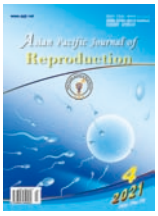

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Proposed age-stratified reference intervals of FSH derived from normozoospermic men

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ABSTRACT

Objective: To demonstrate that serum follicle-stimulating hormone (FSH) in men rises with age, and to explore FSH reference intervals of age-related partitioning.

Methods: Men aged 20-50 years (n=1 190) underwent semen analysis according to World Health Organization (2010) methods. Serum was frozen prior to measurement of FSH by using the Siemens ADVIA Centaur® XP immunoassay system. FSH central 95% intervals after logarithmic transformation based on age were derived from 1 037 normozoospermic men. These were then applied to oligozoospermic and azoospermic men. Men producing azoospermic semen samples were further classified as having non-obstructive azoospermia by clinical diagnostic criteria, including genetic analysis and surgical exploration.

Results: Serum FSH in normozoospermic men increased with age (P<0.05), and reference intervals were determined with 10-year brackets: 21-30 years [(1.0-8.2) IU/L], 31-40 years [(1.4-9.5) IU/L], 41-50 years [(1.9-12.0) IU/L]. The proportion of oligozoospermic men with normal FSH concentrations was less than the normozoospermic men, which in turn was lower among azoospermic men (both P<0.01). The azoospermic men were further broken down according to the nature of the azoospermia as either obstructive or non-obstructive azoospermia, and 86.4% (38/44) men with non-obstructive azoospermia had elevated serum FSH concentrations whereas only 6.7% (1/15) men with obstructive azoospermia had high FSH levels, and this was significantly different (P<0.01).

Conclusions: FSH concentrations increase in men between 20-50 years, and clinical interpretation of serum FSH results in men must be made by using age-based reference intervals.

KEYWORDS: Male infertility; FSH; Reference intervals; Age; Obstructive azoospermia; Non-obstructive azoospermia

1. Introduction

Infertility is a global health issue affecting an estimated 15% of couples[1], and males are found to be solely responsible for 20%-30% of infertility cases and contribute to 50% of cases overall[2]. Semen analysis remains the main screening test for male fertility on the basis that it is the quality of the ejaculated semen that appears most relevant[3] but serum follicle stimulating hormone (FSH) has long been known to be an important biochemical marker of testicular function[4-6].

Different assay platforms measuring FSH can give significantly different results when analysing the same sample[7-9] but this is not always the case[10], and a far more pressing issue is the selection of an appropriate reference interval for clinical interpretation.

Significance

There is an increase in serum FSH in men as they get older. Accordingly, it is important to have age-stratified reference intervals but this has not been done previously. Men used to provide data for the reference intervals should have healthy testicular function, and so the present study has used normozoospermic men. The usefulness of the proposed reference intervals was demonstrated by applying them to oligozoospermic and azoospermic men.

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Reference intervals commonly use the central 95% of a healthy population[11] but should take into account normal physiological changes such as age[12]. A number of studies have demonstrated that serum FSH in men rises with age[13–15] and yet many FSH reference intervals applied do not have age-related partitioning for men of reproductive age[8,9,16–19].

This study analysed retrospectively the serum FSH concentrations and semen quality at the initial consultation of men attending a clinic for the investigation of their fertility, and aimed to: 1) determine the effect of age upon serum FSH in men producing normozoospermic semen samples, 2) derive an age-based reference interval for these men, 3) apply these reference intervals to men producing oligozoospermic and azoospermic semen samples, and 4) validate further the age-based reference intervals on men with obstructive azoospermia and non-obstructive azoospermia.

2. Materials and methods

2.1. Study population

A total of 1 190 men were enrolled who were sexually mature and aged >20 years. Hence, changes around puberty were excluded as a pre-analytical issue. They attended for initial fertility assessment between January 2010 - July 2018 where semen collection and analysis were performed on the same date as blood collection. Semen samples were excluded if they 1) were not fresh semen (*i.e.* epididymal, testicular, and cryopreserved samples); 2) had a semen volume of <1.5 mL; 3) had recorded days of abstinence outside of 2-7 days; 4) were samples that were not initial assessments; 5) were not collected within the study centre (*i.e.* home collections); and 6) were produced by men taking exogenous hormone medication, or had HIV or any form of cancer. Men producing azoospermic semen samples were identified as having non-obstructive azoospermia by clinical diagnostic criteria including genetic analysis and surgical exploration.

