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# Clinical Role of Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone as Predictive Markers of Sperm Yield by Testicular Biopsy in Azoospermic Patients

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## Abstract

The study aim is to evaluate the predictive rate of positive sperm retrieval of serum concentration of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (TT) in azoospermic patients presenting to High Institute of infertility diagnosis and assisted reproductive technologies. The study records of 65 azoospermic men with who underwent open testicular sperm extraction between February 2017 to April 2019 were studied. The patient's age, hormonal profile for FSH, LH and TT and testicular histopathology were recorded. The sperm retrieval rate according to histological findings norm-spermatogenesis (NS), hypo-spermatogenesis (HS), maturation arrest (MA) and Sertoli cell-only syndrome (SCOS) were recorded as follows: 100%, 75%, 44.4%, and 28.6% respectively. The cutoff value of FSH was (≤ 14.56 mIU/L) with an accuracy level of 73.0%, a sensitivity (66.7 %) and a specificity (79.3 %). The cutoff value of the serum level of LH was (≤ 9.37 mIU/L) with an accuracy level of 78.4%, a sensitivity level of 77.7 % and a specificity level of 72.4 %.

**Keywords:** Testicular biopsy; Histopathology; Reproductive hormones; Sperm retrieval.

## 1. Introduction

Azoospermia is the second major etiology of infertility in Azoospermic men after varicocele. It is defined as no sperm found in the seminal fluid sample and it was identified approximately as 1% of males and about 10-15% of infertile men (Jarow, et al [1]). Azoospermia is divided into obstructive azoospermia (OA) due to obstruction in the seminal fluid outflow or non-obstructive azoospermia (NOA) due to inadequate production of spermatozoa) (Jarvi, et al [2]). The spermatogenesis is a process controlled by several factors (endocrine, paracrine, and autocrine). Hormonal effect on spermatogenesis is under control of kisspeptin, gonadotropin-releasing hormone, the gonadotropins, and the testicular steroidogenic path (White [3]). The understanding of the FSH, LH and TT physiological effect is essential for the evaluation of patients complaining of abnormalities of spermatogenesis (Matsumoto, et al [4] Krausz [5]). The principal action of FSH in man is spermatogenesis stimulation. The FSH performing its physiological action by binding to FSH receptor present in the Sertoli cells of the testis. The FSH triggers many transcription factors that are participated in germ cell multiplication, given metabolic assistance to germ cells, aid transferring of glucose (converted to lactate to give energy), and permits the transfer in excretion which is very important for supporting spermatogenesis (Nieschlag, et al [6]). The principal action of LH in men is acting upon the Leydig cells of testis to synthesis testosterone which is essential hormone in the man

(White [3]). In Leydig cells, synthesis and secretion of specific proteins which regulate steroid synthesis and testosterone production. There is no receptor of LH on the Sertoli cells and germ, so, spermatogenesis is indirectly controlled by LH throughout raising testosterone concentration inside the testes (Ismael, et al [7]). Males with inactivating mutations of LH-β gene or LH receptor lead to defect of pubertal development, arrested spermatogenesis or azoospermia, and infertility. These give an explanation of the central function of intra-testicular testosterone levels within normal spermatogenesis (Matsumoto, et al [4]). So both FSH, as well as LH, were supporting spermatogenesis, in addition, they act in different ways depending on spermatogenesis stage, but, FSH is mainly acted upon the sperm maturation, early meiosis as well as transformation of spermatogonia to pachytene spermatocytes. Whereas, LH effect mainly on finishing of meiosis and spermiation. So that LH and FSH were having the main action on spermiogenesis (White [3]). The concentration of intra-testicular testosterone (near the basement membrane of the seminiferous tubules) is too higher than its serum level, this displays its essential function in spermatogenesis (Kerr, et al [8]). Hence, the predictive value of these hormones for detecting sperm of testicular sperm extraction in azoospermia treatment is an important aspect of assisted reproduction (intra-cytoplasmic sperm injection).

## 2. Materials and Methods

The case-control study conducted on 65 azoospermia males in the period from February 2017 to April 2019 at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University in Baghdad, Iraq. All the 65 patients were undergone clinical examination and laboratory investigation such as hormonal, seminal fluid analysis as well as testicular biopsy with histopathological diagnosis. A blood sample was taken for all patients for assessment serum levels of LH, FSH, and testosterone. Written informed consent was obtained from all patients.

