8th WORLD CONGRESS of PEDIATRIC SURGERY
WOFAPS
Turkish Association of Pediatric Surgeons
12-15 November 2025
Titanic Mardan Palace Hotel
ANTALYA, TÜRKİYE

INHIBIN-B LEVELS IN CHILDREN WITH CRYPTORCHIDISM WHO UNDERWENT ORCHIDOPEXY AFTER 18-MONTHS OF AGE: A SINGLE CENTRE CROSS-SECTIONAL STUDY

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BACKGROUND

- Cryptorchidism is the most common anomaly in sexual development in boys. While orchidopexy is expected to stimulate an increase in inhibin B levels – particularly when performed early, delayed intervention may impair this response, reflecting reduced germ cell function and compromised fertility potential. In developing countries, children often present beyond the recommended 18-month surgical window, yet data on hormonal outcomes in this context remain scarce.

METHODOLOGY

- This cross-sectional observational study carried out at Kenyatta National Hospital (KNH) evaluated serum inhibin B levels six months after orchidopexy in 69 boys with delayed surgery. Participants were categorized into age-specific inhibin B percentiles. Statistical analyses included Kruskal–Wallis, Mann–Whitney U, Chi-square tests, and multiple linear regression.

RESULTS

- The median age at surgery was 6.75 years. Overall, 65.2% of participants had inhibin B levels at or below the 25th percentile, indicating reduced Sertoli cell function, while 34.8% showed preserved activity. Bilateral cryptorchidism occurred in 34.8%, and 22.2% required staged repair. Age at surgery was the only independent predictor of postoperative inhibin B levels ($P < 0.05$).

CONCLUSION

- Delayed orchidopexy is associated with lower postoperative inhibin B levels, highlighting diminished testicular function. Early correction remains crucial for preserving fertility potential in boys with cryptorchidism.

- **KEY WORDS:** Inhibin B, Cryptorchidism, Orchidopexy, Paediatric Urology

INTRODUCTION

BACKGROUND

- Cryptorchidism is commonly used interchangeably with undescended testis. Descent of the testis occurs in two distinct phases: transabdominal (gestation weeks 8-15) and inguinoscrotal (gestational weeks 25-35). A true undescended testis adheres to its customary descent trajectory but experiences a hindrance on its journey to the scrotum. In a recent 8-month study at the Paediatric filter clinic of KNH, cryptorchidism was found to have a prevalence of 5.4% among the 679 patients included in the research [1]. Clinical decisions regarding management are guided by the testes' presence and location. Palpable testis call for surgical interventions such as orchidolysis and orchidopexy while for non-palpable testis, diagnostic laparoscopy stands as the simplest and most precise method for locating an intra-abdominal testis. Surgical options for such cases include the staged Fowler-Stephens orchidopexy [2] or the Shehata technique [3].

- Braga and her colleagues while conducting a comprehensive review of cryptorchidism in Africa, express concern over the paucity of African publications pertaining to the condition [4]. They refer to West African population studies and an institutional study conducted in Tanzania. A common theme that prevails throughout the region is the issue of delayed surgery due to late presentation. In their account of the local situation in Congo, Alumenti and team reported that 78.9% of their patients aged 2 years or more [5]. Ilkul and his co-authors, during the analysis of cryptorchidism cases at Consolata Nyeri Hospital over an 8-year timeframe, reported a median age of presentation of 9 years [6]. A more recent prospective cross-sectional study carried out in 2022 at the paediatric surgical outpatient clinic (PSOPC) in KNH by Said M et al. with focus on patients seen with cryptorchidism reported the mean age of presentation was 5 years [7]. Treatment timing influences adult spermatogenesis, hormone production, and tumour risk [8]. Undescended testes, subjected to histological analysis at 12-18 months, exhibit diminishing germ cells and Leydig cells [9].

- Existing guidelines advise timing of surgery with a dual purpose: preserving fertility potential and minimizing the risk of complications such as malignancy and torsion. It is advisable to start treatment at 6 months; undescended testes seldom descend later [10]. In addition, any intervention achieving scrotal placement should conclude by 12 – 18 months. The region lacks research on fertility potential after surgical intervention for undescended testes and more importantly when done beyond the recommended timeframe.

