International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Preethi SP et al. Int J Reprod Contracept Obstet Gynecol. 2026 Jun;15(6):1938-1944
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20261458

Original Research Article

Clinical impact of thymosin alpha-1 as an adjuvant in enhancing frozen embryo transfer outcomes: a self-controlled study

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Received: 18 February 2026
Revised: 27 April 2026
Accepted: 29 April 2026

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ABSTRACT

Background: Endometrial immune milieu when studied appeared to be dysregulated in 81.7% of women with recurrent implantation failure (RIF). Thymosin alpha 1, a small peptide secreted from the thymus gland has the ability to favourably alter the maternal immune milieu conducive for embryo implantation and maintenance of pregnancy. This study plans to evaluate the impact of empirical use of Thymosin alpha 1 as an adjuvant in improving the pregnancy outcomes in women with more than two failed frozen embryo transfers (FETs).
Methods: Women who experienced two prior failed FETs despite good-quality embryos underwent endometrial preparation for their next FET cycle using hormone replacement therapy (HRT) supplemented with exogenous Thymosin alpha 1. Pregnancy outcomes were then compared to those from the immediately preceding cycle.
Results: In this cohort of 27 patients with two prior FET failures, addition of Thymosin alpha 1 in the subsequent FET cycle significantly improved implantation rates (63.4%; $p<0.001$) biochemical and clinical pregnancy rates (66.7%; $p<0.001$), and ongoing pregnancy rates (59.3%; $p<0.001$). No significant difference was observed in mean Thymosin dose between pregnant and non-pregnant women (22 mg vs. 20.3 mg; $p=0.373$).
Conclusions: The study provides encouraging evidence that thymosin alpha 1 could be an useful adjunct for improving pregnancy outcomes in women with RIF.

Keywords: Thymosin alpha 1, Recurrent implantation failure, Frozen embryo transfer

INTRODUCTION

Recurrent implantation failure (RIF) is prevalent in 10-15% of couples undergoing artificial reproductive techniques (ART).[1] Successful implantation hinges on a precise equilibrium between pro-inflammatory and anti-inflammatory responses at the feto-maternal interface. Imbalances in CD4 T-helper lymphocytes (Th), i.e. Th1, Th2, Th17, and regulatory T cells (Treg) leading to elevation in pro-inflammatory cytokines and increased Th1/Th2 cytokine ratios have been suggested as one of many contributing factors to RIF.[2] Endometrial immune profiles appeared to be dysregulated in 81.7% of the RIF patients.[3] Thymosin alpha 1, a peptide secreted by the thymus gland has the potential to increase the Treg cells, thus reducing the Th1/Th2 ratio thereby favouring embryo implantation by improving the maternal-foetal immune tolerance. In addition to this, by promoting a positive Th2 balance conducive for maintenance of pregnancy, Thymosin alpha 1 could provide an answer to 30-50% of women with unexplained RIF.[4] When performed, immuno-profiling investigations significantly tend to increase the cost of ART. This study plans to evaluate the impact of empirical use of Thymosin alpha 1 as an adjuvant in improving the pregnancy outcomes in women with two or more failed FETs.

METHODS

This was a self-controlled retrospective study done between November 2024 and January 2026 at the Institute of Reproductive Medicine, Madras Medical Mission,

Chennai, Tamil Nadu, India. This study included 27 women with two prior failed FETs who underwent subsequent FET with thymosin alpha 1.

Inclusion criteria

Women with previous two failed FETs despite good quality embryos, endometrial thickness on progesterone start day is 8 mm and age ≤40 years were included in the study.

Exclusion criteria

Women with endometritis, congenital uterine anomalies and hydrosalpinx, women with pre-transfer serum progesterone <12 ng/ml, women with uncontrolled diabetes mellitus/ hypertension, renal or hepatic profile derangement, autoimmune diseases and vascular/haematological disorders were excluded from the study.

Data collection

Baseline demographics and clinical history of women were recorded. All participants had normal karyotype. Autoimmune and APLA profile-negative. Endometrial tissue CD-138-negative.

