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Testicular Tissue Biopsies Sperms Yield of Non-Obstructive Azoospermic Men: Predictive Factors

Najat Mutar[1], Thulfiqar Ibrahim Raheem[2], Siham Mahmood Al-Rehemi[3], Ali Ibrahim Al-Dulaimi[4-6]

[1]Department of Anatomy, Faculty of Medicine, University of Kufa, Najaf, Iraq; e-mail: najat.alsallami@uokufa.edu.iq, ORCID: 0009-0004-9180-1029
[2]Department of Urology, Iraqi Board of Medical Specializations, Al-Sadr Medical City, Najaf, Iraq; e-mail: thu.urologist84@gmail.com, ORCID: https://orcid.org/0009-0003-6299-8216
[3]Department of Anatomy, Faculty of Medicine, University of Kufa, Najaf, Iraq; e-mail: sihamm.alrehemi@uokufa.edu.iq, ORCID: 0000-0002-0832-7935
[4]Department of Anatomy, Faculty of Medicine, University of Kufa, Najaf, Iraq; e-mail: aliir.abbas@uokufa.edu.iq, ORCID: 0000-0003-4409-2050
[5]Department of Anatomy, College of Medicine, University of Al-Ameed, Karbala, Iraq
[6]Al-Kafeel Super-Specialty Hospital, IVF Center, Karbala, Iraq
Corresponding author: Ali Ibrahim Al-Dulaimi; e-mail: aliir.abbas@uokufa.edu.iq

ABSTRACT:

Background: Most testicular sperm retrieval procedures in non-obstructive Azoospermia (NOA) fail. Reliable pre-operative predictors remain undefined, and Iraqi data are scarce.

Objective: To evaluate clinical and hormonal predictors of successful testicular sperm retrieval in Iraqi men with NOA.

Methods: This prospective cross-sectional study enrolled 85 men with NOA undergoing conventional testicular sperm extraction at Al-Sadr Medical City in Najaf, Iraq (November 2025–May 2026). Age, body mass index (BMI), infertility duration and serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin and Inhibin B were compared between retrieval groups using Mann–Whitney U tests, univariate logistic regression and receiver operating characteristic analysis.

Results: Sperm were retrieved in 29/85 men (34.5%). Successful retrieval was associated with older age (33.62 ± 8.39 vs. 29.96 ± 5.85 years, p = 0.042), lower FSH (7.04 ± 4.48 vs. 11.59 ± 8.30 mIU/mL, p = 0.008) and lower LH (4.67 ± 2.35 vs. 7.61 ± 5.19 mIU/mL, p = 0.037). Discrimination was fair (area under the curve: FSH 0.676, LH 0.639). BMI, infertility duration, testosterone, prolactin and Inhibin B were not predictive.

Conclusions: FSH and LH were associated with retrieval outcome but discriminated only fairly. They should inform counselling, not exclude men from surgery.

INTRODUCTION

Infertility is a common condition with substantial personal, social and economic consequences. The World Health Organization (WHO) estimates that approximately 17.5% of adults — around one in six — experience infertility at some point in their lives, with a period prevalence of 12.6%; lifetime prevalence in the Eastern Mediterranean Region is estimated at 10.7% [1]. A male