- Overall, it's expected that inhibin-B levels will increase following orchidopexy with a more substantial rise expected when surgery is performed at a younger age [11]. Failure to surge during the postoperative period could indicate an insufficient quantity of germ cells within the testis and hence increased infertility risk [12].

- The study involved an evaluation of children with cryptorchidism who underwent surgery at KNH with a focus on the age at which the surgery was done and will analyse post-operative inhibin-B levels.

OBJECTIVES:

1. To determine the age at surgery in children with cryptorchidism who underwent orchidopexy after the age of 18-months at KNH.
2. To determine Inhibin-B levels at 6-month follow-up in children with cryptorchidism who underwent orchidopexy after 18-months at KNH.

METHODOLOGY

Study Design

- Cross-sectional observational study

Study Site

- The study was conducted at Kenyatta National Hospital (KNH), paediatric surgical unit. KNH is the largest Tertiary referral Hospital in Kenya with a bed capacity of 1,800 and 24 theatres out of which 2 theatres are dedicated to paediatric surgery. The paediatric surgical division, under the leadership of 10 consultants, is located on the 4th floor and is partitioned into 3 sections – A general ward, a nursery, and an 8-bed NICU.

Study Population

- The study encompassed all patients with cryptorchidism from ages 18months – 13 years who are surgically managed at KNH.

Inclusion and Exclusion Criteria

- Inclusion Criteria

- All children with cryptorchidism aged 18months -13yrs six months post-orchidopexy at KNH whose parent/guardian will have consented for the study.

- Exclusion Criteria

1. Patients with disorders of sexual differentiation (DSD).
2. Syndromic children or children with any chromosomal anomaly.
3. Patients on hormonal therapy.
4. Patients with anorchia discovered during surgery.

Sample Size Calculation

1. The Cochran formula was used to calculate the sample size.

$$n = \frac{(Z\ score)^2 \times p(1-p)}{e^2}$$

n= Sample size
Z= Statistic for level of confidence (95% confidence interval Z value is 1.96)
P= Proportion of patients > 2 years of age whose Inhibin-b levels normalised after orchidopexy according to Shankar et al. in a similar study conducted in India was 92% [13].
e= Desired level of precision (5%)
Substituting into the above formula the desired sample size shall be **113 patients.**

Sampling Technique

- Consecutive sampling of all patients who met the inclusion criteria was done until the desired sample size was achieved.

Data Collection

- The parents and guardians of all patients who meet the inclusion criteria were approached and recruited. The researcher briefed them about the purpose, risks, and benefits of the study. An informed consent form was then administered.

- The patients were recruited from the Paediatric Surgical Outpatient Clinic (PSOPC) and the Paediatric Surgical Ward (4A) and encompassed all those who had completed the 6-month post-operative period.
- Once included in the study, the post-operative samples were collected 6-months after surgery under standard phlebotomy principles between 8am – 11am as serum inhibin -B levels have peak values in the early morning [14]. This visit was timed to coincide with the PSOPC review visit.

- The 6-month interval is supported by Da Ma et al. who conducted a comparable study. During the subsequent monitoring periods of 1-month, 6-months, and 12 months , a statistically significant elevation compared to pre-op levels was exclusively observed from the 1-month follow-up and then the levels plateau during the 6-month and 12-month interval [15].

- The samples were labelled and left to stand for 2-5 minutes, stored at room temperature until the time of analysis. They were collected on the same day by a designated rider and transported to the biochemistry unit in Metropolis laboratory which is a globally certified KANAS CAP ISO 15189:2012 and locally accredited by the Kenya Medical Laboratory Technicians and Technologists Board.

- The samples were analysed as a batch using commercially available enzyme-linked immunosorbent assay kits from M/S Beckman Coulter, UK.

Data Collection

- Data accuracy was verified, imported into Microsoft Excel for cleaning, and analysed using IBM SPSS Statistics for Windows, Version 26.0. It was then analyzed using a combination of descriptive, bivariate, and multivariate statistical techniques. Descriptive statistics, including means, medians, and percentile distributions, were used to summarize continuous variables such as Inhibin B levels and age at surgery.

