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Original Article

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Morphometric Analysis of Human Testes in Relation to Spermatogenic Failure: A Cadaveric Study

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ABSTRACT

Background: Spermatogenic failure, a major histological correlate of male infertility, is conventionally graded using the Johnsen scoring system. Whether gross testicular morphometry reliably reflects histological spermatogenic status remains unclear when assessed by direct cadaveric measurement. This study correlated gross and histomorphometric testicular parameters with spermatogenic status in human cadaveric testes.

Methods: This descriptive cross-sectional study examined 100 formalin-fixed adult human testes (50 right, 50 left). Gross length, width, thickness, weight and testicular volume (ellipsoid formula) were recorded, followed by histological processing and Johnsen scoring (1–10) of seminiferous tubules, measurement of tubule diameter and germinal epithelium thickness, and qualitative assessment of interstitial fibrosis and Leydig cell hyperplasia. Specimens with Johnsen score ≤6 were categorised as showing spermatogenic failure. Data were analysed using the Mann–Whitney U, Kruskal–Wallis and Chi-square tests and Spearman's rank correlation.

Results: Spermatogenic failure was present in 61/100 (61.0%) specimens. Germinal epithelium thickness (32.23±6.93 vs 62.73±10.48 µm) and tubule diameter (194.18±39.52 vs 250.83±36.57 µm) were markedly reduced in the failure group (both $p<0.001$) and correlated strongly with Johnsen score ($\rho=0.692$ and 0.540). Gross length, width, thickness and weight did not differ significantly between groups (all $p>0.15$); testicular volume showed only a weak, borderline association ($p=0.045$). Interstitial fibrosis was strongly associated with spermatogenic failure ($p<0.001$); Leydig cell hyperplasia was not ($p=0.715$).

Conclusion: Histomorphometric parameters of the seminiferous tubule, rather than gross testicular dimensions, are the principal correlates of spermatogenic status in cadaveric testes, reaffirming the limited standalone value of gross testicular size in predicting spermatogenic competence.

Key-words: Heliothis armigera, Argemone mexicana, Ethanol, acetone, Epithelial lining, Epithelial cells, vacuoles, Gut lining, Gut wall

INTRODUCTION

Male infertility is a substantial and growing global health burden. Estimates from the Global Burden of Disease framework place the worldwide prevalence of male infertility at over 56 million cases, representing a rise of nearly 77% since 1990 [1].

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Despite this substantial burden, male-factor contributions to couple infertility continue to receive comparatively little clinical and research attention relative to female infertility.

The seminiferous tubules, which constitute the bulk of the testicular parenchyma, are the functional site of spermatogenesis, and their histological integrity is the single most direct determinant of male fertility potential [2]. Since 1970, the semi-quantitative testicular biopsy score count described by Johnsen has remained the reference method for grading spermatogenesis on a 1–10 scale, ranging from complete absence of germ cells (score 1) to full, qualitatively normal spermatogenesis

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with abundant mature spermatozoa (score 10) [3]. The score continues to guide clinical decision-making in andrology and has recently been the subject of automated, artificial-intelligence-assisted scoring efforts, underscoring its continued relevance as the histological benchmark against which other morphometric indices are compared [4].

Large retrospective series of testicular biopsies performed for the clinical work-up of infertility have described the relative frequencies of hypospermatogenesis, germ cell maturation arrest and the Sertoli-cell-only pattern [5-7], but these cohorts are drawn exclusively from men already presenting with infertility, and biopsy specimens are small and non-representative of the testis as a whole. Cadaveric dissection, by contrast, allows the entire testis to be examined and gross morphometric parameters to be measured directly, without the confounding of antemortem ultrasonographic estimation or orchidometric approximation, both of which have shown only moderate agreement with true testicular volume [8].