In patients with previous two FER cycles failed, in the subsequent cycle, endometrial preparation was done using hormone replacement therapy (Oestradiol valerate). After getting informed written consent, Inj. Thymosin alpha 1 was started on the day 2-3 of menses, 3.2 mg subcutaneous alternate day dosing till progesterone start day. No immuno-profiling had been done prior to administration of thymosin alpha 1.

When endometrial thickness on serial ultrasound monitoring was ≥8 mm, daily exogenous natural progesterone injection (100 mg intramuscular) had been started. Serum progesterone levels were measured after 2 days of progesterone administration. Patients were individualised and good quality, stage specific embryos were transferred. Luteal support was given and pregnancy when positive was followed up till 12 weeks.

The FER cycle immediately preceding the Thymosin alpha 1 study cycle was used as the control, provided it had been performed within the preceding one year.

Outcome measured

Biochemical pregnancy rate

Beta HCG: ≥50 IU sixteen days post FER.

Implantation rate

Visualisation of a gestational sac on ultrasound about 5 weeks after embryo transfer.

Clinical pregnancy rate

Transvaginal ultrasound confirmation of fetal heart rate at 7 weeks.

Early pregnancy loss

Miscarriage before 12 weeks.

Ongoing pregnancy rate

Pregnancy beyond 12 weeks.

Statistical analysis

Continuous variables are expressed in mean and standard deviations; categorical variables are expressed in frequency and percentage. Differences between the groups are analysed using paired t test and Wilcoxon sign ranked test. Pregnancy outcome comparisons were analysed using McNemar's exact test, Mann Whitney U test, Logistic regression analysis and Pearson correlation. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

Twenty-seven patients with at least previous two failed FETs were included in this study after consideration of inclusion and exclusion criteria. Mean age of women was 35.6 years with standard deviation of 3.6 years. The average body mass index (BMI) was 30.9 kg/m^2 with standard deviation (SD) of 5.93 kg/m^2 among which 63% of women were of BMI >30 kg/m^2. The mean duration of infertility was 9.2 years (SD=4.5 years) and 92 % (n=25) were nulliparous women (Table 1).

Among 27 women, 44.4% (n=12) women had normal ovaries and uterus while 55.6% had some pelvic pathology (Figure 1). Fibroids seen were <2 cm in size, 1-2 in number and well away from the endometrial cavity or endo-myometrial junction.

Between thymosin alpha 1 cycle and control cycle without thymosin alpha 1, no significant difference was seen in cumulative oestradiol dose used for endometrial preparation, duration of endometrial preparation or endometrial thickness on progesterone start day (Table 2). Except for the addition of thymosin alpha 1, there was no difference in endometrial preparation between study and control cycles. Luteal support in both thymosin alpha 1 cycle and control cycle were similar.

Against zero biochemical pregnancy rate of control cycle, biochemical and clinical pregnancy rate of thymosin alpha 1 cycle was 66.7% (n=18; exact McNemar's test, p<0.001) and implantation rate was 63.4% (Exact McNemar's test, p<0.0001). One woman underwent missed miscarriage at 8 weeks of pregnancy and other women had spontaneous abortion at 10 weeks, thus concluding an ongoing

pregnancy rate (>12 weeks) of 59.3% (n=16; exact McNemar's test, p<0.0001) (Figure 2).

In study group using thymosin alpha 1, clinical pregnancy rates did not differ significantly across age groups (p=0.53), BMI categories (p=0.48), type of infertility (p=0.48), cause of infertility (female vs male factor; p=0.22), previous obstetric history (nulligravida, previous abortions, previous live birth; p=0.22), number of previous FER failures (p=0.57) or presence and type of pelvic pathology (p=0.24). Duration of infertility was only variable significantly associated with pregnancy outcome (p=0.0007), with patients having 6-10 years of infertility demonstrating a higher clinical pregnancy rate compared with those with >10 years of infertility (Table 3).

The mean duration of infertility among women with positive clinical pregnancy was 9.0 years (SD=3.6) and among non-pregnant women was 9.7 years (SD=5.9). There was a very weak negative linear association between duration of infertility and pregnancy positivity rates although the corelation was not statistically significant (Pearson correlation: r=-0.07: p=0.721).