- Participants were stratified into percentile-based Inhibin B categories to evaluate fertility potential, using thresholds derived from the age-specific normative data published by Thomas Kelsey et al. in their meta-analysis [16].

- Bivariate analyses were conducted to assess associations between Inhibin B levels and categorical clinical variables. Non-parametric tests were used due to the skewed distribution of hormonal data. These included the Kruskal-Wallis test for comparing age across multiple Inhibin B groups, and the Mann-Whitney U test for comparing Inhibin B levels between participants undergoing staged versus single-stage orchidopexy. Chi-square tests were used to assess the relationships between Inhibin B group classification and categorical variables such as testicular laterality and surgical approach.

- A multiple linear regression model was employed to assess the independent effects of age at surgery, laterality, and staged surgical approach on post-operative Inhibin B levels.

Ethical considerations

- Institutional approval was sought and granted while ethical approval was obtained from Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KNH-UON ERC) REF Number P58/02/24. Written informed consent was obtained from all parents or guardians.

RESULTS

A). PATIENT CHARACTERISTIC SUMMARY

Table 1. Distribution of Age, Laterality & surgical approach Among Study Participants.

PATIENT CHARACTERISTIC	VALUE
Median Age (Years)	6.75
Mean Age (Years)	6.0
Age Standard Deviation (years)	3.43
Right Laterality	22
Left Laterality	23
Bilateral Laterality	24
Staged surgery : Yes	13

