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Original Research Article

Recorded Pregnancy Outcomes Following Short-Course Thymosin Alpha 1 as Adjunctive Therapy in Women with Implantation Failure: A Single-Centre Retrospective Cohort Study

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Abstract

Background: Evidence supporting thymosin alpha 1 as an adjunct in assisted reproduction is limited and is derived mainly from small, uncontrolled studies. This study described pregnancy outcomes and documented adverse events among women with implantation failure who received a short course of thymosin alpha 1 in routine clinical practice.

Methods: This single-centre retrospective cohort study reviewed records of women older than 25 years with clinician-recorded implantation failure who received thymosin alpha 1 (Guficin Alfa) 3.2 mg on alternate days over 13 days. Demographic and infertility characteristics, previous embryo-transfer failures, the recorded binary pregnancy outcome, and treatment-related adverse events were summarized descriptively. Wilson 95% confidence intervals (CIs) were calculated for key proportions.

Results: Thirty-nine women were included. Mean age was 33.10 ± 5.90 years, and the mean number of previous embryo-transfer failures was 2.03 ± 1.35. Ovarian factors were recorded in 14 (35.9%) women and pregnancy- or implantation-related factors in 12 (30.8%). A positive pregnancy outcome was documented in 37 of 39 women (94.9%; 95% CI, 83.1%-98.6%); the source dataset did not distinguish biochemical, clinical, ongoing pregnancy, or live birth. No treatment-related adverse event was documented in routine records.

Conclusion: A high proportion of this treated cohort had a recorded positive pregnancy outcome. Because the study lacked an untreated comparator, used a non-standardized outcome, and did not control for prognostic or treatment-related confounding, the findings do not establish effectiveness. Prospective controlled studies using standardized recurrent implantation failure definitions and live-birth outcomes are required.

Keywords: Assisted Reproductive Technology; Embryo Transfer; Implantation Failure; Pregnancy Outcome; Retrospective Studies; Thymosin Alpha 1.

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Introduction

Successful implantation requires coordinated interaction among a developmentally competent embryo, a receptive endometrium, and the maternal systemic and local environment. Recurrent implantation failure (RIF) is commonly used to describe repeated failure to achieve pregnancy after embryo transfer, but definitions vary by the number and quality of embryos transferred, maternal age, embryo ploidy, and the outcome used. The European Society of Human Reproduction and Embryology recommends an individualized assessment based on the cumulative predicted chance of implantation rather than a single universal numerical threshold. [1]

Potential contributors include embryo aneuploidy, uterine abnormalities, endometrial factors, endocrine and metabolic disease, thrombophilia, male factors, and immune dysregulation.2 Although immune mechanisms are biologically plausible, evidence for immune testing and empiric immunomodulatory treatment in unexplained implantation failure remains uncertain. Current recommendations therefore emphasize investigation and treatment supported by a clear rationale and adequate clinical evidence. [1,3]

Thymosin alpha 1 is a synthetic form of a naturally occurring 28-amino-acid peptide with immunomodulatory activity. It can influence innate and adaptive immune responses, including

dendritic-cell function and T-lymphocyte activity. [4] In-vitro findings suggest effects on T-helper-cell subsets and regulatory T cells, but these observations were obtained outside reproductive medicine and should not be assumed to translate directly into improved implantation.[5]

Published reproductive evidence consists largely of small observational cohorts and case reports with heterogeneous eligibility criteria, thymosin schedules, and pregnancy endpoints. [6-10] The objective of the present study was therefore to describe recorded pregnancy outcomes and treatment-related adverse events in women with clinician-recorded implantation failure who received short-course thymosin alpha 1 as an adjunct to routine assisted reproductive treatment.

Materials and Methods

Study design and setting: This retrospective, observational, single-centre cohort study was conducted at Ahalya Nursing Home and IVF Center, Guntur, Andhra Pradesh, India. Medical records from a one-year period were reviewed. Reporting was structured according to core recommendations for observational studies.

Participants and eligibility: Records were eligible when the woman was older than 25 years, had clinician-recorded implantation failure, received thymosin alpha 1 as part of assisted reproductive treatment, completed the documented regimen, and had an available pregnancy outcome. Records were excluded if the clinician had not diagnosed implantation failure, another immunomodulatory treatment was administered concurrently, thymosin alpha 1 was discontinued before completion, or key outcome information was unavailable.

