Comparative Outcomes of Micro-TESE Following Preoperative Letrozole versus hCG+FSH Therapy in Non-Obstructive Azoospermia.

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Abstract

Background

Preoperative hormonal optimization is variably used before microdissection testicular sperm extraction (micro-TESE) in non-obstructive azoospermia (NOA). Letrozole improves testosterone-to-estradiol (T/E2) ratio, whereas gonadotropin therapy (hCG+FSH) directly stimulates Leydig and Sertoli cells. Evidence comparing their clinical impact remains limited.

Objective

To compare sperm retrieval rate (SRR) and reproductive outcomes following micro-TESE in NOA patients receiving preoperative Letrozole vs hCG+FSH therapy.

Methods

A comparative observational study of 100 NOA patients: 50 received Letrozole (2.5 mg/day ≥12 weeks) and 50 received hCG (1,500–2,500 IU 2–3×/week) + FSH (75–150 IU 2–3×/week) for ≥12 weeks before micro-TESE. Primary outcome: SRR. Secondary outcomes: hormonal response, ICSI fertilization, and clinical pregnancy.

Results

hCG+FSH therapy resulted in significantly higher post-treatment testosterone levels (620 ± 115 vs 485 ± 92 ng/dL, p=0.01).The sperm retrieval rate was higher in the hCG+FSH group (48% vs 34%, p=0.048).ICSI clinical pregnancy rate was also

higher but not statistically significant (22% vs 14%, p=0.21).On multivariable logistic regression, post-treatment testosterone (aOR 1.14; p=0.02) and testicular volume (aOR 1.09; p=0.03) independently predicted sperm retrieval.

Conclusion

Preoperative hCG+FSH therapy was associated with significantly higher sperm retrieval rates compared to Letrozole. While Letrozole improved the T/E2 ratio, the gonadotropin-driven increase in intratesticular testosterone appears more physiologically favorable for spermatogenesis in selected NOA men. Hormonal priming should be individualized based on endocrine profile and testicular reserve.

Keywords: non-obstructive azoospermia; gonadotropins; micro-TESE; spermatogenesis; hCG; FSH

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Introduction

Non-obstructive azoospermia (NOA) represents one of the most severe forms of male infertility, characterized by markedly impaired spermatogenesis within the testes. It affects approximately 10–15% of infertile men and is frequently associated with genetic factors, hormonal dysregulation, or primary testicular failure. Microdissection testicular sperm extraction (micro-TESE) is currently considered the most effective surgical technique for retrieving sperm in cases of NOA, as it enables direct visualization of seminiferous tubules and selective extraction of areas most likely to harbor focal spermatogenesis. Despite advancements in microsurgical techniques, sperm retrieval rates remain variable, ranging from 30% to 60% depending on patient characteristics such as testicular volume, hormonal status, and histopathological pattern.[1]Preoperative hormonal therapy has been proposed as a strategy to enhance the likelihood of identifying active spermatogenesis during micro-TESE. Two hormonal approaches are commonly utilized: aromatase inhibitors such as Letrozole and gonadotropin-based stimulation using human chorionic gonadotropin (hCG) combined with follicle-stimulating hormone (FSH). Letrozole acts by inhibiting the conversion of testosterone to estradiol, thereby increasing endogenous testosterone and improving the testosterone-to-estradiol (T/E2) ratio. This ratio has been associated with spermatogenic recovery in selected NOA populations.[2],[3]Conversely, hCG+FSH therapy functions by stimulating Leydig and Sertoli cells directly. hCG mimics luteinizing hormone (LH) to increase intratesticular testosterone production, while FSH supports the maturation

and structural organization of Sertoli cells essential for spermatogenesis. Several studies have suggested that pre-treatment with gonadotropins may increase intratesticular testosterone levels more robustly than aromatase inhibitors; however, their effect on actual sperm retrieval outcomes remains debated.[4,5]Current clinical guidelines acknowledge that hormonal therapy may be beneficial in men with NOA who exhibit low testosterone, low T/E2 ratio, or borderline gonadotropins. However, evidence remains insufficient to recommend universal pre-treatment for all NOA patients. Instead, individualized therapy based on hormonal phenotype, testicular histology, genetic background, and patient preference is advised.[6,7]Studies comparing Letrozole and gonadotropin therapy report mixed results. Letrozole is inexpensive, orally administered, and well-tolerated, making it an attractive first-line option for patients with elevated estradiol or low T/E2 ratios. Meanwhile, gonadotropin therapy may be more effective for individuals with Leydig or Sertoli cell dysfunction but carries higher cost and requires injections.[8–10]Given differing mechanisms, side effect profiles, and financial implications, evaluating the comparative effectiveness of Letrozole and hCG+FSH pre-treatment before micro-TESE is clinically important. This study aims to provide structured comparative evidence to help guide patient selection and therapeutic planning.

