 women who tested positive for serum beta HCG, cardiac activity was present in 8 (88.9%) women.

Age, BMI and past history of implantation failure did not affect the beta HCG positivity (Table 2).

Patients between the age of 35 to 39 years were significantly found to have positive cardiac activity after the embryo transfer on day 5 (Table 3).

There was no association between beta HCG levels and serum progesterone levels (Table 4).

There was no association between cardiac activity and serum progesterone levels (Table 5).

The mean Th1/Th2 ratio in patients with positive cardiac activity (n = 8) was 9.5±4.3, as compared to Th1/Th2 ratio in patients with negative cardiac activity (n = 1) 7.1.

DISCUSSION

The endometrium is an important site for the implantation and maturation of fertilized eggs. The endometrium undergoes repetitive proliferation, maturation (decidualization) and exfoliation changes every menstrual cycle. At the same time, the number and type of endometrial immunocompetent cells vary during the menstrual cycle. At the implantation stage, the immunocompetent cells occupy approximately half of the endometrial cells. Immunocompetent cells normally eliminate pathogenic microorganisms to protect the body; however, they also promote immune tolerance to accept the fetus during pregnancy. The immunocompetent cells in the uterus can perform both these functions. With the establishment of pregnancy, stimuli from the trophoblast (placenta) and fetus can also change the immune environment of the uterus, and pregnancy can be maintained only when the immune system is well adapted to the stimuli of some hormones and the fetus. Immunity for the establishment of pregnancy is not simple because multiple immunocompetent cells are involved in establishing and maintaining pregnancy[6].

In the uterine environment, a particular form of natural killer (NK) cells with aunique transcriptional profile, the uterine NK (uNK) cells, represents the most abundantlymphocyte population, especially in the endometrium. In fact, most of the immunecells present in the uterus usually display a unique phenotype. Peripheral blood NKcells express CD56+CD16+ at their membrane surface and are characterized by a highlycytotoxic profile. However, uNK cells are less toxic since they do not express CD16on their membrane surface. During the menstrual cycle, levels of uNK cells startto increase in the mid-secretory phase, which could explain their importance in embryoimplantation[8].

RIF caused by immunologicalfactors can be managed using several innovative therapeutic options. Among them, administration of thymosinalpha 1 (Tα1) can be suggested as atreatment for patients suffering from RIF. Tα1 is a 28-amino-acid peptide expressed in

the thymus. It was originally used as an immune booster and has been used in the treatment of immunodeficiency diseases. As an immunomodulatory compound, Tα1 can significantly upregulate the production of CD4+T and CD8+T cells[9].

Th1 immunity, characterized by immune-inflammatory responses, becomes dominant during the peri-implantation period and the "controlled" Th1 immunity benefits the invading trophoblasts rather than harm. Quickly after the placental implantation, the early inflammatory Th1 immunity is shifted to the Th2 anti-inflammatory immune responses. The predominant Th2 immunity, which overrules the Th1 immunity at the placental implantation site, protects a fetus by balancing Th1 immunity and accommodate fetal and placental development. In the present study, the Th1/Th2 cell ratio was higher in women with viable pregnancy[10]. In a study of women who experienced RPL (*N* = 26), Th1/Th2 cell ratios were significantly higher in the peripheral blood than those of healthy fertile controls[11]. In another study of women with RPL (n = 44), IVIg treatment reduced Th1 cell levels, transcription factor expression and type 1 cytokine levels, while increasing Th2 cell levels with the substantial decrease in Th1/Th2 cell ratios. Women with IVIg treatment had a significantly higher live birth rate (87.5%, 28 out of 32) when compared to that in controls (41.6%, 5 out of 12)[12].

In a study conducted by Kaufmann *et al*.[13], the authors found that the Thymosin α_1 levels from pregnancies that remained viable were significantly higher than those from pregnancies that spontaneously aborted. Preovulation thymosin α_1 levels also tended to be lower in pregnancies that subsequently aborted. Thymosin β_4 levels were similar between the two groups[13]. Partially purified thymosin has also been found to stimulate the secretion of luteinizing hormone-releasing factor from medial basal hypothalami. In addition, luteinizing hormone is released from pituitary glands superfused in sequence with hypothalami. This suggests a direct effect of the endocrine thymus on the hypothalamus and a potentially important role for thymic peptides in reproductive function[14]. In the present study, all the women having serum progesterone levels more than 0.8 pg/mL had a viable fetus showing cardiac activity. This finding was not found to be statistically significant, possibly due to the low sample size. However, the findings from this study suggest a biological plausibility given that fact that it is cost effective.

CONCLUSION

The immunomodulatory effect of Thymosin alpha 1 may play an important role in the management of recurrent implantation failure. Further studies such as randomized controlled trials are warranted to determine the effectiveness of Thymosin alpha 1 in preventing recurrent implantation failure.

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