Whether gross testicular size — as opposed to histomorphometric indices such as seminiferous tubule diameter and germinal epithelium thickness — reliably reflects the underlying spermatogenic status has not been extensively examined in a general (non-infertility-clinic) cadaveric population using direct measurement of both parameters in the same specimens. The present study was therefore undertaken to characterise the gross and histomorphometric testicular parameters of 100 human cadaveric testes, to grade spermatogenesis using the Johnsen scoring system, and to determine which parameters, if any, correlate with and discriminate spermatogenic failure.

MATERIALS AND METHODS

Place of the study- This study was conducted in the Department of Anatomy, Shri Balaji Institute of Medical Science, Raipur, Chhattisgarh, India, over a period of eight weeks.

Research design- This was a descriptive cross-sectional cadaveric study. Cadavers were sourced through the institution's routine body-donation and unclaimed-body dissection programme; no cadaver was procured or examined for the specific purpose of this study outside the department's existing ethically approved dissection protocol. One hundred formalin-fixed adult human testes (50 right-sided, 50 left-sided, from separate cadavers) were studied. Age at death and probable cause of death were recorded from available cadaver records and grouped into five decadal bands (20–30, 31–40, 41–50, 51–60 and 61–70 years).

Inclusion criteria

- Adult human testes obtained through the institution's body-donation and unclaimed-body dissection programme, with grossly unremarkable external morphology.

Exclusion criteria

- Testes showing gross traumatic disruption.
- Prior orchidectomy.
- Testicular tumour.
- Advanced autolysis precluding reliable measurement.

Gross morphometry- Testicular length, width and thickness were measured using a digital vernier caliper (precision 0.01 mm), and testicular weight was recorded on an electronic balance (precision 0.01 g). Testicular volume was calculated using the ellipsoid formula (volume = 0.71 × length × width × thickness), which has been validated against true water-displacement volume in surgically excised human testes [8] and has also been applied in cadaveric testicular morphometric studies [10].

Histological processing and Johnsen scoring- A mid-transverse block from each testis was fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin. For each specimen, a minimum of ten round-to-oval seminiferous tubule cross-sections were examined under a light microscope fitted with a calibrated ocular micrometer. Seminiferous tubule diameter (mean of two orthogonal diameters per tubule) and germinal epithelium thickness were measured and averaged per specimen [2]. Each tubule was assigned a Johnsen score (1–10) according to the original Johnsen criteria [3], and the mean score across evaluated tubules was taken as the specimen's Johnsen score; scoring was performed with the observer blinded to the gross morphometric data. A schematic representation of the Johnsen scoring concept used to guide grading in this study is shown in Fig. 3.

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This is an illustrative diagram constructed by the authors and does not depict an actual photomicrograph of any specimen.

Specimens with a mean Johnsen score of ≤6 were categorised as showing spermatogenic failure, and those with a score of ≥7 as showing preserved spermatogenesis, consistent with thresholds used in prior histopathological classification of testicular biopsies to separate clinically preserved spermatogenesis from significant impairment [3,5]. Each specimen was additionally assessed qualitatively for the presence or absence of interstitial fibrosis and Leydig cell hyperplasia.

Statistical Analysis- Data were analysed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. As most continuous variables were non-normally distributed, data are presented as mean ± standard deviation (range), and non-parametric tests were applied throughout. The Mann–Whitney U test was used for two-group comparisons, the Kruskal–Wallis H test for comparisons across age groups, Pearson's Chi-square test for categorical variables, and Spearman's rank correlation for associations between continuous variables. A two-tailed p-value <0.05 was considered statistically significant.

Ethical approval- Prior approval was obtained from the Institutional Ethics Committee (IEC Approval No. SBIMS/IEC/Certi./189/2026, dated 05.07.2026) and the study was conducted in accordance with the National Ethical Guidelines for Biomedical and Health Research Involving Human Participants.[9]

RESULTS

Of the 100 cadaveric testicular specimens examined (50 right, 50 left), the age-group and cause-of-death distribution is summarised in Table 1.