2.2. Semen analyses

Methods for the analysis of semen[20] and lower reference limits for the interpretation of results[21] were those currently recommended by the World Health Organisation (WHO). Semen samples included in the study were classed as either normozoospermic with the three parameters of sperm concentration, motility and morphology all above the lower reference limits, or oligozoospermic (reduced sperm concentration but normal motility and morphology), and azoospermic (an absence of sperm in the semen). The laboratory participated in an external quality assurance scheme to ensure acceptable performance in terms of accuracy and precision for sperm concentration, sperm motility and sperm morphology (EQASRM, PO Box 162, Northlands, Western Australia 6905).

2.3. Blood collection and FSH measurement

All blood collections were done within the PIVET Medical Centre on the same day as semen sample collection and analysis. Bloods were non-fasting samples collected into plain tubes, allowed to clot and the serum were aspirated and frozen prior to analysis. The Siemens ADVIA Centaur® XP immunoassay system was the platform used to analyse all blood samples in this study. Internal quality controls at three levels (Liquichek Immunoassay Plus Control, Trilevel, Bio-Rad) were run on each day the FSH analyses were made, and clinical samples were analysed only if the quality control samples were within defined ranges. Participation in an external quality assurance scheme (RIQAS-Randox Immunoassay Quality Assurance Scheme) also confirmed acceptable performance of the FSH assay. The assay laboratory is accredited on an annual basis by the National Australian Testing Authority.

2.4. FSH reference intervals

The FSH reference intervals were stratified according to age[12], namely 21-30, 31-40 and 41-50 years, and each age bracket had more than 120 samples[22]. The serum FSH concentrations for each age bracket were tested with the Kolmogorov-Smirnov test to determine if it matched the characteristics of a normal distribution and, if not, concentrations were transformed to $\log_{10}$ values and retested for normality. The reference intervals in the present study were determined by calculating the 95% centile range after logarithmic transformation as the upper and lower limits of two standard deviations for men producing normozoospermic semen samples and thereby having normal spermatogenesis.

2.5. Statistical analysis

The association between age and serum FSH concentration was analysed by using Spearman's ρ (rank correlation coefficient). The effect of age was assessed by using the Kruskal-Wallis test with *post–hoc* analysis by the Dunn method adjusted by the Benjamini–Hochberg procedure, whilst proportions were compared by using *Chi* square (χ^2) analysis with *post–hoc* analyses using the Bonferroni correction. Associations and differences were considered significant if $P<0.05$.

2.6. Ethics approval, Assisted Reproductive Technology (ART) Unit accreditation, and patient consent

Ethics approval had been obtained to use data contained in the clinical database for retrospective research, and all patient information was de-identified to protect the identity of all subjects. Specifically, the reporting of this data was approved under Curtin University Human Ethics Committee approval No. RD_25–10 general approval for retrospective data analysis in 2010, updated

in 2015, and again further updated in August 2020. The ART Unit was accredited by both the Reproductive Technology Accreditation Committee, a committee within the Fertility Society of Australia, as well as the Reproductive Technology Council of Western Australia. Consent forms permitting the retrospective use of data received approval under both regulatory bodies.

3. Results

3.1. Study population

Table 1 shows the age and semen parameters for the normozoospermic (n=1 037), oligozoospermic (n=94) and azoospermic (n=59) men in the study. There was a significant difference in age between normozoospermic, oligozoospermic and azoospermic groups (Kruskal-Wallis test; P<0.05), with *post-hoc* testing confirming the oligozoospermic men were younger than their normozoospermic counterparts (P<0.05). The azoospermic men were excluded from the comparisons of semen quality on the basis that their samples were, by definition, devoid of sperm. The normozoospermic men had significantly higher sperm concentrations in their semen than the oligozoospermic men (Kruskal-Wallis test; P<0.01) although this was to be expected by definition according to the lower reference limit for sperm concentration set by the WHO[20]. Whilst all oligozoospermic men had normal sperm morphology and progressive motility above the lower reference limit of the WHO 5th edition manual[20], their proportion of normal forms and sperm with progressive motility were nevertheless both lower than for the normozoospermic men (Kruskal-Wallis test; both P<0.01).