There was no significant difference in the number of embryos transferred between control cycle and thymosin alpha 1 cycle (46 vs 39; mean: 1.7 vs 1.4; Paired t test: p=0.07). Transfer of embryos of same stage or advanced stage after extending the culture by one more day between control and thymosin alpha 1 cycle did not significantly impact the clinical pregnancy outcome (Table 4).

Table 1: Demographic variables of study group.

Variables	No. of women	Proportion (%)
Age (in years)		
<35	10	37.04
35-37	7	25.93
38-40	10	37.04
BMI (kg/m^2)		
18.5-24.9	4	14.81
25-29.9	6	22.22
30-34.9	10	37.04
≥35	7	25.93
Type of infertility		
Primary	10	37.04
Secondary	17	62.96
Cause of infertility		
Female factor	15	55.56
Male factor	12	44.44
Duration of infertility		
≤5 years	6	22.22
6-10 years	14	51.85
>10 years	7	25.93
Obstetric history		
Nulligravida	10	37.04
Previous abortions	15	55.56
Previous live birth	2	7.41
No. of previous FER failures		
2	17	62.96
3-4	9	33.33
≥5	1	3.70

Table 2: Variables involved in pre- embryo transfer endometrial preparation.

Variables	Control cycle	Thymosin alpha 1 cycle	P value*
Cumulative estradiol dose (mg)	99.5±35.9	107.2±47.7	0.47
Duration of endometrial preparation (Days)	15.07±3.93	15.4±1.97	0.66
Endometrial thickness (mm)	8.27±0.53	8.33±0.61	0.58

*Paired t test.

Mean number of doses of thymosin used in study cycle-7 (range: 5-9), with single dose accounting to thymosin alpha 1 of 3.2 mg. No significant difference was observed in mean thymosin alpha 1 dose used between women who achieved clinical pregnancy and those who did not, indicating no detectable dose-response effect within this small sample (Table 5). On multiple logistic regression analysis, after adjusting for age, BMI and weight, dose of thymosin alpha 1 used did not show statistically significant association with clinical pregnancy outcome (p=0.29).

Table 3: Clinical pregnancy outcome among different demographic variables.

Variables	Positive	Negative	P value¥
Age (in years)			
<35	8 (80%)	2 (20%)	0.526
35-37	4 (57.1%)	3 (42.9%)	
38-40	6 (60%)	4 (40%)	
BMI (kg/m^2)			
18.5-24.9	4 (100%)	0 (0%)	0.478
25-29.9	4 (66.7%)	2 (33.3%)	
30-34.9	6 (60%)	4 (40%)	
≥35	4 (57.1%)	3 (42.9%)	
Type of infertility			
Primary	8 (80%)	2 (20%)	0.481
Secondary	10 (58.8%)	7 (41.2%)	
Cause of infertility			
Female factor	8 (53.3%)	7 (46.7%)	0.218
Male factor	10 (83.3%)	2 (16.7%)	
Duration of infertility			
≤5 years	2 (33.3 %)	4 (66.7%)	0.0007*
6-10 years	14 (100 %)	0 (0%)	
>10 years	2 (28.6%)	5 (71.4%)	
Obstetric History			
Nulligravida	8 (80%)	2 (20 %)	0.223
Previous abortions	8 (53.3%)	7 (46.7 %)	
Previous live birth	2 (100%)	0 (0%)	
No. of previous FER failures			
2	12 (70.6%)	5 (29.4%)	0.572
3-4	5 (55.6%)	4 (44.4 %)	
≥5	1 (100%)	0 (0%)	
Pelvic pathology			
None	8 (66.7%)	4 (33.3%)	0.242
PCOM	4 (57.1%)	3 (42.9%)	
Fibroid	4 (100%)	0 (0%)	
Fibroid + adenomyosis	4 (100%)	0 (0%)	