Table 1: Demographic and specimen characteristics (n=100)

Characteristic	Category	n (%)
Age group (years)	20–30	21 (21)
	31–40	16 (16)
	41–50	19 (19)
	51–60	19 (19)
	61–70	25 (25)
Side	Right	50 (50)
	Left	50 (50)
Probable cause of death	Cardiac death	27 (27)
	Road traffic accident	25 (25)
	Poisoning	23 (23)
	Unknown	25 (25)

Overall descriptive gross and histomorphometric parameters are presented in Table 2. The mean Johnsen score for the series was 5.85 ± 2.61 (range 2–10); its distribution is illustrated in Fig. 1, which shows a bimodal-tending spread with a substantial cluster of specimens at the lower end of the scale. Applying the pre-specified threshold of Johnsen score ≤6, spermatogenic failure was identified in 61/100 specimens (61%), while 39/100 (39%) showed preserved spermatogenesis. Interstitial fibrosis was present in 25/100 specimens (25%) and Leydig cell hyperplasia in 42/100 specimens (42%).

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Table 2: Descriptive gross and histomorphometric parameters (n=100)

Parameter (n=100)	Mean ± SD	Range (min–max)
Testicular length (cm)	4.33 ± 0.51	3.4 – 5.3
Testicular width (cm)	2.70 ± 0.43	2.0 – 3.5
Testicular thickness (cm)	2.41 ± 0.33	1.8 – 3.0
Testicular weight (g)	16.97 ± 4.11	10.7 – 24.2
Testicular volume (mL)*	19.91 ± 4.56	12.5 – 32.6
Seminiferous tubule diameter (µm)	216.28 ± 47.23	122.4 – 316.1
Germinal epithelium thickness (µm)	44.13 ± 17.17	20.4 – 79.8
Johnsen score	5.85 ± 2.61	2 – 10

**Testicular volume was calculated from each specimen's own measured length, width and thickness using the validated ellipsoid formula 0.71×length×width×thickness.*

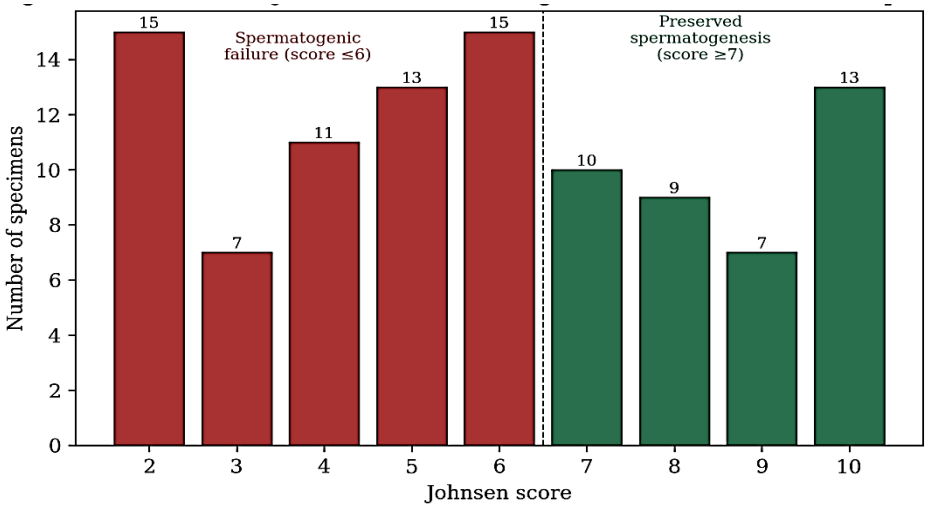

Fig. 1: Distribution of Johnsen scores among 100 cadaveric testicular specimens

Gross testicular length, width, thickness and weight did not differ significantly between the spermatogenic-failure and preserved-spermatogenesis groups (all p>0.15, Table 3). Testicular volume showed a small difference that reached only borderline statistical significance (20.50 ± 4.42 vs 18.97 ± 4.67 mL; U=1474.0, p=0.045), with the failure group showing marginally *higher*, not lower, mean volume — a finding discussed further below. In contrast, seminiferous tubule diameter and germinal epithelium thickness were both markedly and highly significantly reduced in the spermatogenic-failure group (Table 3).