B). DESCRIPTIVE STATISTICS

i). Inhibin B levels by age at surgery

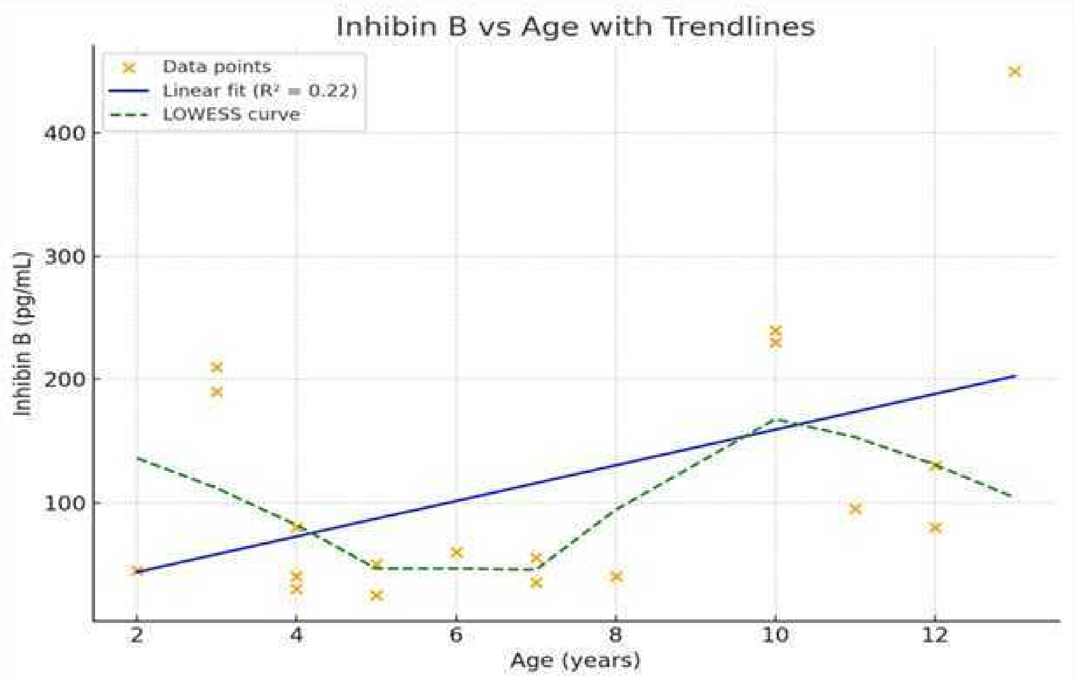

Figure 1: Scatter plot showing Inhibin B levels by Age

- To explore the relationship between Inhibin B levels and age at surgery, a scatter plot was generated to visualize overall trends. A simple linear regression line was fitted, demonstrating a general positive association between increasing age and inhibin B levels. Additionally, a locally weighted scatterplot smoothing (LOWESS) curve was overlaid to better capture non-linear variations. The LOWESS curve highlighted a more detailed pattern: a gradual decline in Inhibin B from early childhood to mid-childhood, a modest rise around 10 years of age, and a tapering thereafter. This confirms that Inhibin B levels fluctuate with age in a non-linear manner rather than following a simple linear trajectory.

ii). Percentile Classification Analysis

Table 2. Participant Classification Based on Serum Inhibin B Percentile Bands

Percentile	Number of Patients	Percentage (%)
<2.5th	20	28.99
2.5th – 10th	11	15.94
10th – 25th	14	20.29
25th – 50th	8	11.59
50th – 75th	1	1.45
75th – 90th	5	7.25
90th – 97.5th	6	8.7
>97.5th	4	5.8

Table 3. Clinical Interpretation of Serum Inhibin B Percentiles according to Thomas Kelsey et al.

Percentile Range	Interpretation
Below 2.5th percentile	High concern for impaired fertility
2.5th – 10th percentile	Moderate concern for impaired fertility
10th – 25th percentile	Borderline / Mildly decreased fertility risk
Above 25th percentile	Normal fertility potential

Table 4. Summary of Participants at or below the 25th Percentile

Metric	Value
Number of participants	45
Mean Inhibin B	44.9 pg/mL
Median Inhibin B	35.0 pg/mL
Most common laterality	Bilateral
Staged surgeries	10 out of 45

Table 5. Summary of Participants above the 25th Percentile

Metric	Value
Number of participants	24
Mean Inhibin B	217.2 pg/mL
Median Inhibin B	204.0 pg/mL
Most common laterality	Right
Staged surgeries	3 out of 24

- A total of 24 participants (34.8%) had Inhibin B levels above the 25th percentile, suggesting preserved Sertoli cell function and a potentially favorable fertility profile. The majority had unilateral undescended testes and underwent single-stage surgery.

- The remaining 45 participants (65.2%) had Inhibin B levels at or below the 25th percentile, indicating varying degrees of impaired Sertoli cell activity. This group was predominantly composed of patients with bilateral disease and included a higher proportion of staged surgical interventions.

C). BIVARIATE ANALYSIS

i). Age at surgery and Inhibin B Levels

Table 6. Group statistics for age

GROUP	MEAN AGE (YEARS)	MEDIAN AGE (YEARS)	SD
Low	5.06	5.0	2.37
Normal	7.65	7.0	3.51
High	8.87	10.0	3.66