¥Chi square test, *significant

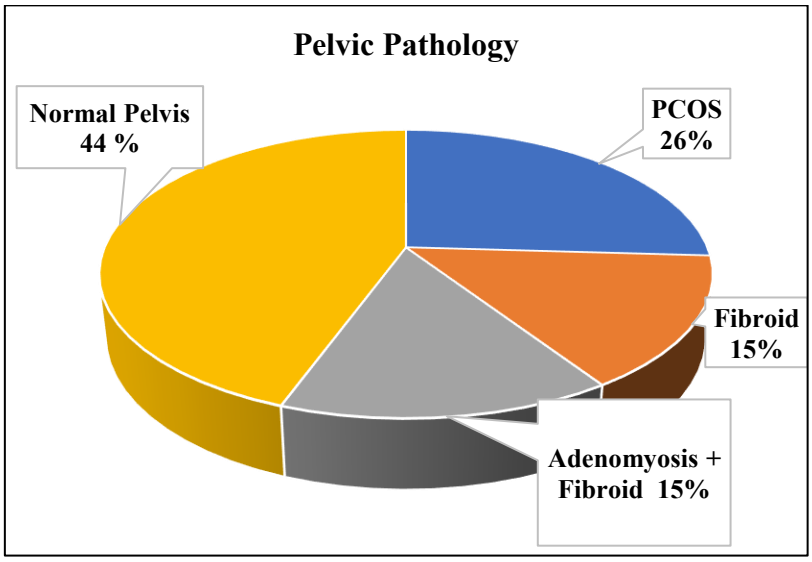

Figure 1: Distribution of pelvic pathology among study group.

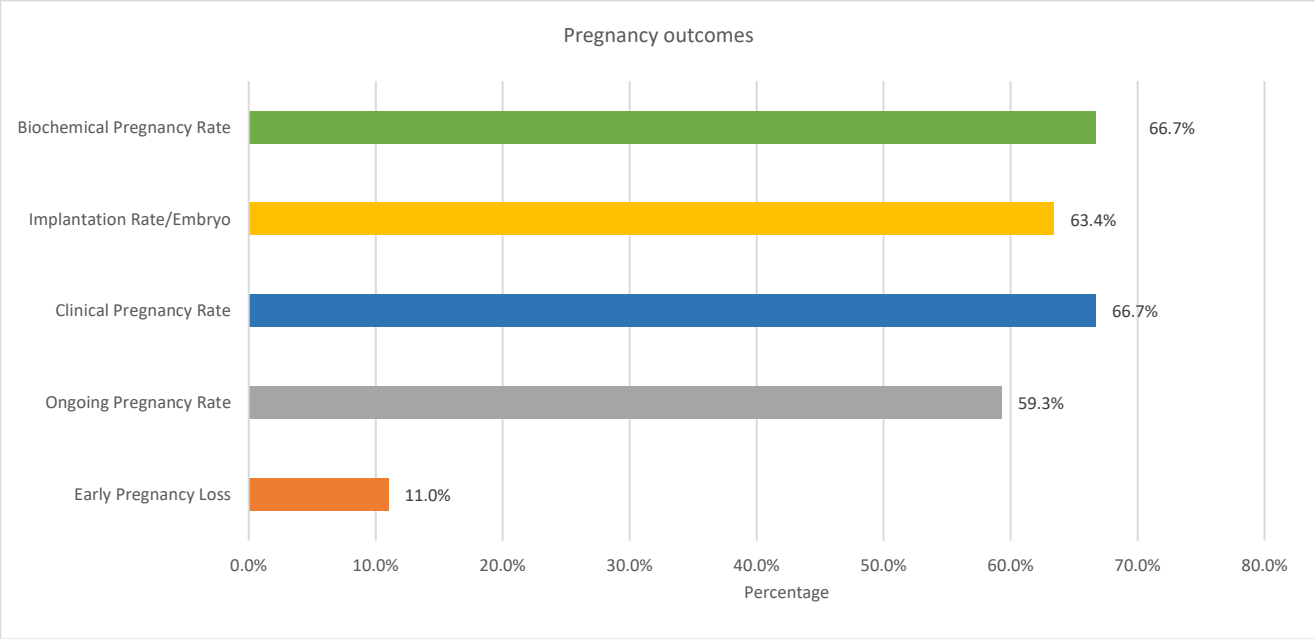

Figure 2: Pregnancy outcome of FER using thymosin alpha 1.

Table 4: Clinical pregnancy rate with change in stage of embryo transferred between cycles.