factor contributes to approximately half of all cases [2]. Azoospermia, the complete absence of spermatozoa in the ejaculate after examination of a centrifuged pellet on two separate occasions, represents the most severe expression of male factor infertility [3]. It affects approximately 1% of all men and 10–15% of infertile men [5]. Azoospermia is broadly classified as obstructive, in which spermatogenesis is preserved but sperm transport is impeded, or non-obstructive (NOA), in which spermatogenesis itself is impaired. NOA accounts for the majority of cases and is the more difficult to treat [2-5]. The introduction of intracytoplasmic sperm injection (ICSI) transformed the outlook for these men, since a single viable spermatozoon recovered from testicular tissue may achieve fertilization. Surgical sperm retrieval — by conventional testicular sperm extraction (TESE) or by microdissection TESE (micro-TESE) — is therefore the decisive step in the management pathway [2, 6]. However, retrieval fails in a substantial proportion of men. A systematic review and meta-analysis of 15 studies comprising 1890 patients reported a mean sperm retrieval rate (SRR) of 35% for conventional TESE, with micro-TESE approximately 1.5 times more likely to succeed [6]. A more recent meta-analysis of 34 publications reported a mean positive retrieval rate of 45% following micro-TESE [7]. A failed retrieval carries real costs. The patient undergoes an operation with attendant risks of haematoma, infection and testicular devascularisation; the couple may have committed to a synchronized ovarian stimulation cycle that is then abandoned; and the psychological impact of a negative result delivered on the day of oocyte retrieval is considerable. In health systems where assisted reproduction is largely self-funded, as is the case in Iraq, the financial burden falls directly on the family. A pre-operative test that could stratify the probability of success would therefore have clear clinical value. Because spermatogenic failure disrupts the hypothalamic–pituitary–gonadal axis, serum gonadotropins have long been proposed for this purpose. Loss of the seminiferous epithelium reduces Sertoli-cell secretion of Inhibin B, removing selective negative feedback on pituitary FSH, so that FSH rises as spermatogenesis deteriorates [8-9]. On this reasoning, low FSH and high Inhibin B should mark residual spermatogenesis and predict successful retrieval. The evidence, however, is inconsistent. Some series report significantly lower FSH in men with successful retrieval [4, 10], whereas others find endocrine parameters and testicular volume to be unhelpful [11-13]. A meta-analysis of diagnostic accuracy studies found that serum Inhibin B achieved a pooled sensitivity of only 0.65 and specificity of 0.83 and concluded that it cannot serve as a stand-alone predictor [14]; a 2024 meta-analysis similarly found neither FSH (pooled odds ratio [OR] 1.03, 95% confidence interval [CI] 1.00–1.06) nor Inhibin B (OR 1.01, 95% CI 1.00–1.02) to predict retrieval significantly [7]. The explanation is biological: NOA testes frequently contain focal islands of active spermatogenesis, so sperm may be recovered despite markedly elevated FSH, and no hormonal threshold excludes spermatogenesis with certainty [5, 15]. Accordingly, current European Association of Urology (EAU) and American Urological Association/American Society for Reproductive Medicine (AUA/ASRM) guidance does not endorse the use of hormonal thresholds to deny a man a retrieval attempt [2, 16]. Data from Iraq remain limited. Published Iraqi work has characterized the genetic contribution to Azoospermia, including Y-chromosome microdeletions and karyotype abnormalities [17–19], and the histopathological spectrum of testicular biopsies [20], but studies formally evaluating the predictive performance of the routine hormonal panel against retrieval outcome are few [21]. This matters because the case-mix presenting to Iraqi centers may differ from that of the largely European and East Asian cohorts on which the international literature is based: consanguineous marriage is common in Iraq, with reported rates of 47–60% [22], which may plausibly alter the distribution of genetic causes of spermatogenic failure. The present study was therefore designed to address this gap. We evaluated whether routinely available clinical parameters (age, BMI, duration of infertility) and serum hormonal parameters (FSH, LH, testosterone, prolactin, Inhibin B) are associated with, and can discriminate, successful testicular sperm retrieval in a consecutive series of Iraqi men with NOA undergoing conventional TESE in Najaf.

MATERIALS AND METHODS

Study design and setting

This was a prospective cross-sectional study conducted at Al-Sadr Medical City, in Najaf, Iraq. Patients were recruited consecutively between November 2025 and May 2026. The manuscript was prepared in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [23], and the diagnostic accuracy component in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD) 2015 guidance [24].

Ethical approval and consent

The study protocol was reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine, University of Kufa, Najaf, Iraq (Reference number MEC-98). Initial ethical approval authorizing the commencement of recruitment was

granted in October 2025, and the committee issued its final approval letter on 23 June 2026. All participants were enrolled after initial ethical approval had been obtained, and no data were collected before that date. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from every participant before enrolment, after a full explanation of the study purpose, procedures and the voluntary nature of participation. Participants were informed that refusal would not affect their clinical care.

Participants

Inclusion criteria

Men aged 18 years or older with a confirmed diagnosis of NOA who were scheduled for testicular sperm retrieval and who provided written informed consent.

Exclusion criteria

Obstructive Azoospermia; previous testicular sperm retrieval; active genital tract infection; current or recent (within 6 months) use of exogenous androgens, gonadotropins, anti- estrogens or aromatase inhibitors; previous chemotherapy or scrotal/pelvic radiotherapy; untreated hyperprolactinemia due to a pituitary adenoma; and incomplete clinical or laboratory records.