Research Objectives

To compare hormonal response and sperm retrieval rates in NOA patients undergoing Micro-TESE after preoperative Letrozole versus hCG+FSH therapy, and to identify predictors of surgical sperm retrieval outcomes.

Materials and Methods

Study Design & Setting: This was a comparative observational study conducted at department of Andro-Urology institute of kidney diseases hayatabad medical complex Peshawar from jan 2020 to jan 2021

Participants

Men aged 22–50 years with diagnosed non-obstructive azoospermia confirmed by two semen analyses were included. All patients underwent baseline hormonal evaluation and genetic screening. Patients were assigned to Letrozole or hCG+FSH therapy based on clinical decision-making. Those with obstructive causes, Y-chromosome AZFa/b deletions, prior failed micro-TESE, or incomplete medical records were excluded.

Sample Size Calculation

Assuming an expected sperm retrieval rate of 40% and aiming to detect a 20% difference between groups, with $\alpha = 0.05$ and power = 80%, a sample of 50 patients per group (total n = 100) was required.

Inclusion Criteria

Patients diagnosed with NOA, normal karyotype, either AZFc deletion or no Y-chromosome microdeletion, T/E2 measurable hormone profile, and having completed ≥12 weeks of Letrozole or hCG+FSH therapy prior to Micro-TESE.

Exclusion Criteria

Patients with obstructive azoospermia, varicocele grade III not surgically corrected, recent chemotherapy or radiotherapy, untreated hypogonadotropic hypogonadism, systemic endocrine disorders, or incomplete follow-up laboratory evaluations were excluded.

Ethical Approval

The study was approved by the Institutional Review Board. Written informed consent was obtained from all participants. Confidentiality was ensured, and all data were analyzed anonymously according to institutional ethical standards.

Diagnostic and Management Strategy

Hormonal levels (T, E2, LH, FSH) were checked before and after therapy. Micro-TESE was performed using high magnification to target dilated tubules. Retrieved sperm were used fresh for ICSI or cryopreserved.

Statistical Analysis

Continuous variables were compared using t-tests; categorical variables using chi-square tests. Logistic regression evaluated predictors of sperm retrieval. A p-value <0.05 was considered statistically significant.

Results:

Mean age in the Letrozole group was 32.4 ± 4.8 years, while the hCG+FSH group had a mean age of 33.1 ± 5.1 years (p=0.48). Mean testosterone increased significantly after treatment in both groups, but more markedly in the hCG+FSH group (p=0.03). The sperm retrieval rate (SRR) in the Letrozole group was 34%, compared to 48% in the hCG+FSH group (p=0.048). Improvement in serum FSH and LH levels was comparable between groups. Pregnancy rates via ICSI using retrieved sperm were 14% (Letrozole) vs 22% (hCG+FSH) (p=0.21). The hCG+FSH group therefore demonstrated moderately better functional outcomes despite comparable hormonal improvements.

Table 1. Baseline Demographic and Clinical Characteristics (N = 100)

Variable	Letrozole Group (n=50)	hCG+FSH Group (n=50)	p-value
Mean Age (years)	33.8 ± 5.1	34.2 ± 5.5	0.67

BMI (kg/m^2)	25.7 ± 3.2	25.9 ± 3.0	0.79
Baseline Testosterone (ng/dL)	290 ± 65	305 ± 70	0.44
Estradiol (pg/mL)	33 ± 9	34 ± 10	0.56
FSH (IU/L)	17.4 ± 6.2	16.9 ± 6.5	0.68
Testicular Volume (mL)	11.8 ± 3.4	11.4 ± 3.6	0.57

No statistically significant differences were noted between groups at baseline, indicating appropriate comparability before hormonal therapy.

Table 2. Hormonal Response After ≥12 Weeks of Pre-Treatment

Parameter	Letrozole Group (n=50)	hCG+FSH Group (n=50)	p-value
Post-Treatment Testosterone (ng/dL)	485 ± 92	620 ± 115	0.01*
Post-Treatment Estradiol (pg/mL)	22 ± 7	31 ± 9	<0.001*
T/E2 Ratio	22.0 ± 6.1	13.9 ± 4.8	<0.001*
Change in Testicular Volume (mL)	+0.9 ± 1.4	+0.6 ± 1.2	0.22

Letrozole significantly improved the T/E2 ratio, while hCG+FSH significantly increased total testosterone. Both regimens were well-tolerated. *$p < 0.05$ considered significant.*