Change in stage (Control cycle→ thymosin alpha 1 cycle)	N	Pregnancy positive	Pregnancy negative	P value (Exact multinomial McNemar test
Embryo stage advanced	14	10	4	0.775
Embryo stage unchanged	13	8	5	

Table 5: Thymosin alpha 1 dose on clinical pregnancy outcome.

Clinical pregnancy	N	Total dose of thymosin alpha 1 (mg)	P value (Mann-Whitney U test)
Positive	18	22.0±3.94	0.373
Negative	9	20.3±5.06	

DISCUSSION

Feto-maternal immune tolerance operates at three interconnected levels: the local maternal decidual interface, systemic maternal immune modulation, and the foetal component. IL-2, IFN-γ, TNF-α and TNF-β are pro-inflammatory cytokines predominantly produced by Th1 cells. Together, IL-2 and IFN-γ can synergistically enhance the cytotoxic activity of T lymphocytes and natural killer (NK) cells, promoting trophoblast destruction and embryo loss.[5] In contrast, IL-4, IL-6 and IL-10 are mainly secreted by Th2 cells. IL-4 and IL-10, in concert with regulatory T cells (Treg), help maintain immune homeostasis and create a permissive environment for embryo implantation and maintenance of pregnancy.[6]

During early implantation, a controlled pro-inflammatory bias is required to support trophoblast invasion and uterine vascular remodelling, after which a shift toward Th2-type, anti-inflammatory cytokine is necessary to sustain immune tolerance and foetal growth.[7] Excessive early pro-inflammatory activity can result in implantation failure, whereas inadequate anti-inflammatory dominance in later phase after implantation may contribute to subsequent early pregnancy loss.

In patients with RIF, elevated pro-inflammatory cytokines, reduced anti-inflammatory cytokines, and an increased Th1/Th2 cytokine ratio have been reported, with a sensitivity of 96.55% and specificity of 87.50% and women who achieved clinical pregnancy showed a progressive shift toward Th2 dominance by 5 weeks.[8] Dominant Th1-type immune responses in peripheral blood lymphocytes may contribute to recurrent spontaneous miscarriage or repeated implantation failure in IVF cycles.[9]

Targeted modulation of the maternal immune environment using agents such as intravenous immunoglobulins, hydroxychloroquine, intralipids, or thymosin alpha 1 may help optimise the endometrial immune milieu and thereby support successful embryo implantation.

In this study cohort of 27 women with at least two prior FER failures, baseline characteristics reflected a relatively advanced infertile population, with mean age 35.6 years, mean BMI in the borderline obese range, and long-standing infertility (mean 9.2 years). Importantly, endometrial preparation between the thymosin alpha 1 and control cycles was comparable with respect to estradiol dose, duration of preparation, and endometrial thickness, and luteal support protocols were identical. This indicates that differences in outcome are unlikely to be driven by disparities in conventional endometrial preparation.

Studies indicate that thymosin alpha 1 concentrations were markedly higher during the preovulatory phase and early gestation in pregnancies that progressed to viability, compared to those that ended in spontaneous abortion.[10]

The key observation in the present study is marked contrast in pregnancy outcomes: while the control cycles yielded no biochemical pregnancies, thymosin alpha 1 cycles achieved a clinical pregnancy rate of 66.7% and implantation rate of 63.4%. For a population with repeated previous failures, these rates are clinically impressive and suggest that thymosin alpha 1 may help overcome an implantation barrier that was not corrected by standard hormonal preparation alone. Although the absence of pregnancy in the control cycles exaggerates the apparent effect size, the within-patient comparison strengthens the impression of a real signal.

There are currently limited studies on benefits of thymosin alpha 1 in reproductive medicine. A study analysed the effect of thymosin alpha in 14 patients with RIF.[11] Of the 14 participants, 64.3% had biochemical pregnancy and 88.9% of them showed cardiac activity in ultrasound showing positive outcomes similar to the present study.