Diagnosis of Azoospermia and classification as non-obstructive. Azoospermia was diagnosed in accordance with the sixth edition of the WHO laboratory manual for the examination and processing of human semen [3]. Two semen samples were obtained at least four weeks apart after 2–7 days of sexual abstinence, produced by masturbation into a sterile, wide-mouthed container, and examined within one hour of production after liquefaction at 37 °C. When no spermatozoa were seen on initial wet-preparation microscopy, the entire sample was centrifuged at 3000 × g for 15 minutes and the resulting pellet resuspended and examined in its entirety by phase-contrast microscopy at ×400 magnification. Azoospermia was confirmed only when no spermatozoa were identified in the centrifuged pellet of both samples. Classification as non-obstructive rather than obstructive was made on combined clinical, endocrine and operative grounds, consistent with AUA/ASRM guidance [16]: a history compatible with primary spermatogenic failure, reduced testicular consistency or volume on clinical examination, palpable and normal vasa differentia, normal semen volume and pH, and an elevated or high-normal serum FSH. Men in whom the clinical picture indicated obstruction (normal testicular volume with normal FSH, or absent vasa) were excluded.

Clinical assessment

All participants underwent a standardized history and physical examination performed by the same clinical team. Recorded variables were age (completed years), duration of infertility (years of unprotected regular intercourse without conception), previous medical, surgical and drug history, and history of cryptorchidism, orchitis, varicocele, trauma or genital surgery. Body weight was measured to the nearest 0.1 kg using a calibrated digital platform scale with the participant lightly clothed and without shoes, and height to the nearest 0.5 cm using a wall-mounted stadiometer with the head in the Frankfort horizontal plane. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and interpreted using WHO cut-off values (underweight <18.5; normal 18.5–24.9; overweight 25.0–29.9; obese ≥30.0 kg/m^2) [25].

Hormonal assays

A single venous blood sample (5 mL) was drawn from an antecubital vein between 08:00 and 10:00 h following an overnight fast, in order to limit the influence of the diurnal rhythm of testosterone. Samples were collected into plain gel-separator tubes, allowed to clot at room temperature for 20–30 minutes, and centrifuged at 3000 rpm for 10 minutes. Serum was separated, divided into aliquots and stored at −20 °C until analysis; repeated freeze–thaw cycles were avoided. To minimize the effect of pulsatile gonadotropin secretion, all samples were obtained on a single occasion under identical conditions, and all assays for a given Analyte were performed in the same laboratory using a single reagent lot where possible. Serum FSH, LH, total testosterone and prolactin were measured by chemiluminescent immunoassay on an automated analyzer (Mindray CL-900i, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), following the manufacturer's instructions. Results were expressed in mIU/mL for FSH and LH, ng/mL for total testosterone, and ng/mL for prolactin. Serum Inhibin B was measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available human Inhibin B kit, in accordance with the manufacturer's protocol, with results expressed in pg/mL. Samples and standards were assayed in duplicate on the same plate, absorbance was read at 450 nm on a microplate reader, and concentrations were derived from a four-parameter

logistic standard curve. Internal quality-control material at two concentrations was analyzed with each run for every Analyte, and runs were accepted only when control values fell within the manufacturer's stated ranges. Assay 7performance was monitored throughout; intra- and inter-assay coefficients of variation were within the limits specified by the respective manufacturers.

Testicular sperm retrieval

All procedures were performed by the same experienced surgical team. Conventional testicular sperm extraction was carried out under spinal or general anaesthesia. After skin preparation and draping, transverse or median raphe scrotal incision was made and the tunica vaginalis opened to deliver the testis. Multiple small incisions were made in the tunica albuginea at separate sites, avoiding subtunical vessels, and small fragments of seminiferous tubules were excised from each site. Where no spermatozoa were identified on the first side, the contralateral testis was sampled during the same session. Haemostasis was secured and the tunica albuginea, tunica vaginalis, dartos and skin were closed in layers. Each tissue fragment was placed immediately into pre-warmed HEPES-buffered culture medium and transferred to the adjacent andrology laboratory. Specimens were mechanically minced with fine sterile needles and glass slides to disperse the seminiferous tubules and release germ cells, and the resulting suspension was examined under an inverted microscope at ×200 and ×400 magnifications by an experienced embryologist [21, 26]. Definition of the outcome. The outcome was binary. Retrieval was recorded as positive when morphologically identifiable spermatozoa were observed in the processed testicular tissue, figure (1), and as negative when no spermatozoon was identified after systematic examination of the entire specimen from all sampled sites. The embryologist assessing the specimen was not involved in the hormonal analyses, and hormonal results were not used to guide the extent of surgical sampling.