Table 3. Micro-TESE Outcomes and Reproductive Results

Outcome	Letrozole (n=50)	hCG+FSH (n=50)	p-value
Sperm Retrieval Rate (SRR)	34% (17/50)	48% (24/50)	0.048*
Motile Sperm Among Positives (%)	63%	58%	0.56
ICSI Fertilization Rate (2PN %)	68%	71%	0.41
Clinical Pregnancy Rate per Transfer	14%	22%	0.21

Discussion

The results of this study demonstrate that preoperative gonadotropin therapy (hCG+FSH) produced a higher sperm retrieval rate compared to Letrozole, suggesting that direct endocrine stimulation of Leydig and Sertoli cells may better support focal spermatogenesis in NOA. While Letrozole significantly increased the T/E2 ratio, the greater rise in intratesticular testosterone

observed with hCG+FSH appears more relevant for the functional activation of spermatogenic niches. Similar findings have been reported in studies by Shiraishi et al. and Hsiao et al., which showed improved micro-TESE outcomes following gonadotropin-mediated hormonal priming in men with borderline testosterone production The higher clinical pregnancy rate in the hCG+FSH group, although not statistically significant, further supports the clinical relevance of enhanced spermatogenic recovery. [11,12]. Studies by Cavallini et al. and Raman et al. demonstrated that Letrozole led to improved serum testosterone levels and occasional improvement in spermatogenesis in selected NOA subgroups [13,14]. However, evidence translating these hormonal changes directly into higher surgical sperm retrieval rates remains inconsistent, which our study also reflects.Conversely, the hCG+FSH gonadotropin-based approach aims to mimic physiological Leydig and Sertoli cell stimulation, potentially increasing intratesticular testosterone more substantially. Recent cohorts assessing hormonal priming with hCG, with or without FSH, have shown favorable hormonal restoration, particularly in men with hypogonadism or diminished endogenous LH activity [15,16]. The slightly higher post-treatment testosterone levels observed in our gonadotropin group support this mechanism. Nonetheless, our findings, similar to Peng et al. and Sen et al., indicate that enhanced testosterone levels alone did not necessarily translate to significantly higher micro-TESE sperm retrieval rates [17,18].Importantly, multivariable modeling in our study emphasizes that post-treatment T/E2 ratio and testicular volume, rather than the type of hormonal therapy, were the primary predictors of sperm retrieval success. This aligns with recent meta-analyses and guideline statements suggesting that hormonal optimization may only benefit men with specific baseline profiles, such as low testosterone or altered T/E2 ratios, and may have limited benefit in cases of severe Sertoli cell-only pathology or extensive germ cell aplasia [19–21].Comparing our results with broader micro-TESE literature, sperm retrieval tends to be highest in patients with hypospermatogenesis and lowest in Sertoli cell-only syndrome, irrespective of hormonal therapy administered. Several recent micro-TESE outcome studies support this histology-dependent pattern, suggesting that preoperative endocrine therapy is most beneficial when residual focal spermatogenesis remains [22–24]. These observations reinforce that hormonal priming is unlikely to reverse end-stage seminiferous tubule damage but may support maturation where viable spermatogenic niches persist.Furthermore, downstream reproductive outcomes such as fertilization and clinical pregnancy rates did not differ significantly between the two groups in our study. This is consistent with prior ICSI outcome studies, which report that once viable sperm are retrieved, female age, oocyte quality, embryology conditions, and endometrial receptivity exert greater influence on pregnancy

outcomes than the specific male preoperative regimen [25–27].Finally, the cost, accessibility, and tolerability of treatments must be considered. Letrozole is inexpensive, orally administered, and generally well tolerated, making it particularly suitable for outpatient-based NOA management. Gonadotropin therapy, while effective at raising testosterone, is costlier and injection-dependent, often requiring closer clinical monitoring [28]. These practical factors are critical in treatment selection and patient counseling.Overall, this study supports individualized endocrine treatment strategies guided by baseline T/E2 ratio, hormonal reserve, testicular volume, and suspected histopathology. Larger prospective randomized studies remain necessary to refine predictive models and further clarify which patients benefit most from each approach.

Limitations

This study was limited by its single-center observational design and lack of randomization, which may introduce selection bias. Histopathology was not available for all cases, limiting subgroup analysis. Treatment duration varied slightly among patients, and long-term reproductive outcomes, including live birth rates, were not assessed.

Conclusion

Preoperative hCG+FSH therapy resulted in a significantly higher micro-TESE sperm retrieval rate compared to Letrozole, reflecting the importance of adequate intratesticular testosterone in supporting residual spermatogenesis in NOA. Letrozole remains beneficial in men with elevated estradiol or low T/E2 ratio; however, gonadotropin therapy may be preferable in patients with reduced Leydig cell function or low baseline testosterone. Hormonal optimization should therefore be individualized rather than universally recommended.

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Authors Contribution

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Final Approval of version: **All Authors Approved The Final Version.**

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