### *2.1 Statistical Analysis*

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. The following statistical tests were used: Chi-square test was used to evaluate the association between any two categorical variables provided that less than 20% of cells have an expected count of less than 5. However, Fischer exact test was used instead when the chi-square test was not valid (in case that more than 20% of cells have an expected count of less than 5). Independent samples t-test was used to evaluate the difference in mean of numeric variables between any two groups provided that these variables were normally distributed; otherwise, the Mann Whitney U test would be used instead if those variables were not normally distributed. One-way analysis of variance (ANOVA) was used to evaluate difference in mean of numeric variables among more than two groups provided that these numeric variables were normally distributed, but Kruskal Wallis test was chosen in case of non-normally distributed variables. One-way ANOVA was followed by pos hoc LSD test to evaluate individual differences in mean values between any two groups among groups tested primarily using One-way ANOVA; whereas, Kruskal Wallis test was followed by Mann Whitney U test for the same purpose in case of non-normally distributed numeric variables. Spearman correlation was used to evaluate the correlation between any 2 numeric variables and the results were expressed as correlation coefficient (r) and the level of significance (P). In order to detect the cutoff value that predicts a positive finding, receiver operator characteristic (ROC) curve analysis was used with its corresponding area under the curve (AUC), accuracy level, sensitivity, specificity and level of significance (P). The level of significance was considered at P-value of equal or less than 0.05. The level of high significance was considered at P-value of equal or less than 0.01.

## *3. Results*

The mean age of 65 azoospermia cases was recording 33.37 ± 6.99 years and categorized into 21 (32.3%), 33 (50.8%) and 11 (16.9%) as being < 30 years, 30-40 years and > 40 years as in table 1. Infertility duration ranged from one to 22 years and the median and interquartile range (IQR) was 7.00 (6.00) years as in table 1. Histological findings were in the form of norm-spermatogenesis (NS), hypo-spermatogenesis (HS), maturation arrest (MA) and Sertoli cell-only syndrome (SCOS). 10 (15.38 %), 16 (24.6%), 18 (27.7%) and 21 (32.3%), respectively as in table 2, whereas the sperm retrieval rate according to histological findings were as following: 100%, 75%, 44.4% and 28.6% respectively as in figure 1. Serum FSH level

***Table (1):*** *General characteristic of the evaluated patients*

| **Characteristic** | **Value** |
|---|---|
| **Age (years)** | |
| **Range (min.-max.)** | 26 (22-48) |
| **Mean ±SD** | 33.37 ±6.99 |
| **< 30 years, *n* (%)** | 21 (32.3 %) |
| **30-40 years, *n* (%)** | 33 (50.8 %) |
| **> 40 years, *n* (%)** | 11 (16.9 %) |
| **Duration (years)** | |
| **Mean ±SD** | 7.6±3.845 |

Min.: minimum; max.: maximum; SD: standard deviation, *n*=no. cases, Total Sample size=65

***Table (2):*** *Classification of Azoospermic cases according to histological findings*

| **Histology** | **Total *n* = 65** | **Obstructive azoospermia *n* = 10** | **Non-obstructive azoospermia *n* = 55** | ***P**** |
|---|---|---|---|---|
| **Hypo-spermatogenesis, *n* (%)** | 16 (24.6) | 0 (0) | 16 (29.1) | 0.046 S |
| **Norm-spermatogenesis, *n* (%)** | 10 (15.4) | 10 (100) | 0 (0.0) | <0.001 HS |
| **Maturation Arrest, *n* (%)** | 18 (27.7) | 0 (0) | 18 (32.7) | 0.029 S |
| **Sertoli only syndrome, *n* (%)** | 21 (32.3) | 0 (0) | 21 (38.2) | 0.014 S |

*n*: number of cases; *: Fischer exact test; S: significant at $P \leq 0.05$; HS: highly significant at $P<0.0$.