Table 3: Comparison of gross and histomorphometric parameters by spermatogenic status (Mann–Whitney U test, two-tailed)

Parameter	Spermatogenic failure (n=61)	Preserved spermatogenesis (n=39)	U	p-value
Length (cm)	4.32 ± 0.52	4.34 ± 0.50	1188	0.994
Width (cm)	2.75 ± 0.45	2.63 ± 0.41	1380.5	0.178
Thickness (cm)	2.45 ± 0.31	2.35 ± 0.36	1390.5	0.156

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Weight (g)	16.66 ± 4.17	17.45 ± 4.02	1048.0	0.319
Volume (mL)*	20.50 ± 4.42	18.97 ± 4.67	1474.0	0.045
Tubule diameter (µm)	194.18 ± 39.52	250.83 ± 36.57	374.0	8.43×10^{-9}
Epithelium thickness (µm)	32.23 ± 6.93	62.73 ± 10.48	0.0	4.35×10^{-17}

Spearman correlation of each continuous parameter with Johnsen score is shown in Table 4. Germinal epithelium thickness showed the strongest correlation with Johnsen score (ρ=0.692, $p=1.58\times10^{-15}$), followed by seminiferous tubule diameter (ρ=0.540, $p=6.47\times10^{-9}$). Gross linear dimensions showed negligible-to-weak correlation with Johnsen score, and testicular volume showed a weak *negative* correlation (ρ=−0.198, p=0.048).

Table 4: Spearman correlation of morphometric parameters with Johnsen score (n=100)

Parameter	Spearman ρ	p-value
Length (cm)	0.007	0.946
Width (cm)	−0.118	0.244
Thickness (cm)	−0.182	0.070
Weight (g)	0.071	0.485
Volume (mL)*	−0.198	0.048
Tubule diameter (µm)	0.540	6.47×10^{-9}
Epithelium thickness (µm)	0.692	1.58×10^{-15}

Interstitial fibrosis was strongly associated with spermatogenic failure: all 25 specimens showing fibrosis fell within the failure group, and none within the preserved-spermatogenesis group ($\chi^2=19.18$, df=1, $p=1.19\times10^{-5}$). Leydig cell hyperplasia showed no significant association with spermatogenic status (42.0% vs 38.5% prevalence in the failure and preserved groups respectively; $\chi^2=0.134$, df=1, p=0.715). Neither side of the body ($\chi^2=0.000$, df=1, p=1.000) nor probable cause of death ($\chi^2=0.754$, df=3, p=0.861) was significantly associated with spermatogenic failure.