A research work conducted in 2021 concluded that after having controlled the confounding variables like maternal age and the type of embryo, the implantation rate in women who had 0, 1, 2, and 3 or more previous failed ET cycles were 45.8%, 35.9%, 31.2%, 21.0%, respectively ($p<0.001$).[12] Using this benchmark implantation rates of 31.2% for women with two prior FER failures and 21.0% for those with three or more failures (without thymosin alpha 1), the implantation rate in our cohort receiving thymosin alpha 1 was 56% for women with only two previous FER failures (n=17, binomial exact test $p\approx0.008$) and 66.7% for those with three or more failures (n = 10, binomial exact test $p<0.001$). These findings indicate a statistically significant increase in implantation rates with Thymosin alpha 1 compared with the expected rates in similar patients not treated with this drug.

Within the thymosin alpha 1 cycles, clinical pregnancy rates did not differ significantly across age groups, BMI strata, type and cause of infertility, obstetric history, number of prior FER failures, or the presence of pelvic pathology. This uniformity suggests that, within the constraints of small numbers, the beneficial effect of thymosin alpha 1 was not restricted to a particular demographic or etiologic subgroup.

Lack of effect of age and BMI is noteworthy but should be interpreted cautiously. In larger studies, both factors are strong determinants of ART success. In this dataset, distribution of pregnant and non-pregnant women across categories is too sparse to detect anything but very large differences.

The number of embryos transferred and embryo developmental stage were comparable between the thymosin alpha 1 and control cycles, limiting the likelihood that improved embryo quality or quantity alone explains the observed outcome difference. Transfers in which embryo stage was upstaged that is, from cleavage to morula or morula to blastocyst in the thymosin cycle did not show a significant trend toward higher pregnancy rates ($p\approx0.775$), indicating that improvements in embryo stage alone did not have an impact on the pregnancy outcome in patients with RIF. Importantly, however, pregnancies also occurred when embryo stage was unchanged, suggesting that thymosin alpha 1 may primarily act through modulation of the endometrial or immunologic environment rather than through embryo selection bias.

No dose-response relationship was observed for thymosin alpha 1. The mean cumulative dose was similar between women who conceived and those who did not, and logistic regression adjusting for BMI and weight confirmed absence of a significant association. This could mean that the dosing regimen used in the study already lies on the plateau of the dose–response curve, with a “threshold” effect rather than a graded response. Alternatively, the sample may simply be too small to detect subtle differences. Without a range of substantially different doses in a larger population, firm conclusions about optimal dosing cannot be drawn.

The high implantation rate observed after thymosin alpha 1 in this study supports the hypothesis that modifying immune milieu around the time of implantation can convert prior failures into successful outcomes. A recent case report of a woman with unexplained RIF and documented Th1 dominance also described successful pregnancy after intensive pre-transfer thymosin alpha 1 therapy, after multiple previous failed transfers despite using other immunomodulatory adjuvants.[13]

While future studies incorporating detailed immune profiling are required to confirm benefits of thymosin alpha 1, current work reflects its empirical use, chosen specifically to avoid increasing the overall cost of ART for patients.

Limitations

The major limitation of this study is the small sample size and retrospective design. Additionally, the study does not report incidence of anomalies, live-birth rate, obstetric

complications, or neonatal outcomes beyond early ongoing pregnancy. For an immune modulator, safety data are critical, particularly with respect to miscarriage, preeclampsia, and foetal growth.

Strength and future prospects

The primary strength of this self-controlled study lies in its design, which minimizes confounding variables. The striking improvement-from 0% pregnancy rate in control cycles to substantially higher implantation, biochemical, clinical, and ongoing pregnancy rates with Thymosin alpha 1 cycles warrants validation through larger, adequately powered prospective randomized controlled trials. Future studies incorporating endometrial receptivity assays, preimplantation genetic testing of embryos, and immune dysregulation profiling would enhance methodological rigor.

CONCLUSION

This study offers promising evidence that thymosin alpha 1 may serve as a valuable adjunct therapy for women experiencing RIF, especially when standard factors like embryo quality and endometrial thickness are favourable yet implantation has failed.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Preethi SP, Shankar K, Veerasigamani G, Vittal RG, Ranji G. Clinical impact of thymosin alpha 1 as an adjuvant in enhancing frozen embryo transfer outcomes: a self-controlled study. Int J Reprod Contracept Obstet Gynecol 2026;15:1938-44.