The source protocol described RIF as failure to achieve clinical pregnancy after at least three IVF or intracytoplasmic sperm injection cycles involving good-quality embryos. However, the summarized dataset contained fewer previous embryo-transfer failures for some women. The cohort is therefore described conservatively as women with clinician-recorded implantation failure; diagnostic status was not reclassified post hoc.

Treatment exposure and routine care: All included women received thymosin alpha 1 (Guficin Alfa; Gufic Biosciences Ltd.) at a dose of 3.2 mg on alternate days over 13 days as an adjunct to the treating clinician's usual ART protocol. The extracted dataset did not contain sufficiently detailed information on route of administration, timing relative to embryo transfer, fresh versus frozen transfer, endometrial preparation, embryo stage or ploidy, number of embryos transferred, luteal support, or other components of routine care for adjusted analysis.

Variables and outcomes: Data were abstracted from electronic records, paper case files, laboratory reports, ultrasonography records, and ART-cycle documentation. Variables included age, duration of marriage, recorded duration and principal cause of infertility, number of previous embryo-transfer failures, thymosin alpha 1 regimen, pregnancy outcome, and treatment-related adverse events documented during routine care. The primary outcome was the binary pregnancy category recorded in the source dataset (positive or negative). The available summary did not specify the laboratory threshold, assessment time, ultrasonographic confirmation, gestational age, or whether a positive result represented biochemical pregnancy, clinical pregnancy, ongoing pregnancy, or live birth. Safety assessment was based on adverse events documented in routine records; there was no prospective active-surveillance procedure.

Bias, missing data, and sample size: All eligible treated records identified during the study period were included. No a priori sample-size calculation was performed because the analysis used the available census of treated patients. No values were imputed. The descriptive analysis used the recorded denominator for each variable. The retrospective design, treatment selection by clinicians, incomplete measurement of prognostic factors, and absence of an untreated group were considered potential sources of selection, information, and confounding bias.

Statistical Analysis: Continuous variables are reported as mean ± standard deviation (SD) and, where available, median with interquartile range (IQR). Categorical variables are summarized as frequency and percentage. Wilson 95% CIs were calculated for the recorded pregnancy proportion and the proportion with documented treatment-related adverse events. No between-group hypothesis testing or adjusted modelling was performed.

Ethical considerations: The study was reported as having ethical approval under approval number GUF-THY-01-2026. No patient-identifying information is presented in this manuscript.

Results

Participant Characteristics: Thirty-nine women met the documented eligibility requirements and were included in the analysis. The mean age was 33.10 ± 5.90 years; median age was 34 years (IQR, 30-35.5). Mean duration of marriage was 9.64 ± 5.95 years, with a median of 8 years (IQR, 6-13). Infertility duration was 5-10 years in 24 (61.5%) women, longer than 10 years in 12 (30.8%), and shorter than 5 years in 3 (7.7%). The mean number of previous embryo-transfer failures was 2.03 ± 1.35, and the median was 2 (IQR, 1-3) (Table 1).

Table 1: Demographic and infertility characteristics (N=39)

Characteristic	Value
Age, years, mean ± SD	33.10 ± 5.90
Age, years, median (IQR)	34 (30-35.5)
Duration of marriage, years, mean ± SD	9.64 ± 5.95
Duration of marriage, years, median (IQR)	8 (6-13)
Infertility duration <5 years, n (%)	3 (7.7)
Infertility duration 5-10 years, n (%)	24 (61.5)
Infertility duration >10 years, n (%)	12 (30.8)
Previous embryo-transfer failures, mean ± SD	2.03 ± 1.35
Previous embryo-transfer failures, median (IQR)	2 (1-3)

IQR: interquartile range; SD: standard deviation.

Recorded causes of infertility: Ovarian factors were the most frequently recorded principal cause, occurring in 14 (35.9%) women. These included diminished ovarian reserve, reduced antral follicle count, polycystic ovary syndrome, and endometriosis. Pregnancy- or implantation-related factors, including recurrent pregnancy loss and implantation failure, were recorded in 12 (30.8%) women. The cause categories supplied for the analysis accounted for 37 participants; two participants were therefore retained as not classified in the summarized source data (Table 2).