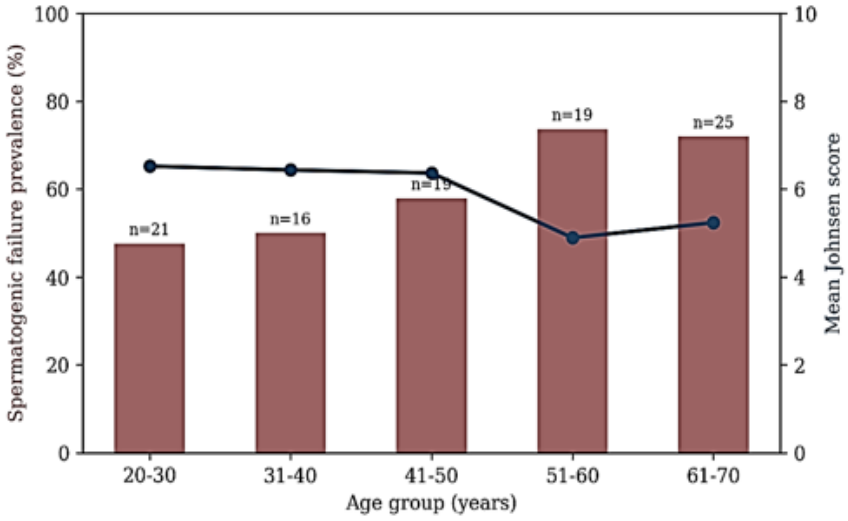

Fig. 2: Age-group distribution of spermatogenic failure and mean Johnsen score (Kruskal–Wallis p=0.150)

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Spermatogenic failure prevalence increased numerically across successive age groups, from 47.6% in the 20–30 year group to 73.7% in the 51–60 year group and 72.0% in the 61–70 year group (Fig. 2); however, this trend did not reach statistical significance for either Johnsen score (Kruskal–Wallis H=6.738, df=4, p=0.150) or testicular volume (H=6.029, df=4, p=0.197) across the five age groups, likely reflecting the limited size of individual age subgroups (n=16–25 per group).

Schematic illustration of the Johnsen scoring system showing representative histological grades of spermatogenesis (scores 2, 4, 6, 8, and 10), from Sertoli cell-only tubules to complete spermatogenesis with abundant spermatozoa (Fig. 3).

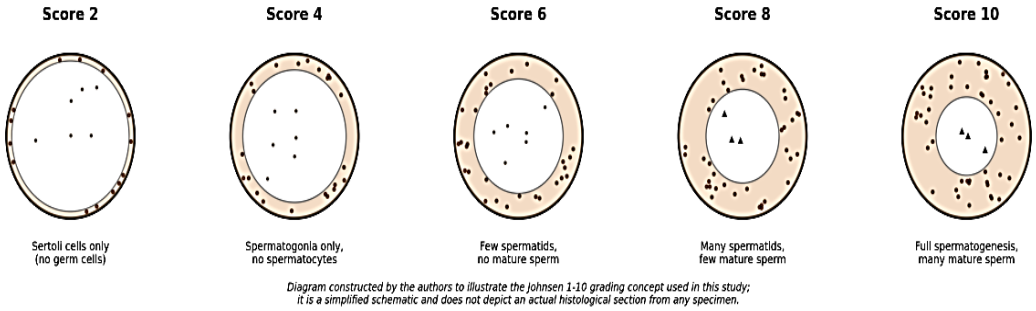

Fig. 3: Schematic representation of the Johnsen scoring criteria used to guide grading in this study (illustrative diagram, not a photomicrograph)

DISCUSSION

This cadaveric morphometric study of 100 human testes found that histomorphometric parameters of the seminiferous tubule — germinal epithelium thickness and tubule diameter — were strongly and highly significantly associated with spermatogenic status, whereas gross testicular dimensions (length, width, thickness, weight) showed no meaningful association, and testicular volume showed only a weak, borderline effect. This pattern is biologically coherent: germinal epithelium thickness and tubule diameter are direct histological correlates of the cellularity of the seminiferous epithelium that the Johnsen score itself grades [2,3], so a strong relationship between these parameters and Johnsen score is an internally consistent finding rather than an independent validation of a separate predictive marker.

The overall prevalence of spermatogenic failure in this general cadaveric series (61.0%) is higher than the pattern typically reported in men undergoing testicular biopsy specifically for the work-up of infertility, where normal or near-normal spermatogenesis is often reported in only a minority and Sertoli-cell-only or maturation-arrest patterns predominate [5,6]. This difference likely reflects the different sampling frame. Infertility-clinic biopsy series are drawn from a population already selected for suspected testicular pathology. In contrast, the present cadaveric series represents a broader age range of the general population, in which age-related involutional changes are expected to contribute increasingly to impaired spermatogenesis [11,12].