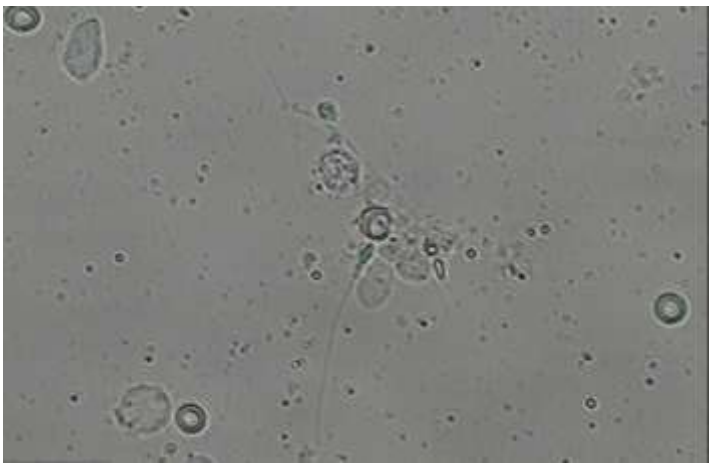

Figure 1: Minced testicular tissue under microscope (power 40X) revealed presence of mature sperms

Sample size

All eligible consecutive patients presenting during the recruitment period were included, giving a final sample of 85 men. No formal a priori sample-size calculation was performed; the sample was determined by the number of eligible patients available within the study window. This is acknowledged as a limitation.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics

A total of 85 patients were included in the study. The mean age was 31.21 ± 7.00 years (range 20–58 years), and the mean body mass index (BMI) was 26.64 ± 4.78 kg/m². The mean duration of infertility was 6.79 ± 4.95 years. The mean serum levels of

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FSH, LH, testosterone, prolactin and Inhibin B were 10.02 ± 7.50 mIU/mL, 6.59 ± 4.62 mIU/mL, 3.70 ± 1.47 ng/mL, 17.16 ± 5.51 ng/mL and 46.44 ± 46.82 pg/mL, respectively, table (1).

Table 1: Baseline clinical and hormonal characteristics of the study participants (n=85)

Variable	Mean ± SD	Range (min-max)
Age (years)	31.21± 7.00	20.00-58.00
BMI (kg/m²)	26.64 ± 4.78	18.00-40.90
Duration of infertility (years)	6.79 ± 4.95	1.00-30.00
FSH (mIU/mL)	10.02 ± 7.50	1.72-35.08
LH (mIU/mL)	6.59 ±4.62	1.30-22.00
Testosterone (ng/mL)	3.70 ± 1.47	1.05-9.20
Prolactin (ng/mL)	17.16 ± 5.51	2.10-37.92
Inhibin B (pg/mL)	46.44 ± 46.82	0.10-255.66

Comparison according to sperm retrieval outcome

Testicular spermatozoa were successfully retrieved in 29 of the 85 men (34.5%). Patients with positive testicular sperm retrieval were significantly older than those with negative retrieval outcomes (33.62 ± 8.39 vs. 29.96 ± 5.85 years, p = 0.042). Serum FSH and LH levels were significantly lower in the positive retrieval group than in the negative retrieval group (FSH: 7.04 ± 4.48 vs. 11.59 ± 8.30 mIU/mL, p = 0.008; LH: 4.67 ± 2.35 vs. 7.61 ± 5.19 mIU/mL, p = 0.037). No statistically significant differences were observed between the two groups regarding BMI, duration of infertility, testosterone, prolactin or Inhibin B levels all p>0.05, table (2).

Table 2: Comparison of demographic and hormonal characteristics according to testicular sperm retrieval outcome

Variable	Positive retrieval (n=29) Mean ± SD	Negative retrieval (n=56) Mean ± SD	p-value
Age (years)	33.62 ± 8.39	29.96 ± 5.85	0.042*
BMI (kg/m²)	26.98 ± 3.99	26.47 ± 5.18	0.232
Duration of infertility (years)	7.76 ± 6.33	6.29 ± 4.03	0.479
FSH (mIU/mL)	7.04 ± 4.48	11.59 ± 8.30	0.008*
LH (mIU/mL)	4.67 ± 2.35	7.61 ± 5.19	0.037*
Testosterone (ng/mL)	3.66 ± 1.23	3.71 ± 1.59	0.959
Prolactin (ng/mL)	17.24 ± 3.77	17.12 ± 6.25	0.550
Inhibin B (pg/mL)	44.27 ±39.58	47.56 ± 50.47	0.760

Univariate logistic regression

Univariate binary logistic regression analysis demonstrated that age, FSH and LH were significantly associated with successful testicular sperm retrieval. Increasing age was associated with higher odds of successful sperm retrieval (OR = 1.08, 95% CI 1.01–1.16, p = 0.029). In contrast, higher FSH (OR = 0.89, 95% CI 0.81–0.98, p = 0.015) and higher LH levels (OR = 0.82, 95% CI 0.71–0.95, p = 0.010) were associated with lower odds of successful sperm retrieval. BMI, duration of infertility, testosterone, prolactin and Inhibin B levels were not significantly associated with the retrieval outcome all p>0.05, table (3).