3.2. Men producing normozoospermic samples

Figure 1 shows the scatterplot of serum FSH concentration for normozoospermic men against their age, and there was a significant positive association (Spearman's ρ = 0.17, P<0.01). The men were then arranged into the three age groups as shown in Table 2, namely 21-30, 31-40, and 41-50 years. The FSH concentrations for each of these three groups were not normally distributed (Kolmogorov-Smirnov test; all P<0.05 thus rejecting the null hypothesis that the values were sampled from a population that follows a Gaussian distribution). The FSH values were then transformed to $\log_{10}$ values and these were found to be normalized with each null hypothesis then being accepted. Further analysis on the normalized data (Table 2) showed that the serum FSH concentrations for men aged 21-30, 31-40 and 41-50 years were different (Kruskal-Wallis test; P<0.005), with *post-hoc* analysis confirming there were progressive and significant rises from men aged 21-30 years to 31-40 years to 41-50 years (all P<0.05). Calculation of the upper and lower limits of 2 standard deviations of the geometric transformation gave the 95th centile range and enabled stratified reference intervals to be obtained for these three age brackets, namely 21-30 years [(1.21-9.26) IU/L], 31-40 years [(1.37-10.05) IU/L], and 41-50 years [(1.57-10.89) IU/L].

3.3. Qualitative measures of serum FSH according to semen quality

The stratified reference intervals and the proportion of normozoospermic, oligozoospermic and azoospermic men that were above or below these intervals are shown in Table 3. Just under 5% of

Table 1. The population and semen quality of the 1 190 men in the study.

Parameters	Semen quality		
	Normozoospermia (n=1 037)	Oligozoospermia (n=94)	Azoospermia (n=59)
Age, years	35.0 (31.0-40.0)[a]	33.0 (30.0-38.0)[b]	35.0 (32.0-40.0)[a]
Sperm concentration, $\times10^6$/mL	65.0 (42.0-98.0)[a]	8.0 (4.4-12.0)[b]	-
Sperm normal forms, %	6 (5-8)[a]	5 (4-6)[b]	-
Sperm progressive motility, %	65.0 (58.0-70.0)[a]	58.0 (50.0-67.0)[b]	-

-: None. Values are expressed as median (interquartile range) and comparisons are made by using the Kruskal-Wallis test with or without *post-hoc* testing as appropriate. Different superscripts in a row are significantly different (P<0.01). WHO[20] limits of normality: concentration (≥15×10^6/mL), morphology (≥4% normal forms) and progressive motility (≥32%).

Table 2. The serum concentrations of FSH according to age in 10-year brackets for men producing a normozoospermic semen sample at their first assessment (IU/L).

Serum FSH concentration[1]	Age group		
	21-30 years (n=209)	31-40 years (n=617)	41-50 years (n=211)
Geometric mean	3.35[a]	3.71[b]	4.14[c]
Limits of 2sd	1.21-9.26	1.37-10.05	1.57-10.89

[1]Serum FSH concentrations with different superscript in a row are significantly different, all P<0.05. Geometric scales are tested for normality with the Kolmogorov-Smirnov test, and reference intervals are subsequently represented as the limits of 2 geometric standard deviations (sd) after data transformation. FSH: follicle-stimulating hormone.

Table 3. The identification of men with abnormal serum FSH concentrations according to their semen quality [*n*(%)].

Category*	Semen quality		
	Normozoospermia (*n*=1 037)	Oligozoospermia (*n*=94)	Azoospermia (*n*=59)
Low FSH	20 (1.9%)	3 (3.2%)	0 (-)
Normal FSH	987 (95.2%)[a]	78 (83.0%)[b]	20 (33.9%)[c]
High FSH	30 (2.9%)	13 (13.8%)	39 (66.1%)

*Serum FSH concentrations for each man were compared with the age-appropriate reference interval and then were described as low, normal or high in Table 2. Statistical comparisons are made with χ^2 test, and the results with different superscript in a row are significantly different, all $P<0.01$. FSH: follicle-stimulating hormone.