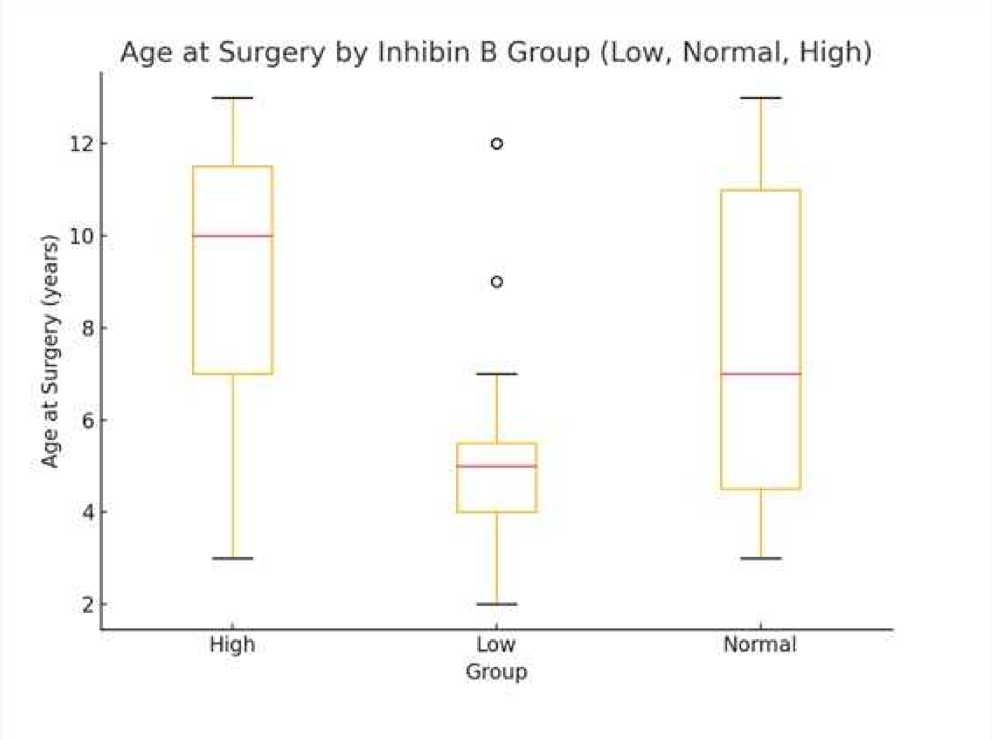

Figure 2. Box Plot Showing Age at Surgery by Inhibin B Group

- The box plot in figure 2 illustrates the distribution of age at surgery across three Inhibin B groups: Low, Normal, and High. Notably, participants in the High Inhibin B group had the highest median age at surgery, followed by the Normal group, while those in the Low group underwent surgery at a younger age on average. The median age for the High group was approximately 10 years, compared to 7 years in the Normal group and 5 years in the Low group. Despite conventional expectations that earlier surgery is associated with better hormonal outcomes, this figure demonstrates that higher Inhibin B levels were paradoxically observed in boys who had surgery later.

ii). Laterality and Inhibin B Levels

Chi-square Test: Laterality vs Inhibin B Group

- A Chi-square test was conducted to determine if there was an association between laterality (Right, Left, Bilateral) and Inhibin B group classification (Low, Normal, High). The results indicated no statistically significant association (Chi2-statistic = 1.94, p-value = 0.747, degrees of freedom = 4).

Kruskal-Wallis Test: Laterality vs Inhibin B Levels

- A Kruskal-Wallis test was performed to assess if serum Inhibin B levels differed according to laterality. The results also indicated no statistically significant difference between groups (H-statistic = 0.22, p-value = 0.895).

iii). Staged Surgery and Inhibin B Levels

Chi-square Test: Staged Surgery vs Inhibin B Group

- A Chi-square test was conducted to assess the association between staged surgery (Yes/No) and Inhibin B group classification (Low, Normal, High). No statistically significant association was found (Chi2-statistic = 0.43, p-value = 0.807, degrees of freedom = 2).

Mann-Whitney U Test: Staged Surgery vs Inhibin B Levels

- A Mann-Whitney U test was performed to evaluate differences in serum Inhibin B levels between patients undergoing staged versus one-stage surgery. The analysis demonstrated no statistically significant difference (U-statistic = 327.5, p-value = 0.580).

D). MULTIVARIATE ANALYSIS

Predictors of Inhibin B Levels

- A multiple linear regression model was constructed to evaluate the combined effect of age at surgery, testicular laterality, and whether surgery was staged on post-operative Inhibin B levels. The model was statistically significant ($F(4, 64) = 10.24$, $p < 0.001$) and explained approximately 39% of the variance in Inhibin B levels ($R^2 = 0.39$).

- Of the independent variables, only age at surgery was found to be a statistically significant predictor ($\beta = +19.09$, $p < 0.001$), indicating that for each additional year of age, the Inhibin B level increased by an average of 19.1 pg/mL. Neither testicular laterality (left or right vs bilateral) nor staged surgery was independently associated with Inhibin B levels after adjusting for age (all $p > 0.05$).