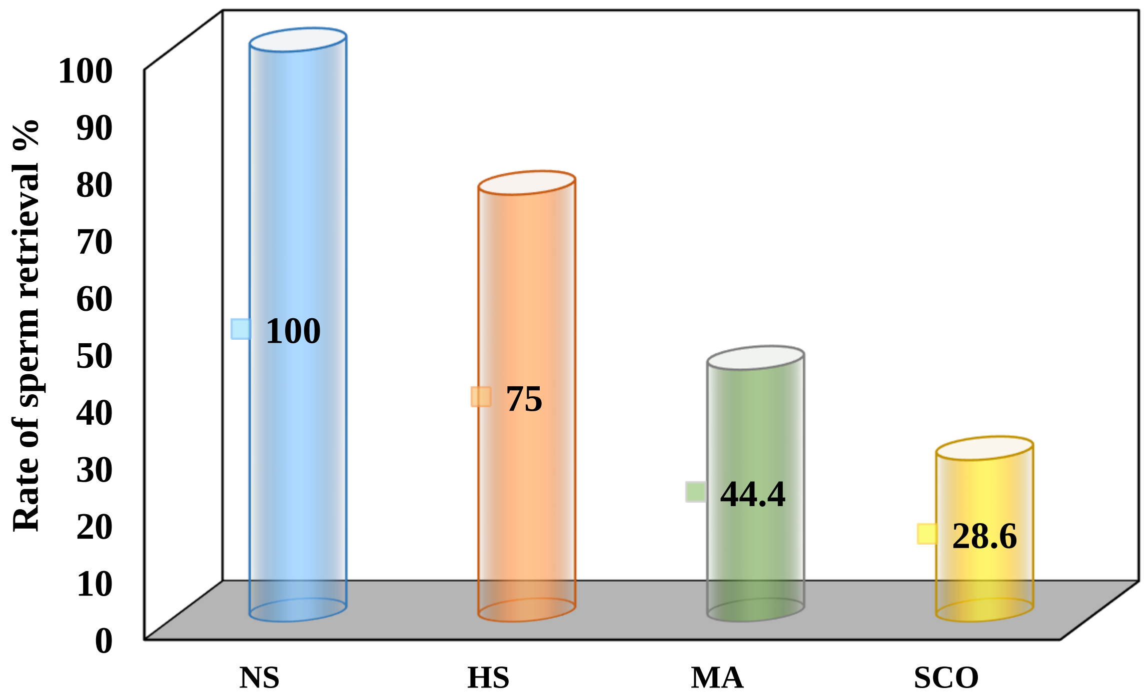


*Figure (1): Sperm retrieval rate according to histological findings; NS: normospermatogenesis; HS: hypospermatogenesis; MA: maturation arrest; SCO: Sertoli cell-only syndrome*

*Table (3): Serum hormonal levels in patients according to histological findings*

| Histology | FSH (mIU/L) Median (IQR) | LH (mIU/L) Median (IQR) | Testosterone (ng/ml) Median (IQR) |
|---|---|---|---|
| **Norm-spermatogenesis** | 6.86 (5.15) C | 5.51 (3.96) C | 2.91 (2.11) A |
| **Hypo-spermatogenesis** | 16.26 (26.89) B | 10.47 (12.35) A | 3.58 (2.60) A |
| **Maturation arrest** | 17.31 (12.61) B | 7.48 (16.30) B | 3.50 (3.64) A |
| **Sertoli cell only syndrome** | 25.60 (16.55) A | 11.11 (12.99) A | 3.40 (2.82) A |
| ***P **** | 0.003 HS | 0.029 S | 0.951 NS |

FSH: follicle-stimulating hormone; LH: luteinizing hormone; IQR: interquartile range; HS: highly significant at $P \leq 0.01$; S: significant difference at $P \leq 0.05$; NS: not significant at $P \leq 0.05$; *: Kruskal Wallis test; Capital letters were used to indicate level of significance of differences in hormonal levels between successive histological groups; similar letters indicate insignificant difference whereas different letters indicate significant difference at $P \leq 0.05$ so that (A) is the highest value followed by (B) then (C).