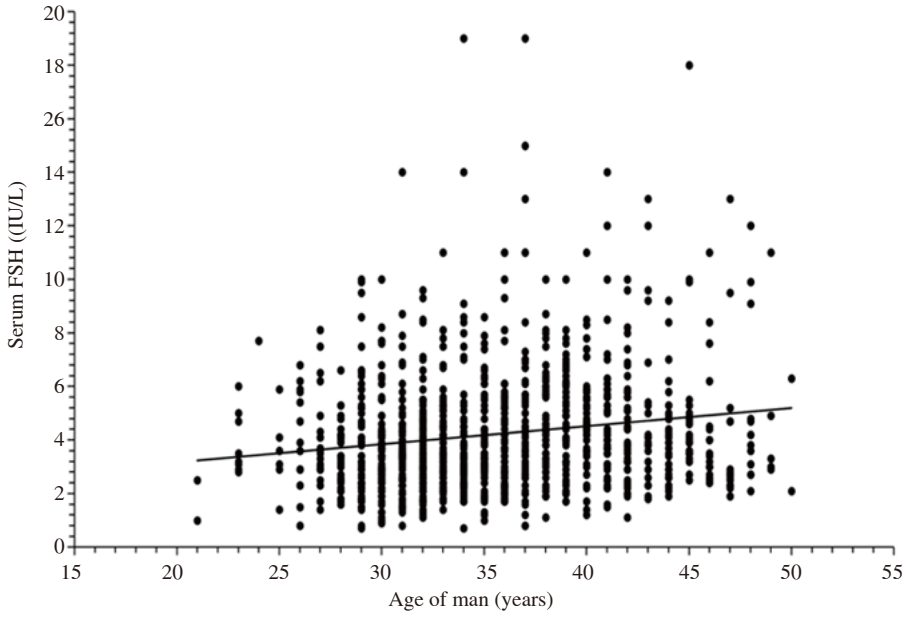

Figure 1. The scattergram of serum FSH concentrations against age for the 1 037 normozoospermic men. A trendline shows the overall change in pattern, and there is a significant positive association between FSH and age (Spearman's $\rho = 0.170$, $P<0.01$). FSH: follicle-stimulating hormone.

men producing normozoospermic samples had FSH concentrations outside of the reference intervals, consistent with the intervals being derived from 95% of the men. The proportion of men with normal FSH concentrations in the three age groups was different overall (χ^2 test; $P<0.05$) and post hoc testing showed that the proportion of oligozoospermic men with normal FSH concentrations was less than the normozoospermic men, which in turn was less in the azoospermic men (χ^2 test; both $P<0.01$). The azoospermic men were further broken down according to the nature of the azoospermia as either obstructive azoospermia or non-obstructive azoospermia. A total of 86.4% (38/44) men with non-obstructive azoospermia had elevated serum FSH concentrations whereas only 6.7% (1/15) men with obstructive azoospermia had high FSH levels showing significant difference (χ^2 test; $P<0.01$).

4. Discussion

Reference intervals are usually derived from a group of healthy individuals against which others can be deemed unhealthy if the analyte under investigation is outside of the reference interval[11]. In this study dealing with the measurement of serum FSH as part of the investigation of male infertility, the reference group were sexually mature men producing normozoospermic semen samples, with men who had possible semen abnormalities through disease or medication being excluded. The identification of such a reference group of men based upon the quality of their semen is not without difficulty as the performance of semen analysis itself has long been known through external quality assurance schemes to potentially have poor accuracy and precision[23], and more recent findings have shown some counting chambers to give erroneous results when counting human sperm[24]. Furthermore, the reference limits for semen analysis have changed over the years[25–27], confounding the definition of men whose semen can be categorized within normal limits. The laboratory in this study therefore used the methods[20] and lower reference limits[21] most recently recommended by the WHO for semen analysis and the interpretation of results. The laboratory also participated in an external quality assurance scheme to demonstrate acceptable performance in the measurement of sperm concentration, sperm motility and sperm morphology.