Table 7. Multivariate Regression Model Summary

Variable	Coefficient (β)	Standard Error	p-value
Intercept	-1.0802	34.270	0.975
Age	**1{Citation}9.0894**	**3.107**	**< 0.001**
Laterality: Left	-44.4313	32.217	0.173
Laterality: Right	-7.3766	32.459	0.821
Staged Surgery: Yes	-31.1471	35.992	0.390

CHAPTER FIVE: DISCUSSION

- Late presentation and subsequently delayed orchidopexy remain common challenges in the management of cryptorchidism across many low-middle income countries (LMICs) [5–7]. In our study, the median age at surgery was 6.75 years, with a mean age of 6.0 years (SD =3.43), which significantly exceeds the globally recommended surgical window of 6 to 18 months [17,18].This delay likely reflects systemic challenges such as late referral, lack of awareness, or healthcare access limitations—factors particularly relevant in LMICs [6].

- Inhibin B as a glycoprotein primarily secreted by Sertoli cells in the testes serves a reliable marker of Sertoli cell function and, indirectly, of spermatogenic activity. Its production is closely linked to the presence and maturation of germ cells, which provide paracrine signals that stimulate Sertoli cell activity [12]. While testicular histology using biopsies serves as a broadly endorsed approach for prognosticating future fertility, measurement of serum Inhibin B has been used by different studies as a non-invasive substitute [11,12,19].

- Reference ranges for serum Inhibin B have been defined across all paediatric age groups [20–23]. Thomas Kelsey et al. in their meta-analysis were able to further establish age-specific Inhibin B percentiles, with values below the 2.5th percentile indicating high concern for impaired fertility, 2.5th-10th percentile indicating moderate concern, 10th-25th percentile suggesting borderline risk, and values above the 25th percentile reflecting normal fertility potential [16]

- In our study, majority of the cohort (45 out of 69 participants, 65.2%) exhibited Inhibin B levels at or below the 25th percentile for their age. This distribution raises concerns regarding the functional integrity of Sertoli cells and overall testicular endocrine activity in this population. The mean and median Inhibin B levels for this group were 44.9 pg/mL and 35.0 pg/mL, respectively—substantially lower than their counterparts in the higher percentiles—highlighting the degree of hormonal compromise observed.

- The relatively high proportion of bilateral cases (34.8%) in this subgroup is clinically significant, as bilateral cryptorchidism is associated with a greater risk of testicular dysgenesis and compromised fertility potential. This is consistent with findings from Peter Lee et al. where bilateral cases exhibited an infertility rate approximately 3.5 times that of the unilateral group and more than six times that observed in controls [24].

- The clinical characteristics of this subgroup further revealed that most had unilateral undescended testes, with the right side being most commonly affected. Additionally, only a minority (3 out of 24) underwent staged orchidopexy. These patterns may suggest that unilateral involvement and the ability to undergo a single-stage surgical correction are associated with better endocrine outcomes. However, further statistical analysis did not support these associations.

- An unexpected finding in this cohort was that patients classified into the High Inhibin B group underwent surgery at a significantly later median age (10-years) compared to those classified into the Low (5-years) and Normal groups (7-years).

- Several possible explanations exist for this observation. Later surgical timing may have reflected selection bias, where milder forms of cryptorchidism, such as palpable, partially descended, or otherwise less impaired testes, were managed less urgently and inherently maintained better Sertoli cell function. Additionally, physiological age-related increases in Inhibin B production, particularly approaching puberty, may account for the elevated hormone levels observed in older children irrespective of intrinsic testicular health [25].The small sample size of the High group (n=15) must also be considered, as a few outliers with extremely elevated Inhibin B values could disproportionately influence group statistics.

- The multivariate regression analysis revealed that age at the time of surgery was the only independent factor significantly associated with Inhibin B levels post-orchidopexy. This finding supports prior bivariate observations and reinforces the role of age-related physiological maturation in modulating Sertoli cell function.

- Overall, these findings highlight age at surgery as a critical determinant of hormonal outcomes and underscore the importance of timely intervention.

CONCLUSION

- This study underscores the persistent challenge of delayed presentation and late orchidopexy in LMICs, with the median age at surgery substantially exceeding international recommendations. The use of Inhibin B as a non-invasive biomarker provided valuable insights into post-operative Sertoli cell function and potential fertility outcomes.