***Table (4):*** *Serum hormone levels according to sperm retrieval outcome*

| Hormone | Positive sperm retrieval ***n*** **= 26** | Negative sperm retrieval ***n*** **= 29** | ***P*** * |
|---|---|---|---|
| **FSH (mIU/L), Median (IQR)** | 10.55 (17.53) | 24.3 (15.59) | 0.002 HS |
| **LH (mIU/L), Median (IQR)** | 5.86 (5.43) | 13.2 (13.51) | <0.001 HS |
| **Testosterone (ng/ml), Median (IQR)** | 3.45 (2.99) | 3.40 (2.68) | 0.885 NS |

FSH: follicle-stimulating hormone; LH: luteinizing hormone; IQR: interquartile range; HS: highly significant at $P \leq 0.01$; NS: not significant at $P \leq 0.05$; *: Mann Whitney U test

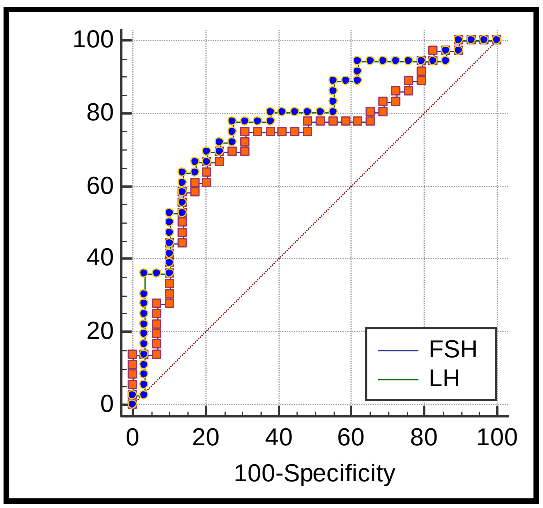


***Figure (2):*** *Receiver Operator Characteristic (ROC) curve to identify serum FSH and LH cutoff values that predict positive sperm retrieval*

***Table (5):*** *ROC curves characteristics*

| Characteristic | FSH | LH |
|---|---|---|
| **Cutoff value (mIU/L)** | ≤ 14.56 | ≤ 9.37 |
| **Area under the curve (AUC)** | 0.730 | 0.784 |
| **Accuracy** | 73.0 % | 78.4 % |
| **95 % confidence interval (CI)** | 0.605 - 0.833 | 0.664 - 0.876 |
| ***P*** | <0.001HS | <0.001HS |
| **Sensitivity** | 66.7 % | 77.7 % |
| **Specificity** | 79.3 % | 72.4 % |

FSH: follicle-stimulating hormone; LH: luteinizing hormone; HS: highly significant at $P \leq 0.01$

was significantly lower in men with NS “6.86 (5.15)” mIU/L (P <0.003), while no significant difference was seen in serum level FSH between men with HS and MA, 16.26 (26.89) versus 17.31 (12.61) mIU/L (P > 0.05). The highest serum FSH was seen in men with Sertoli cell-only syndrome, as shown in table 3. The LH concentration is significantly lowest in men with Norm-spermatogenesis (NS) “5.51 (3.96)” mIU/L. However, it is significantly highest in men with SCOS and HS, 11.11 (12.99) mIU/L and 10.47 (12.35) mIU/L, respectively. The level of serum LH in men with MA was 7.48 (16.30) mIU/L as in table 3. Serum testosterone (TT) level shows no significant variation according to histological findings (P > 0.05) as in table 3. The FSH concentration was significantly lower (P = 0.002) in males with successful sperm extraction than in males with failed sperm extraction, 10.55 (17.53) mIU/L versus 24.3 (15.59) mIU/L. whereas, serum LH was significantly lower (P < 0.001) in males have successful sperm finding than males with failed sperm finding, 5.86 (5.43) mIU/L versus 13.2 (13.51) mIU/L. Serum TT was not significantly different (P = 0.885) between males with positive sperm finding from that of males with negative sperm finding, 3.45 (2.99) mIU/L versus 3.40 (2.68) mIU/L, respectively as shown in table 4. The FSH level cutoff value was recording ≤ 14.56 mIU/L with an accuracy level of 73.0%, the sensitivity level of 66.7% and a specificity level of 79.3%. A cutoff value of serum level of LH was (≤ 9.37 mIU/L) with an accuracy level of 78.4%, a sensitivity level of 77.7% and a specificity level of 72.4% as shown in table 5 and figure 2.