Age-related increases in serum FSH during reproductive life have been reported previously[13–15], although some reports only show an increase in serum FSH from the 5th[28] or 6th[29] decade of life. Nevertheless, the reality is that the vast majority of reference intervals available do not take age into account[8,16–19]. This should be seen as a major limitation in the use of FSH as a biomarker of testicular function, with a possible loss of sensitivity in the detection of men with testicular deficiency.

The measurement of FSH in men is a fundamental investigation since it is considered to be an indicator of germinal function[30], and hence elevated serum FSH concentrations can be seen in some men with seminal abnormalities such as azoospermia, oligozoospermia, and the presence of varicocoeles[31]. More specifically, a raised concentration of FSH is considered a reliable indicator of germinal epithelial damage and is usually associated with azoospermia or severe oligozoospermia[5,32,33]. The present study reported 13.8% of oligozoospermic men to have elevated FSH levels. This is particularly important as the oligozoospermic group will inevitably have a range of underlying pathologies and the measurement of FSH helps to identify the sub-group of men with a degree of germinal epithelial damage. In further examining the group of azoospermic men, non-obstructive azoospermia was an important sub-category as these men had no obstruction within the male reproductive tract and would therefore contain a high proportion of men with extremely low levels or absence of spermatogenesis[34]. The present study showed a high proportion of men with non-obstructive azoospermia to have high FSH concentrations outside of the age-related reference intervals when compared with men with obstructive azoospermia. Low serum FSH can be a good predictor of the surgical recovery of sperm from men with non-obstructive azoospermia[35] such that motile sperm, even in extremely low numbers, can be used for intracytoplasmic sperm injection and generate pregnancies[36]. Nevertheless, others were able to collect surgically sperm even in the presence of high FSH concentrations[37]. Whether age-stratified reference intervals can help further improve the reliability of the predictive capacity of sperm retrieval linked to circulating FSH values remains to be seen.

The use of a set of age-related reference intervals for FSH from just one laboratory based upon a group of reference subjects from one location is technically only valid for that laboratory. An argument therefore exists for the use of the development of common reference intervals by laboratories using the same analytical platform[38], and choosing a number of suitable laboratories to collaborate in the production of such common age-based reference intervals could prove beneficial. The choice of laboratories is important as there are a number of pre-requisites for the establishment of such a common reference interval at the pre-analytical and analytical stages[39]. In the case of serum FSH interval ranges, the contributing laboratories should at least use 1) current WHO methodology to analyse semen and interpret results for the identification of normozoospermic men, and 2) the Siemens Centaur assay system for measuring serum FSH. The development of these reference intervals would be extremely valuable to users of the Siemens Centaur automated analyser and an improved alternative to the expected results offered by the company Information for Users document[40] and the numbers should be large enough, and then continuous reference intervals could be determined as found useful in other disciplines[41].

The limitations of the study were that patients were from only one geographical location, and the FSH analyses were made on one assay platform.

In conclusion, the present study has shown that male serum FSH concentrations increase with age making the use of age-stratified reference intervals important in the detection of men with abnormal testicular function, and the development of common reference intervals for users of the Siemens ADVIA Centaur XP automated analyser world-wide should be considered. FSH above method and age-specific reference intervals should alert the clinician to increased risk of underlying semen abnormalities and causes of male infertility.

Conflict of interest statement

The authors declare that they have no conflict of interest

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Authors' contributions

All authors have contributed positively to this manuscript. Emily-Jane Waller and Phillip Matson conceived and designed this study, undertook statistical analysis and wrote the initial draft of the manuscript. John Yovich and Jason Conceicao facilitated data collection and patient management. All authors contributed significantly to the proofreading, revision and final approval of the manuscript.

References

[1] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015; **13**(1): 37.

[2] Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril* 2002; **77**(5): 873-882.

[3] McLachlan RI, Gordon Baker HW, Clarke GN, Harrison KL, Matson PL, Holden CA, et al. Semen analysis: Its place in modern reproductive medical practice. *Pathology (Phila)* 2003; **35**(1): 25-33.