Table 3: Univariate logistics regression analysis of predictors of successful sperm retrieval

Variable	OR	95% CI	p-value
Age (years)	1.08	1.01-1.16	0.029*
BMI (kg/m^2)	1.02	0.93-1.12	0.635
Duration of infertility (years)	1.06	0.97-1.16	0.202
FSH (mIU/mL)	0.89	0.81-0.98	0.015*
LH (mIU/mL)	0.82	0.71-0.95	0.010*
Testosterone (ng/mL)	0.98	0.72-1.33	0.882
Prolactin (ng/mL)	1.00	0.93-1.09	0.925
Inhibin B (pg/mL)	1.00	0.99-1.01	0.757

Receiver operating characteristic analysis

Receiver operating characteristic (ROC) curve analysis demonstrated that FSH and LH significantly discriminated between successful and unsuccessful testicular sperm retrieval outcomes. FSH showed the highest discriminatory performance, with an area under the curve (AUC) of 0.676 (95% CI 0.559–0.792, p = 0.008), followed by LH with an AUC of 0.639 (95% CI 0.521–0.757, p = 0.037). Age also showed statistically significant discrimination when analyzed in the direction of the study hypothesis (AUC = 0.633, 95% CI 0.500–0.766, p = 0.046), older age being associated with successful sperm retrieval; because the lower limit of this confidence interval reaches 0.500, the finding should be regarded as marginal. BMI, duration of infertility, testosterone, prolactin and Inhibin B did not demonstrate significant discriminatory ability all p>0.05, table (4).

Table 4: Receiver operating characteristic (ROC) analysis of clinical and hormonal predictors of successful testicular sperm retrieval (n=85)

Variable	AUC	95% CI	p-value
FSH (mIU/mL)	0.676	0.559-0.792	0.008*
LH (mIU/mL)	0.639	0.521-0.757	0.037*
Age (years)	0.633	0.500-0.766	0.046*
BMI (kg/m^2)	0.421	0.295-0.546	0.234
Duration of infertility (years)	0.460	0.325-0.595	0.547
Testosterone (ng/mL)	0.497	0.370-0.623	0.959
Prolactin (ng/mL)	0.468	0.344-0.592	0.631
Inhibin B (pg/mL)	0.488	0.359-0.618	0.862

Diagnostic performance of the FSH and LH thresholds

At a cut-off value of ≤ 7.0 mIU/mL, FSH yielded a sensitivity of 65.5%, a specificity of 60.0%, a positive predictive value (PPV) of 46.3%, a negative predictive value (NPV) of 76.7% and an overall accuracy of 61.9%. LH demonstrated a lower discriminatory ability: at a cut-off value of ≤ 3.65 mIU/mL it achieved a sensitivity of 72.4% but a specificity of only 32.7%, with a PPV of 36.2%, an NPV of 69.2% and an overall accuracy of 46.4%; as detailed in Table 5, this LH threshold does not correspond to the point of maximum Youden index on its ROC curve and is therefore reported as an evaluated rather than an optimized threshold. Because 55 of the 84 patients (65.5%) had a negative retrieval outcome, the overall accuracy of both thresholds lies below the 65.5% that would be obtained by classifying every patient as negative; accuracy is therefore an

uninformative summary measure in this imbalanced sample, and the AUC and the negative predictive value provide a more appropriate basis for interpretation, table (5).

Table 5: Diagnostic performance of the FSH and LH cut-off values for predicting successful testicular sperm retrieval (n=85)

Diagnostic measure	FSH ≤ 7.0 mIU/mL	LH ≤ mIU/mL
Sensitivity (%)	65.5	72.4
Specificity (%)	60.0	32.7
Positive predictive value (%)	46.3	36.2
Negative predictive value (%)	76.7	69.2
Overall accuracy (%)	61.9	46.4
Area under the curve	0.676	0.639

DISCUSSION

Principal findings

In this prospective cross-sectional study of 85 Iraqi men with NOA undergoing conventional multi-site testicular sperm extraction, spermatozoa were recovered in 29 men (34.5%). Three of the eight parameters examined were associated with retrieval outcome: men with successful retrieval were older and had significantly lower serum FSH and LH concentrations. BMI, duration of infertility, testosterone, prolactin and Inhibin B showed no association. Critically, although FSH and LH were statistically significant predictors, their discriminative performance was fair at best (AUC 0.676 and 0.639 respectively), and neither threshold classified patients more accurately than simply assuming that all retrievals would fail. The clinically important message of this study is therefore a cautious one: these hormones carry some prognostic information, but not enough to determine surgical eligibility.