## 4. Discussion

The age was an important factor in the assessment of infertile patients which was nearly the same finding of (Basukarno, et al [9]), who studied 120 patients. There were 66.7% and 33.3% of patients belonged to the obstructive and non-obstructive groups and they had a means of age (36.83 vs 36.62 years) respectively. These observations nearly the same finding of the current observation. (Aggarwal, et al [10]) reported a mean duration of infertility among 40 participants was found 7.225 ± 3.38 years which was approximately similar to the duration of the current study (7.6±3.845 years). In agreement with (Kumar and Gupta [11]) who studied 80 azoospermic males, 9(11.25%) of them were diagnosed as obstructive azoospermia and 71(88.75%) of them were diagnosed as non-obstructive azoospermia patients. Whereas, the current study was recording 10(15.4%) as obstructive azoospermia and 55(84.6%) as non-obstructive azoospermia. However, (Pastore, et al [12]) found that non-obstructive azoospermia affects 49% to 93% of azoospermic males. They were actually harboring either decreased spermatogenesis (HS), MA at initial stage or later stages of spermatogenesis, or SCOS. Whereas, post-testicular obstruction is recorded 7% to 51% of azoospermic males. In such patients, normal spermatogenesis is found in spite of semen lacks of spermatozoa. In comparison to the histopathological finding of (Aggarwal, et al [10]), results were higher than the presented study, the histological grading on open biopsy showed NS in 31(77.5%) of participants, while, 3(7.5%) showed HS, 1(2.5%) showed MA, and five (12.)5% participants had SCOS.

These differences may be due to the type of sperm retrieval procedure or inter-laboratory variation. Regarding the prevalence of sperm retrieval rate (SRR) in the histological types of azoospermia, the study showed highest SRR in obstructive azoospermia (normal spermatogenesis), while lowest SRR in the SCOS type. A similar finding was reported by (Jinno, et al [13]) the percentage of SRRs depending on etiology of azoospermia was 40% for idiopathic azoospermia, and 100% for obstructive azoospermia, undescended testis, and obstruction of ejaculatory duct. In the same line of current study, the sperm recovery rates range from 90% to 100% for obstructive azoospermia using any of standard surgical technique (Miyaoka and Esteves [14]), but the sperm recovery rates for non-obstructive azoospermia range from 30-63% for testicular sperm extraction (TESE), and 43-63% for Micro-TESE; The rate for testicular fine needle aspirations (TEFNA) is approximately 47% (Glina and Vieira [15]). The SRRs from NOA by conventional TESE (38.2%), the spermatozoa were recovered for those with HS, MA and SCOS were 84%, 27.3%, and 6.2%, respectively (Ghalayini, et al [16]), with slight dissimilarity to current study. (Salehi, et al [17]) observation showed the mean of SRR was 48.8%. These rates were 21.6% in the SCOS group, 43.5% in the MA group, and 94% in the HS group. These observations of failed sperm retrieval were recorded a significantly high rate in both MA and SCOS groups compared to HS group, which was approximately same as current observation. (Ku, et al [18]) reported histopathological result of prior needle biopsy illustrates predictive value of sperm given at the time of microsurgical testicular sperm extraction (mTESE), HS (93.3%), early MA (13.3%), late MA (66.7%) and SCOS had SRR of 18.1% [18], they were inconsistent to the presented study which was due to type of sperm retrieval technique. The FSH and LH concentration are significant lower within cases of norm-spermatogenesis than the other type of NOA (MA, HS, and SCOS) while non-significant variation with respect to the serum level of TT. Infertile males have a high level of FSH which is regarded to be an acceptable marker of germinal epithelial impairment as well as reported to be accompanied with azoospermia and severe oligozoospermia (Al-Khateeb, et al [19]). The FSH is essential for stimulation of spermatogenesis in addition to sperm maturation (Oleiwi, et al [20]). Raising the concentration of FSH is associated with increasing degree of severity of defected spermatogensis which was seen in this study. In agreement with (Jinno, et al [13]) the mean FSH and LH concentrations in cases of successful spermatozoa retrieval were significantly lower than those cases of unsuccessful spermatozoon retrieval. Whereas, serum levels of the TT were recorded no significant differences among TESE groups with and without testicular spermatozoa retrieval. (Basukarno, et al [9]) found a significant difference within the serum concentration of TT and FSH between obstructive (norm-spermatogenesis and non-obstructive azoospermia (abnormal spermatogenesis**)**. (Bar-On, et al [21]) stated no statistically significant differences between three groups (SCOS, MA, and NS) of patients regarding the plasma levels of LH and TT. Follicle-stimulating hormone plasma levels were statistically different (P < 0.05) only between the group Sertoli cell-only and the group Full spermatogenesis. [21]In contrast to current study (Aggarwal, et al [10]) reported no significant variation in the means value of serum hormonal profile between OA and NOA. A mean value for serum FSH for participants with OA was 6.60±3.80 mIU/ml and participants with NOA were 10.17±5.55 mIU/ml (P-value = 0.171). The mean value for serum LH level in participants with OA was 6.05±2.82 mIU/ml and for participants with NOA 7.16±2.63 mIU/ml (P-value = 0.878). Serum Testosterone level for participants with OA was 5.38 ± 3.14 ng/ml and mean for participants with NOA was 4.22±2.05 ng/ml (P-value = 0.581). This variation in the result may be variation in the laboratory standardization, technical and personal variation. Based on the endocrinological profiles, (Jinno, et al [13]) SRRs revealed significant differences about 100% if serum level of FSH ≤15 mIU/mL and LH ≤ 2 mIU/mL. Whereas, it records 0% if serum level of FSH > 60 mIU/mL or LH > 12 mIU/mL which was higher than current study was recorded serum level of FSH 10.55 (17.53) mIU/ml and LH 5.86 (5.43) mIU/ml. (Damian, et al [22]) found sperm retrieval through testicular sperm extraction (TESE) were successful on 16(44%) patients, the mean FSH concentration was significantly lower in patients with positive sperm retrieval. The cutoff point was established as < 19.8 UI/L for serum FSH with sensitivities and specificities were recorded 62% and 85% for FSH respectively. Whereas in the presented study cutoff value ≤ 14.56 mIU/L, Sensitivity 66.7% and