[4] Nieschlag E. Care for the infertile male. *Clin Endocrinol (Oxf)* 1993; **38**(2): 123-133.

[5] de Kretser DM, Burger HG, Hudson B. The relationship between germinal cells and serum FSH levels in males with infertility. *J Clin Endocrinol Metab* 1974; **38**(5): 787-793.

[6] Hunter WM, Edmond P, Watson GS, McLean N. Plasma LH and FSH levels in subfertile men. *J Clinl Endocrinol Metab* 1974; **39**(4): 740-749.

[7] Taieb J, Olivennes F, Birr AS, Benattar C, Righini C, Frydman R, et al. Comparison of day 3 FSH serum values as determined by six different immunoassays. *Hum Reprod* 2002; **17**(4): 926-928.

[8] Amballi A, Dada O, Adeleye A, Salu J. Evaluation of the determination of reference ranges for reproductive hormones (prolactin, FSH, LH, and testosterone) using enzyme immuno assay method. *Sci Res Essays* 2007; **2**(4): 135-138.

[9] Sikaris K, McLachlan RI, Kazlauskas R, De Kretser D, Holden CA, Handelsman DJ. Reproductive hormone reference intervals for healthy fertile young men: Evaluation of automated platform assays. *J Clin Endocrinol Metab* 2005; **90**(11): 5928-5936.

[10]Stemp M, McClements A, Sykes P, Chapple V, Matson P. The measurement of oestradiol, progesterone, LH, FSH and hCG for assisted reproduction: A comparison of the Siemens Centaur CP and Roche e411 automated analysers. *Asian Pac J Reprod* 2013; **2**(4): 321-325.

[11]Jones G, Barker A. Reference intervals. *Clin Biochem Rev* 2008; **29**(Suppl 1): S93.

[12]Sikaris KA. Physiology and its importance for reference intervals. *Clin Biochem Rev* 2014; **35**(1): 3.

[13]Nahoul K, Roger M. Age-related decline of plasma bioavailable testosterone in adult men. *J Steroid Biochem* 1990; **35**(2): 293-299.

[14]Neaves WB, Johnson L, Porter JC, Parker Jr CR, Petty CS. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. *J Clin Endocrinol Metab* 1984; **59**(4): 756-763.

[15]Bjørnerem A, Straume B, Midtby M, Fønnebø V, Sundsfjord J, Svartberg J, et al. Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: The Tromsø study. *J Clin Endocrinol Metab* 2004; **89**(12): 6039-6047.

[16]Dighe AS, Moy JM, Hayes FJ, Sluss PM. High-resolution reference ranges for estradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system. *Clin Biochem* 2005; **38**(2): 175-179.

[17]Andersson AM, Petersen JH, Jørgensen N, Jensen TK, Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: Significance of adequate reference values from proven fertile men. *J Clin Endocrinol Metab* 2004; **89**(6): 2873-2879.

[18]Barbotin AL, Ballot C, Sigala J, Ramdane N, Duhamel A, Marcelli F, et al. The serum inhibin B concentration and reference ranges in normozoospermia. *Eur J Endocrinol* 2015; **172**(6): 669-676.

[19]Meeker JD, Godfrey-Bailey L, Hauser R. Relationships between serum hormone levels and semen quality among men from an infertility clinic. *J Androl* 2007; **28**(3): 397-406.

[20]WHO. *WHO laboratory manual for the examination and processing of human semen*. 5th ed. Geneva, Switzerland: World Health Organization; 2010.

[21]Cooper TG, Noonan E, von Eckardstein S, Auger J, Gordon Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010; **16**(3): 231-245.

[22]Horowitz GL, Altaie S, Boyd JC, Ceriotti F, Garg U, Horn P, et al. *Clinical and Laboratory Standards Institute (CLSI) C28–A3: Defining, establishing, and verifying reference intervals in the clinical laboratory*. vol. 28. Annapolis Junction, Maryland, USA: Clinical and Laboratory Standards Institute; 2008, p.1-61.