- A considerable proportion of participants (65.2%) exhibited Inhibin B levels at or below the 25th percentile, raising concerns about compromised testicular endocrine activity particularly with bilateral involvement [24]. However, a notable subset (34.8%) maintained Inhibin B levels above the 25th percentile, suggesting preserved Sertoli cell function in some boys despite delayed intervention.

- Age at surgery emerged as the only independent predictor of post-operative Inhibin B levels, reinforcing the influence of physiological maturation on hormonal profiles, and aligning with findings from previous studies.

- The findings underscore the importance of timely surgical correction. However, the regression model used in this study demonstrated moderate explanatory power (R2 = 0.39) suggesting that additional factors not included in this study – such as testicular position and histology, pubertal timing, testicular size, and individual genetic variability – may also contribute to endocrine outcomes and warrant further investigation.

STUDY LIMITATIONS

- While this study offers valuable insights into the hormonal outcomes of cryptorchidism, several limitations should be acknowledged:

1). Limited Sample Size – The cohort size, particularly in subgroup analyses (eg. High Inhibin B group), was relatively small, which may restrict statistical power and generalizability of the findings.

2). Singe-Time-Point Hormonal Measurement – Serum Inhibin B was assessed only once post-operatively, which does not account for temporal changes or pubertal progression that may influence endocrine function.

3). Absence of Testicular Histology – Without concurrent biopsy data, the relationship between Inhibin B levels and actual germ cell content or testicular architecture could not be established.

4). Lack of Longitudinal Fertility Outcomes – Although Inhibin B is a surrogate marker, this study did not assess definitive fertility endpoints such as semen analysis or paternity rates, limiting long-term clinical extrapolation.

5). Potential confounding variables - Factors such as endocrinopathy, quality of surgical technique or intraoperative complications, perinatal factors, genetic abnormalities, or environmental exposures were not captures and may have influenced hormonal profiles.

RECOMMENDATIONS

1. **Early Surgical Intervention Should Be Prioritized**

Given that age at surgery was the only independent predictor of post-operative Inhibin B levels, these findings reinforce current guidelines recommending early orchidopexy, ideally before 18 months of age, to optimize testicular endocrine outcomes and potentially preserve fertility.

2. Routine Post-Operative Endocrine Monitoring May Be Beneficial

Incorporating serum Inhibin B measurement post-orchidopexy could provide valuable insight into Sertoli cell function, allowing clinicians to identify children at risk for impaired fertility and tailor follow-up accordingly.

3. Broader Hormonal and Clinical Profiling Is Needed

Future studies should include pubertal staging, testicular volume, and hormonal panels (e.g., Follicle Stimulating Hormone (FSH) , Luteinizing Hormone (LH) , testosterone) to complement Inhibin B data and provide a more comprehensive assessment of testicular function.

4. Longitudinal Studies Are Encouraged

Larger prospective studies and long-term follow-up from childhood into adolescence and adulthood is necessary to establish whether Inhibin B levels correlate with eventual fertility outcomes, including semen analysis and paternity rates.

5. Consider Multidisciplinary Care Models

Boys with bilateral disease or poor post-operative hormonal profiles may benefit from early referral to endocrinology or fertility counseling services, particularly in cases with significant hormonal impair.

DECLARATIONS

- **AUTHOR CONTRIBUTIONS:** Brian Bichanga: Conception, design, acquisition of data, analysis of data and draft of the manuscript. Tim Jumbi: Design, analysis of data and draft of the manuscript. Francis Osawa: Design, analysis of the data and draft of the manuscript. Peter Mwika: Conception, design, and draft of the manuscript.

- **FUNDING:** Research funding was provided by the Kenyatta National Hospital Research Department

- **CONFLICT OF INTEREST:** The authors declare that there are no competing interests.

- **ETHICAL APPROVAL** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KNH-UON ERC) REF Number P58/02/24.

- **INFORMED CONSENT:** Informed consent was obtained from all parents or legal guardians.

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