Specificity 79.3%. Consistent with (Wang and Huang [23]) a significant difference among positive and negative sperm recovery groups with respect to mean of FSH and LH concentrations. Areas under the curve (AUC) of both hormones were recorded 0.729 and 0.747 respectively. Cutoff points were counted 11.68 IU/L (FSH) and 4.04 IU/L (LH). (Chen, et al [24]) concluded a raised serum concentration of FSH of >19.4 mIU/mL can be considered as a marker of a trial of testicular sperm retrieval in assisted reproductive techniques. The cutoff point of serum FSH level 19.4 mIU/mL discriminated among positive spermatogenesis and negative spermatogenesis which recording 70% sensitivity. (Karamazak, et al [25]) reported a significant correlation of sperm retrieval rates with FSH and LH concentration. Whereas, (Güneri, et al [26]) found these two hormones with a significant difference among positive and negative TESE groups (P = 0.006, and p = 0.001, respectively). However, (Meijerink, et al [27]) study demonstrated higher plasma concentration of testosterone, while, lower plasma concentration both FSH and LH, with predictive of positive sperm recovery retrieval, which was in contrast to finding of (Kumar and Gupta [11]) a significant difference in FSH and LH was shown among positive and negative sperm retrieval patients.

## *5. Conclusion*

The serum level of FSH and LH could be reliable indicators in the prediction of sperm recovery and differentiated among obstructive and non-obstructive azoospermia. Serum level of testosterone (TT) showed no significant variation according to histological findings, in relevance to this finding, unfortunately, testosterone could not be used in predicting azoospermia classification.

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## Author Contribution

This research was done by Dr. Hussain Khaleefa Kadhem Al Dulaimy with Prof. Dr. Ula M. R. Al-Kawaz and Assist. Prof. Dr. Hayder A. L. Mossa (corresponding author) in male infertility clinics and IVF laboratory at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al Nahrain University.

## Conflict of Interest

The authors declare no conflict of interest.

## Ethical Clearance

The study was approved by the Ethical Approval Committee.

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