Sperm retrieval rate in context

Our SRR of 34.5% corresponds closely to published expectations for conventional TESE. Bernie and colleagues, pooling 15 studies and 1890 patients, reported a mean SRR of 35% (95% CI 30–40%) for conventional TESE, compared with a 1.5-fold higher likelihood of success with micro-TESE [6]. Deruyver et al. similarly reported conventional TESE rates of 16.7–45% against 42.9–63% for micro-TESE [29], and Corona and colleagues reported 37% versus 57% in directly comparative studies [4]. The 2024 metaanalysis by Pozzi et al., confined to micro-TESE, found a mean positive retrieval rate of 45% [7]. Our result therefore sits squarely within the expected range for the technique used, and the difference from micro- TESE series should be interpreted as a technique effect rather than as evidence of a less favorable case-mix. An important local observation supports this interpretation. Allebawi and Al-Janabi reviewed 180 testicular biopsies from Azoospermic men in a Najaf histopathology center over 2020–2022 and found that germ-cell maturation arrest was the commonest pattern (84 cases, 46.67%), followed by germ-cell aplasia/Sertoli-cell only syndrome (54 cases, 30%) [20]. Since maturation arrest and Sertoli-cell-only syndrome are precisely the histological categories associated with the lowest retrieval rates, a pooled SRR in the mid-thirties is what one would predict for a Najaf population undergoing conventional biopsy. This concordance between an independent local histopathological series and our surgical outcome lends coherence to our finding, although we did not perform histopathology ourselves and so cannot demonstrate the link directly.

FSH and LH: statistically significant but only fairly discriminative

Lower FSH and LH in men with successful retrieval is consistent with the underlying endocrinology. FSH acts on Sertoli cells, which in turn secrete Inhibin B; Inhibin B exerts selective negative feedback on pituitary FSH secretion [8-9]. When the seminiferous epithelium is depleted, Inhibin B output falls and FSH rises, so that a high FSH is an indirect marker of germ-cell loss. LH stimulates Leydig-cell testosterone production, and rising LH in the presence of normal or low testosterone indicates compensated Leydig- cell dysfunction, which frequently accompanies advanced tubular damage [9]. Lower gonadotropin

concentrations may therefore mark comparatively preserved testicular architecture and a greater probability of residual focal spermatogenesis. Our effect sizes are consistent with this literature. In univariate regression, each 1 mIU/mL rise in FSH reduced the odds of retrieval by approximately 11% (OR 0.89, 95% CI 0.81–0.98) and each 1 mIU/mL rise in LH by approximately 18% (OR 0.82, 95% CI 0.71–0.95). Le et al., in a Vietnamese cohort of 136 men undergoing conventional multiple TESE — the closest methodological comparator to our study — reported an SRR of 18.4% and found that endocrine tests and testicular volume were not predictive, only testicular density reaching significance [12]. Al-Zubi et al. examined 152 Jordanian men undergoing micro-TESE and likewise found the pre-operative hormonal profile to be of limited predictive value [13]. Conversely, Esteves and colleagues, studying 616 hypogonadal men with NOA, found that the optimal FSH threshold of 9.8 IU/L achieved an AUC of only 0.64, explicitly attributing the poor accuracy to focal spermatogenesis persisting at high FSH [15]. Our FSH AUC of 0.676 lies within the range of these published estimates and, importantly, its 95% CI (0.559–0.792) is wide. The threshold we evaluated (FSH ≤ 7.0 mIU/mL) achieved a sensitivity of 65.5% and specificity of 60.0% — a Youden index of only 0.255. The corresponding LH threshold performed considerably worse, with a specificity of just 32.7% and a Youden index of 0.051; as detailed in the Results, this LH value does not represent the point of maximum Youden index and should be regarded as an evaluated rather than an optimized threshold. Two features of our diagnostic accuracy data deserve emphasis because they temper any enthusiasm for clinical application. First, because 65.5% of our cohort had a negative retrieval, a clinician who predicted failure in every patient would achieve 65.5% accuracy; neither the FSH threshold (61.9%) nor the LH threshold (46.4%) exceeded this no-information rate. Overall accuracy is thus an actively misleading summary statistic in this imbalanced setting, and we present it only for completeness. Second, the asymmetry between the PPV (46.3%) and NPV (76.7%) of the FSH threshold indicates that FSH ≤ 7.0 mIU/mL is more useful for identifying men unlikely to succeed than for confirming those who will. Even this interpretation must be qualified, since predictive values depend on the 34.5% retrieval rate observed here and are not transferable to populations with a different underlying prevalence. Taken together, these observations align with the position of the EAU and AUA/ASRM guidelines, which recommend hormonal evaluation for the diagnostic classification of Azoospermia but do not sanction the use of hormonal thresholds to withhold a retrieval attempt [2, 16]. Our data support that position rather than challenging it.