Table 2: Principal recorded cause of infertility (N=39)

Principal cause	n (%)
Ovarian factor (low AMH, reduced AFC, PCOS, or endometriosis)	14 (35.9)
Pregnancy/implantation factor (RPL or implantation failure)	12 (30.8)
Male factor	3 (7.7)
Tubal factor	2 (5.1)
Unexplained infertility	2 (5.1)
Uterine factor	1 (2.6)
Genetic factor	1 (2.6)
Mixed factor (low AMH and RPL)	1 (2.6)
No abnormality identified on recorded evaluation	1 (2.6)
Not classified in the summarized source data	2 (5.1)
Total	39 (100.0)

AFC: antral follicle count; AMH: anti-Müllerian hormone; PCOS: polycystic ovary syndrome; RPL: recurrent pregnancy loss. Percentages may not total exactly 100 because of rounding.

Pregnancy outcome and documented adverse events: A positive pregnancy outcome was recorded in 37 of 39 women (94.9%; 95% CI, 83.1%-98.6%), while 2 (5.1%) had a negative outcome. No treatment-related adverse event was documented in the routine clinical records (0%; 95% CI, 0.0%-9.0%) (Table 3).

Because pregnancy and safety outcomes were not prospectively adjudicated, these values represent documentation in the available records rather than standardized efficacy or safety endpoints.

Table 3: Recorded pregnancy outcome and treatment-related adverse events (N=39)

Outcome	n (%)	95% CI
Positive pregnancy outcome	37 (94.9)	83.1-98.6
Negative pregnancy outcome	2 (5.1)	-
At least one documented treatment-related adverse event	0 (0.0)	0.0-9.0

CI: confidence interval. Confidence intervals were calculated using the Wilson method.

Discussion

In this retrospective cohort of 39 women treated with short-course thymosin alpha 1, 37 (94.9%) had a positive pregnancy outcome recorded in their medical record, and no treatment-related adverse event was documented. These findings describe outcomes after exposure; they do not quantify a treatment effect.

In the absence of an untreated or active-control group, the observed proportion cannot be separated from the effects of embryo characteristics, endometrial preparation, co-interventions, patient selection, or the underlying probability of pregnancy in a subsequent ART cycle.

The observed pregnancy-positive proportion was higher than that reported by Hirachan and Bakshi, who described a positive beta-human chorionic gonadotropin result in 64.3% and clinical pregnancy in 57.1% of 14 treated women. [6] A single case report also described pregnancy

following a 13-dose course, but a case report cannot distinguish treatment response from chance or concurrent ART care.[7]

Recent Indian observational studies have reported widely varying estimates. Joshi et al. recorded positive reproductive outcomes in all 90 treated patients after substantially longer exposure of at least 118 days.[8] In a self-controlled study of 27 women with two previous failed frozen embryo transfers, Preethi et al. reported biochemical and clinical pregnancy rates of 66.7% and an ongoing-pregnancy rate of 59.3% after adjunctive thymosin alpha 1.[9] Kamaraj et al. reported pregnancy in 60.8% of 102 women with implantation failure.[10] Differences in eligibility, exposure duration, cycle protocols, embryo characteristics, and pregnancy definitions prevent direct comparison with the present series.

The biological rationale for immunomodulation should also be interpreted cautiously. Although maternal immune regulation contributes to implantation, the immune phenotype of implantation failure is heterogeneous, and peripheral or endometrial immune markers do not yet define a single treatable disorder.[1,3] One retrospective study of women with elevated T-helper 1/T-helper 2 ratios reported improved reproductive outcomes after combination immunoregulatory therapy, but that intervention and selected population differ from thymosin alpha 1 monotherapy in an unselected cohort.[11] Thus, mechanistic plausibility does not by itself establish clinical effectiveness.

The number of prior failed transfers is itself prognostically relevant. In a retrospective cohort of 6,376 day-3 transfer cycles, implantation declined progressively as the number of previous failed transfers increased, even after accounting for maternal age and transfer type.[12] Contemporary proposals therefore incorporate age-related euploidy and cumulative implantation probability into the RIF definition.[13] Studies restricted to consecutive euploid single-embryo transfers found that persistent unexplained failure after three transfers was uncommon,[14] and a multicentre analysis of up to five euploid transfers estimated true unexplained RIF to be rare.[15] These findings reinforce the need to report embryo ploidy, number of transferred embryos, and the exact unit of previous failure in the present cohort.