The absence of a meaningful relationship between gross testicular dimensions and spermatogenic status contrasts with the clinical literature on testicular volume, in which ultrasonographic or orchidometric volume is widely used as a proxy for spermatogenic function [8]. Several factors specific to cadaveric material may explain this divergence. First, postmortem interval and fixation can alter testicular turgor and dimensions independently of antemortem histological status. Second, gross volume reflects the combined contribution of seminiferous tubules, interstitium and tunica, and in an involuting or fibrotic testis, interstitial expansion or peritubular fibrosis may partially offset the volume loss that would otherwise accompany germinal epithelial atrophy [11,12], attenuating any net volume–function relationship. The paradoxical direction of the borderline volume association observed here (marginally higher, not lower, volume in the failure group) is consistent with this interpretation and should be regarded as a limited, hypothesis-generating observation rather than a robust finding, given its borderline significance (p=0.045) and the small effect size (ρ=−0.198).

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Interstitial fibrosis was strongly and specifically associated with spermatogenic failure in this series, with every fibrotic specimen falling in the failure group. This finding is consistent with descriptions of testicular involution in ageing men, in which progressive thickening and eventual sclerosis of the tunica propria accompanies germ cell loss and is regarded as a hallmark of the ageing, involuting testis [11,12]. By contrast, Leydig cell hyperplasia showed no significant association with spermatogenic status in this series. Leydig cell hyperplasia is most classically described as a compensatory or paraneoplastic phenomenon in specific contexts — adjacent to germ cell tumours, in Klinefelter syndrome, or in response to focal gonadotropin excess — rather than as a generalised accompaniment of spermatogenic failure of mixed aetiology [13], which may explain why no association was demonstrable in this heterogeneous, general cadaveric sample.

Spermatogenic failure prevalence rose numerically with advancing age, from under half of specimens in the third decade to nearly three-quarters in the sixth and seventh decades, in keeping with the well-described mosaic pattern of testicular involution described in ageing men, ranging from tubules with preserved though reduced spermatogenesis to fully sclerosed tubules [11,12]. That this trend did not reach conventional statistical significance in the present series most plausibly reflects the modest size of individual age subgroups (n=16–25) rather than an absence of a true age effect, and larger multi-decade cadaveric series would be needed to confirm this relationship formally.

This study has several limitations. It is a single-centre, cross-sectional cadaveric study without antemortem semen analysis [14], hormonal profiling or documented fertility history, so "spermatogenic failure" here reflects a purely histological classification and cannot be directly equated with clinical infertility. Postmortem autolysis and fixation, though minimised by standard protocol, may have influenced gross dimensions to a greater extent than histomorphometric parameters measured on processed tissue. The heterogeneous causes of death, while showing no significant association with spermatogenic status in this series, could still carry unmeasured confounding related to chronic illness or systemic hormonal disturbance preceding death. Strengths of the study include the relatively large number of directly measured whole cadaveric specimens (n=100), paired gross and histomorphometric assessment of each specimen, and blinded histological scoring.

CONCLUSIONS

In this cadaveric series, germinal epithelium thickness and seminiferous tubule diameter, but not gross testicular length, width, thickness or weight, correlated strongly with Johnsen-graded spermatogenic status, and testicular volume showed only a weak, borderline relationship. Interstitial fibrosis, but not Leydig cell hyperplasia, was strongly associated with spermatogenic failure. These findings reaffirm that gross testicular size alone is an unreliable surrogate for the histological status of spermatogenesis, and that direct histomorphometric assessment remains necessary where the spermatogenic status of testicular tissue is in question.

Future prospects and implications: Larger multi-centric cadaveric studies incorporating antemortem hormonal and semen data, where available, would help clarify the clinical translatability of these histomorphometric relationships. Integration of automated image-analysis and machine-learning-assisted Johnsen scoring may further standardise histomorphometric assessment and reduce inter-observer variability in future cadaveric and clinical andrology research.

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