The unexpected association with older age

The finding that men with successful retrieval were significantly older (33.62 vs. 29.96 years; OR 1.08 per year, 95% CI 1.01–1.16) runs counter to the intuition that reproductive potential declines with age, and we treat it with corresponding caution. Notably, the same pattern has been reported from the region. Aljubran et al., studying 108 Saudi men with NOA, found that men with successful retrieval were significantly older (40.04 ± 12.22 vs. 33.98 ± 6.18 years, $p = 0.008$) and had significantly lower FSH (7.97 ± 7.11 vs. 18.55 ± 13 mIU/mL, $p < 0.004$) and LH (5.9 ± 4.4 vs. 11.4 ± 7.45 mIU/mL, $p < 0.001$), with age and FSH emerging as independent predictors [10]. The correspondence between that cohort and ours — in the direction of all three significant variables — suggests the observation is unlikely to be a chance artefact of our sample alone. Several non-biological explanations are more plausible than a true protective effect of age. Selection is the most likely: men with severe, early-onset spermatogenic failure (for example Klinefelter syndrome or complete AZF deletions) tend to present younger, following earlier diagnosis, whereas men with milder hypospermatogenesis may attempt natural conception for longer before seeking surgical retrieval. Older men in a fertility clinic may therefore be enriched for phenotypes with residual focal spermatogenesis. Consistent with this, our positive-retrieval group also had a longer, though not significantly different, duration of infertility. A second possibility is referral and treatment-seeking behavior, since in a self-funded system the decision to proceed to surgery may follow years of accumulated resources and prior investigation. Finally, the association was statistically marginal: the lower bound of the age AUC confidence interval reached exactly 0.500, and the OR confidence interval (1.01–1.16) only narrowly excluded unity. We therefore regard the age finding as hypothesis-generating. It should not be used to counsel older men that their prospects are better, and it requires replication in larger cohorts stratified by aetiology and testicular histology before any biological interpretation is entertained.

Inhibin B, testosterone and prolactin

Inhibin B did not differ between groups (44.27 ± 39.58 vs. 47.56 ± 50.47 pg/mL, $p = 0.760$) and showed no discriminative ability (AUC 0.488). Given Inhibin B's role as a direct product of Sertoli cells, this negative result may appear surprising, but it is well supported by the international literature. Toulis et al., in a metaanalysis of diagnostic accuracy studies, found pooled

sensitivity of 0.65 and specificity of 0.83 for serum Inhibin B and concluded that it could not serve as a stand-alone marker of persistent spermatogenesis [14]. Tunc et al. found no significant difference in Inhibin B, FSH or testicular volume between successful and unsuccessful retrievals in 52 men with NOA [30], and Mitchell et al., studying 139 men, found Inhibin B non-predictive and its combination with FSH no better [31]. Most decisively, the 2024 meta-analysis of 34 studies by Pozzi et al. found that pooled Inhibin B did not significantly predict positive retrieval (OR 1.01, 95% CI 1.00–1.02), whereas anti-Müllerian hormone did (OR 0.82, 95% CI 0.73–0.92) [7]. Two further considerations apply to our data specifically. The variability of Inhibin B in our cohort was extreme (mean 46.44 pg/mL, SD 46.82, range 0.10–225.66), giving a coefficient of variation exceeding 100%. Such dispersion substantially reduces the power to detect a between-group difference. In addition, Inhibin B is measured by manual ELISA rather than on standardized automated platforms, and results are not interchangeable between kits; assay-related variability may therefore have contributed to the null finding. Our result should thus be read as a failure to demonstrate an association in this sample, not as evidence that Inhibin B is biologically uninformative. Testosterone ($p = 0.959$) and prolactin ($p = 0.550$) likewise showed no association. For prolactin this is expected, since hyperprolactinemia is an uncommon and generally correctable cause of male infertility rather than a determinant of focal spermatogenesis; men with untreated prolactinoma were in any case excluded. For testosterone, the absence of an association is consistent with the observation that intratesticular rather than serum androgen concentration governs spermatogenesis, and serum testosterone is a poor surrogate for the intratesticular milieu [9]. BMI showed no association with retrieval ($p = 0.232$). Although obesity influences the male reproductive hormonal profile, our cohort had a mean BMI of 26.64 kg/m², in the overweight rather than obese range, with limited representation at the extremes; the study was not powered to detect an effect of severe obesity.