Evidence for empirical immunotherapy remains insufficient for routine IVF practice, and professional guidance recommends caution outside well-designed studies.[16] Regulatory T cells have an established biological role in implantation and maternal-fetal tolerance,[17] while endometrial immune profiling studies have identified heterogeneous local immune patterns among women with repeated failure.[18] Historical observational data also linked low endogenous periconceptional thymosin alpha 1 concentrations with blighted pregnancy, but this association neither defines a therapeutic target nor demonstrates benefit from exogenous treatment.[19] The Lugano consensus similarly emphasized standardized definitions, embryo-related determinants, and avoidance of unproven interventions.[20]

No treatment-related adverse event was documented in this series. This is reassuring at a descriptive level, but the upper confidence limit remains compatible with an event proportion of approximately 9%, and retrospective records can miss mild, transient, or delayed events. A prospective safety framework should define solicited reactions, serious adverse events, timing, severity, attribution, and pregnancy-specific outcomes.

The principal strengths are the use of routine clinical data from a standalone IVF centre and complete binary outcome information for all 39 included women. The analysis also avoids unsupported hypothesis testing and presents uncertainty around the main proportion. Nevertheless, the limitations are substantial: small sample size, single-centre design, no comparator, non-random treatment selection, inconsistent characterization of previous transfer failures, incomplete classification of infertility cause, and absence of standardized pregnancy and live-birth endpoints. Important prognostic variables - including body mass index, ovarian reserve measurements, embryo stage and quality, ploidy status, number of embryos transferred, fresh or frozen cycle, endometrial thickness, luteal support, and concurrent treatments - were not available for adjustment. Adverse events were captured passively. Sponsor funding and the involvement of sponsor-employed authors further underscore the need for independent confirmation.

Future studies should prospectively define implantation failure using an individualized, reproducible framework; prespecify thymosin timing, route, dose, and duration; and use a concurrent standard-care comparator. Clinical pregnancy, ongoing pregnancy, pregnancy loss, and live birth should be reported separately, with denominators based on initiated cycles and embryo transfers. Adequately powered multicentre randomized trials are required before routine use can be recommended.

Conclusion

Among 39 women with clinician-recorded implantation failure who received adjunctive short-course thymosin alpha 1, 37 had a positive pregnancy outcome documented and no treatment-

related adverse event was recorded. The uncontrolled retrospective design and non-standardized endpoint prevent causal interpretation. These data should be considered hypothesis-generating and require confirmation in prospective controlled studies reporting clinical pregnancy and live birth.

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Conflict of Interest: Parekh P and Agarwal Rare employees of Gufic Biosciences Ltd., which markets Guficin Alfa and sponsored the study. Uma Shankar S.N. and S Raj Kumari declare no competing interests.

Ethical Approval: The study received ethical approval under approval number GUF-THY-01-2026.