[23]Matson P. External quality assessment for semen analysis and sperm antibody detection: Results of a pilot scheme. *Hum Reprod* 1995; **10**(3): 620-625.

[24]Zuvela E, Matson P. Accuracy and precision of four types of chamber used to measure sperm concentration: Results of 12 consecutive years' experience from an external quality assurance programme. *Reprod BioMede Online* 2020; **41**(4): 671-678.

[25]WHO. *WHO laboratory manual for the examination of human semen and semen–cervical mucus interaction*. 2nd ed. Cambridge: Cambridge University Press; 1987.

[26]WHO. *WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction*. 3rd ed. Cambridge: Cambridge University Press; 1992.

[27]WHO. *WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction.* 4th ed. Cambridge: Cambridge University Press; 1999.

[28]Isurugi K, Fukutani K, Takayasu H, Wakabayashi K, Tamaoki BI. Age-related changes in serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in normal men. *J Clin Endocrinol Metab* 1974; **39**(5): 955-957.

[29]Tennekoon K, Karunanayake E. Serum FSH, LH, and testosterone concentrations in presumably fertile men: effect of age. *Int J Fertil* 1993; **38**(2): 108-112.

[30]Sultan C, de Paulet Craste B, Audran F, Iqbal Y, Ville C. Hormonal evaluation in male infertility. *Ann Biol Clin (Paris)* 1985; **43**(1): 63-66.

[31]Babu SR, Sadhnani M, Swarna M, Padmavathi P, Reddy P. Evaluation of FSH, LH and testosterone levels in different subgroups of infertile males. *Indian J Clin Biochem* 2004; **19**(1): 45-49.

[32]Franchimont P, Millet D, Vendrely E, Letawe J, Legros JJ, Netter A. Relationship between spermatogenesis and serum gonadotropin levels in azoospermia and oligospermia. *J Clin Endocrinol Metab* 1972; **34**(6): 1003-1008.

[33]Bergmann M, Behre HM, Nieschlag E. Serum FSH and testicular morphology in male infertility. *Clin Endocrinol (Oxf)* 1994; **40**(1): 133-136.

[34]Chen SC, Hsieh JT, Yu HJ, Chang HC. Appropriate cut-off value for follicle-stimulating hormone in azoospermia to predict spermatogenesis. *Reprod Biol Endocrinol* 2010; **8**(1): 108.

[35]Gnessi L, Scarselli F, Minasi MG, Mariani S, Lubrano C, Basciani S, et al. Testicular histopathology, semen analysis and FSH, predictive value of sperm retrieval: supportive counseling in case of reoperation after testicular sperm extraction (TESE). *BMC Urol* 2018; **18**(1): 63.

[36]Kanto S, Sugawara J, Masuda H, Sasano H, Arai Y, Kyono K. Fresh motile testicular sperm retrieved from nonobstructive azoospermic patients has the same potential to achieve fertilization and pregnancy *via* ICSI as sperm retrieved from obstructive azoospermic patients. *Fertil Steril* 2008; **90**(5): 2010. e5-2010. e7.

[37]Ramasamy R, Lin K, Gosden LV, Rosenwaks Z, Palermo GD, Schlegel PN. High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. *Fertil Steril* 2009; **92**(2): 590-593.

[38]Jones GR, Barker A, Tate J, Lim CF, Robertson K. The case for common reference intervals. *Clin Biochem Rev* 2004; **25**(2): 99.

[39]Petersen PH, Rustad P. Prerequisites for establishing common reference intervals. *Scand J Clin Lab Invest* 2004; **64**(4): 285-292.

[40]Siemens Diagnostic Healthcare Inc. *FSH instructions for use.* Rev. U, 2020-06. Tarrytown, NY, USA: Siemens; 2020. [Online]. Available from: https://doclib.siemens-healthineers.com/rest/v1/view?document-id=760438. [Accessed on 5 May 2021].

[41]Asgari S, Higgins V, McCudden C, Adeli K. Continuous reference intervals for 38 biochemical markers in healthy children and adolescents: Comparisons to traditionally partitioned reference intervals. *Clin Biochem* 2019; **73**: 82-89.