Comparison with Iraqi studies

Iraqi data on this question remain sparse, and the present study adds to a small literature. The closest Iraqi comparator is that of Kadhem and Mossa, who studied 65 Azoospermic men undergoing open testicular sperm extraction at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, between February 2017 and April 2019, evaluating FSH, LH and testosterone as predictive markers of sperm yield [21]. Their reported retrieval rates stratified by histology — 100% for normal spermatogenesis, 75% for hypospermatogenesis, 44.4% for maturation arrest and 28.6% for Sertoli-cell-only syndrome — illustrate the point that emerges repeatedly from our own data: histology discriminates far more sharply than any hormone. Our pooled SRR of 34.5% is consistent with a case-mix dominated by the two least favorable histological categories, which is exactly what the Najaf histopathological series of Allebawi and Al-Janabi describes [20]. Genetic studies from Iraq provide further context for the aetiology of NOA in this population. Al-Ouqaili et al. examined 75 infertile Iraqi men in Anbar and reported AZFa sub-region microdeletions in 61.3% by real-time PCR, although only one Azoospermic patient (2.2%) showed a complete AZFa deletion on confirmatory conventional PCR [17]. Abdulwahid and colleagues screened 296 infertile Kurdish men in Erbil and found chromosomal abnormalities in 29 (9.8%), of which Klinefelter syndrome accounted for 20 (69%), together with Y-chromosome microdeletions in 10 of 289 Azoospermic men (3.5%) [18]. A recent scoping review of Arab studies reported an overall Y-chromosome microdeletion frequency of 15.38% among Azoospermic men in the Fertile Crescent, with Iraqi figures spanning a very wide range from 3.5% to 52.83% [19]. Ali et al. examined FSH receptor gene polymorphisms in Iraqi men with NOA, reflecting local interest in the genetic determinants of the FSH axis [32]. This heterogeneity is itself informative: it indicates that the genetic composition of Iraqi NOA cohorts is not well characterized and may vary considerably between centers, which is a further reason to be cautious about generalizing hormonal cut-offs. Since complete AZFa and AZFb deletions predict virtually zero retrieval whereas AZFc deletions are compatible with success in a substantial proportion of men [2, 11], an unmeasured genetic case-mix could materially influence the apparent performance of any hormonal predictor.

Finally, methodological work from Iraq is directly relevant to our surgical protocol. Al-Tameemi and colleagues evaluated repeated sperm extraction using enzymatic and mechanical mincing techniques in Azoospermic patients, addressing precisely the laboratory step by which testicular tissue is processed and examined [26]. The consistency of this processing step across centers is an under-appreciated source of variation in reported SRRs.

Clinical implications

Three implications follow, stated conservatively. First, FSH and LH should be used to inform counselling rather than to determine eligibility. A man with an FSH of 7 mIU/mL may reasonably be told that his prospects are somewhat better than

average; a man with an FSH of 25 mIU/mL should not be told that retrieval is futile, because our data — and the wider literature — show that such men do sometimes have sperm retrieved [5,15]. Neither threshold we examined performed better than the no-information rate, which is a direct argument against using them as decision rules. Second, the fair discrimination of the routine hormonal panel strengthens the case for parameters that were not available to us. Testicular histopathology, testicular volume, karyotype and Y-chromosome microdeletion analysis all carry greater prognostic weight than serum gonadotropins [2, 16, 20-21], and anti-Müllerian hormone has recently outperformed both FSH and Inhibin B in meta-analysis [7]. Where resources permit, incorporating these into the pre-operative assessment is likely to be more productive than seeking better cut-offs for FSH. Third, our SRR of 34.5% with conventional TESE, set against pooled micro-TESE rates of 45–57% [4,6,7], supports the case for developing micro-TESE capability in Iraqi centers, in line with the AUA/ASRM recommendation that micro-TESE should be performed for men with NOA undergoing retrieval [16]. We note this as a service-development argument supported by external evidence, not as a conclusion demonstrated by our own data, since we did not compare techniques.

CONCLUSIONS

FSH and LH were associated with retrieval outcome but discriminated only fairly. They should inform counselling, not exclude men from surgery.

Competing interests

The authors declare that they have no competing interests.

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