References

1. ESHRE Working Group on Recurrent Implantation Failure; Cimadomo D, de Los Santos MJ, Griesinger G, Lainas G, Le Clef N, et al. ESHRE good practice recommendations on recurrent implantation failure. Hum Reprod Open. 2023;2023(3):hoad023. doi:10.1093/hropen/hoad023.
2. Ma J, Gao W, Li D. Recurrent implantation failure: a comprehensive summary from etiology to treatment. Front Endocrinol (Lausanne). 2023;13:1061766. doi:10.3389/fendo.2022.1061766.
3. Genest G, Banjar S, Almasri W, Beauchamp C, Benoit J, Buckett W, et al. Immunomodulation for unexplained recurrent implantation failure: where are we now? Reproduction. 2023;165(2):R39-R60. doi:10.1530/REP-22-0150.
4. Dominari A, Hathaway D 3rd, Pandav K, Matos W, Biswas S, Reddy G, et al. Thymosin alpha 1: a comprehensive review of the literature. World J Virol. 2020;9(5):67-78. doi:10.5501/wjv.v9.i5.67.
5. Yang X, Qian F, He HY, Liu KJ, Lan YZ, Ni B, et al. Effect of thymosin alpha-1 on subpopulations of Th1, Th2, Th17 and regulatory T cells (Tregs) in vitro. Braz J Med Biol Res. 2012;45(1):25-32. doi:10.1590/S0100-879X2011007500159.
6. Hirachan A, Bakshi R. Enhancing embryo implantation success: the role of alpha thymosin in modulating immune response in recurrent implantation failure. Res J Med Sci. 2024;18:306-310. doi:10.36478/makrjms.2024.6.306.310.
7. Rajan A, Sampath A, Supriya SM, Dhanasekaran RK. Successful pregnancy after administration of thymosin alpha-1 in a woman with recurrent implantation failure. Int J Pharm Clin Res. 2024;6(2):73-75. doi:10.33545/26647591.2024.v6.i2b.107.
8. Joshi R, Patil SY, Gawale S, Parekh P, Dhakare TJ, Salunke N, et al. Efficacy of thymosin alpha-1 as an immunomodulatory adjuvant in patients with recurrent implantation failure: a real-world retrospective analysis. Int J Reprod Contracept Obstet Gynecol. 2026;15(4):1272-1277. doi:10.18203/2320-1770.ijrcog20260885.
9. 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(6):1938-1944. doi:10.18203/2320-1770.ijrcog20261458.
10. Kamaraj KSJ, Parekh P, Agarwal R. Role of thymosin alpha-1 in improving pregnancy outcomes in patients with implantation failure. Int J Res Med Sci. 2026;14(7):2945-2948. doi:10.18203/2320-6012.ijrms20262185.
11. Meng S, Zhang T, Li C, Zhang X, Shen H. Immunoregulatory therapy improves reproductive outcomes in elevated Th1/Th2 women with embryo transfer failure. Biomed Res Int. 2022;2022:4990184. doi:10.1155/2022/4990184.
12. Wang Y, Tian Y, Liu L, Li TC, Tong X, Zhu H, et al. The number of previous failed embryo transfer cycles is an independent factor affecting implantation rate in women undergoing IVF/ICSI treatment: a retrospective cohort study. Medicine (Baltimore). 2021;100(9):e25034. doi:10.1097/MD.0000000000025034.
13. Ata B, Kalafat E, Somigliana E. A new definition of recurrent implantation failure on the basis of anticipated blastocyst aneuploidy rates across female age. Fertil Steril. 2021;116(5):1320-1327. doi:10.1016/j.fertnstert.2021.06.045.
14. Pirtea P, De Ziegler D, Tao X, Sun L, Zhan Y, Ayoubi JM, et al. Rate of true recurrent implantation failure is low: results of three successive frozen euploid single embryo transfers. Fertil Steril. 2021;115(1):45-53. doi:10.1016/j.fertnstert.2020.07.002.
15. Gill P, Ata B, Arnanz A, Cimadomo D, Vaiarelli A, Fatemi HM, et al. Does recurrent implantation failure exist? Prevalence and outcomes of five consecutive euploid blastocyst transfers in 123,987 patients. Hum

Reprod. 2024;39(5):974-980. doi:10.1093/humrep/deae040.
16. Practice Committee of the American Society for Reproductive Medicine. The role of immunotherapy in in vitro fertilization: a guideline. Fertil Steril. 2018;110(3):387-400. doi:10.1016/j.fertnstert.2018.05.009.
17. Robertson SA, Care AS, Moldenhauer LM. Regulatory T cells in embryo implantation and the immune response to pregnancy. J Clin Invest. 2018;128(10):4224-4235. doi:10.1172/JCI122182.
18. Ledee N, Petitbarat M, Chevrier L, Vitoux D, Vezmar K, Rahmati M, et al. The uterine immune profile may help women with repeated unexplained embryo implantation failure after in vitro fertilization. Am J Reprod Immunol. 2016;75(3):388-401. doi:10.1111/aji.12483.
19. Kaufmann RA, Welch RA, Mutchnick MG. Low periconceptional maternal serum thymosin alpha 1 levels are associated with blighted pregnancies. Am J Reprod Immunol. 1993;29(3):171-175. doi:10.1111/j.1600-0897.1993.tb00583.x.
20. Pirtea P, Cedars MI, Devine K, Ata B, Franasiak J, Racowsky C, et al. Recurrent implantation failure: reality or a statistical mirage? Consensus statement from the July 1, 2022 Lugano Workshop on recurrent implantation failure. Fertil Steril. 2023;120(1):45-59. doi:10.1016/j.fertnstert.2023.02.014.