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Research Article

Implementation of World Health Organization Recommendations for Semen Analysis: A Survey of Laboratories in the United Kingdom

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The standard method for identification of male fertility status is a semen analysis. This is performed in fertility and pathology laboratories accredited by different bodies in the UK such as the HFEA or UKAS, and is based on whether they perform licenced clinical treatment or diagnostic testing. The WHO laboratory semen analysis criteria provide the most comprehensive guidance for best practice, yet this is not strictly adhered to. Our objective was to determine any differences in semen analyses between laboratories in the UK, based on the regulatory body they are registered with. A cross-sectional survey was sent to NEQAS for andrology registrants ($n = 184$ laboratories), HFEA ($n = 117$ clinics), and individual ARCS members ($n = 682$). Most ARCS members are associated with NEQAS and/or the HFEA. A ~50% laboratory response rate ($n = 106$ included responses) was found. Results were grouped based on accreditation: Group 1, UKAS accredited only ($n = 38$); Group 2, both UKAS accredited and HFEA licenced ($n = 17$); Group 3, HFEA licenced only ($n = 42$); and Group 4, no accreditation ($n = 9$). Over 85% of UKAS accredited laboratories (Groups 1 and 2) state they perform semen analysis according to WHO 2010 recommendations and adhere to best practice guidelines. A significantly fewer number of HFEA only laboratories (<74% Group 3, $p < 0.01$) adhere to both guidelines. Non-HFEA laboratories (Groups 1 and 4) are almost all performing sperm counts according to WHO criteria, while <60% HFEA clinics (Groups 2 and 3) perform counts according to regulation (Group 1 vs. Groups 2 and 3: Fixed sperm, $p < 0.05$; Neubauer chamber: $p < 0.005$). QC is implemented in most laboratories, however there is a significant difference ($p < 0.01$) between non-UKAS (Groups 3 and 4) and UKAS laboratories (Groups 1 and 2). There is a significant difference in semen analysis performance between UKAS and HFEA laboratories with regards to implementation of best practice guidelines and QC procedures. This may have a detrimental effect on result accuracy and consequently lead to patient misdiagnosis and mismanagement.

1. Introduction

Semen analysis is performed as an indicator of male reproductive health and for many decades, it has been the only routine test available for diagnosis of male infertility, affecting approximately 30-million men globally [1]. The test focuses on the assessment of a male ejaculate consisting of the spermatozoa and male accessory gland secretions [2]. These components are measured *in vitro* and evaluated to give an overall indication of male fertility, providing information about testicular and male accessory gland function [2, 3]. This includes measurement of volume, sperm motility, concentration, morphology, vitality, and physical characteristics of the fluid such as appearance, viscosity, pH, and liquefaction [2, 3]. Cellular components other than spermatozoa, such as polymorphonuclear leucocytes as well as antisperm antibodies, may also be determined in a semen analysis, which may be indicative of male accessory gland inflammation and or infection [4, 5].

Semen analysis was first implemented in the 1930s and is generally still used to this day as the first line test to determine treatment for male infertility [6]. It is therefore essential that the semen analysis provides accurate and reliable results that are

properly validated. If the analysis is incomplete or the test is unreliable, this may lead to certain pathologies being overlooked or a misdiagnosis of the patient in terms of their fertility potential, resulting in inappropriate treatment, unnecessary financial cost, and emotional distress [7]. Semen analyses should therefore be performed according to best practice guidelines, with robust quality systems in place to ensure accurate results with well-defined reference limits for the test [7, 8]. This is ascertained in andrology by adhering to the International Organization for Standardization (ISO) 15189:2012 standard [9] and more recently, the ISO 23162:2021 standard [10], ensuring consistent, unbiased, repeatable, and reliable results, thereby maintaining professional quality assurance [11].

Recommendations for semen analysis were first introduced by the World Health Organization (WHO) in 1980 and have been updated on a regular basis [12, 13]. WHO criteria have been recognised internationally as the gold standard for semen analysis, incorporating both ISO 15189:2012 and ISO 23162:2021 [14]. In the UK, the United Kingdom Accreditation Service (UKAS) was established to assess the competency and compliance of laboratories that provide diagnostic testing. UKAS oversees the implementation of the ISO standards and will provide accreditation for those laboratories that meet those standards. Accredited laboratories must demonstrate their ability to meet minimum standards as well as adhere to internal as well as external quality control and must have a quality management system in place [8, 15, 16]. UKAS assesses andrology laboratories against the ISO standards incorporated within the WHO criteria for both preassessment and assessment stages. Unfortunately, in recent years, many pathology services have not included andrology services as part of their UKAS accreditation status, due to the rigorous effort and cost required for accreditation with generally minimal benefit to the umbrella pathology laboratory service [17]. Hence, diagnostic semen analysis is often carried out in UK laboratories with no accreditation for the test. However, according to Green et al. [18], ISO standards are required to maintain minimal standards of competency in pathology, especially for laboratories who have fewer resources available to them, since the benefits of ISO competency and UKAS accreditation are considerable in terms of providing reliable results. Semen analyses are often performed in fertility clinics in the UK, which are required by law to hold a licence to practice by the Human Fertilisation and Embryology Authority (HFEA). This licence determines best practice for assisted conception treatment [19, 20], however there is no requirement to have UKAS accreditation for semen analysis.

The aim of this study was to determine the quality of practice for performing semen analysis in various laboratories around the United Kingdom, including both UKAS and non-UKAS accredited laboratories, as well as HFEA licenced laboratories, and to determine whether routine diagnostic testing for male infertility is fit for purpose.

2. Materials and Methods

2.1. Study Design, Size, and Duration. A cross-sectional email survey was sent out in May 2021 using Jisc Online Surveys (*Supplementary Materials*). The survey was sent to laboratories and clinics in the UK National External Quality Assessment Service (NEQAS) for andrology ($n = 184$), all individual members of the Association of Reproductive and Clinical Scientists (ARCS) ($n = 682$) and all fertility clinics licenced by the HFEA ($n = 117$). The majority of the ARCS members contacted work for a NEQAS participating laboratory. A general introduction to the survey was provided prior to taking part. In order to reduce bias, the survey introduction made clear that the answers should be given by only one individual from each laboratory (even if multiple ARCS members work in that laboratory). This individual should hold a permanent laboratory position at the facility and be familiar with semen analysis (determined by Q2 in the survey). It was noted that the answers provided in this survey should pertain to current laboratory practice, rather than the personal views of the respondent. The survey questions were designed to demonstrate how laboratories in the United Kingdom perform semen analysis and how the results are used to diagnose and manage the patient. Data were collected to determine, (a) the nature of the laboratories involved in conducting semen analyses including their accreditation status; (b) whether laboratories perform semen analyses according to best practice guidelines (WHO 2010 recommendations and ISO 15189:2012 standards; (c) implementation of quality control in the laboratory; and (d) the reference limits used and how the results affect the patient pathway. At the time of sending this survey, the current WHO criteria for semen analysis was published in 2010 (5^{th} edition). A complete list of the questions in the survey is provided in the *Supplementary Materials*. Each question in the survey had to be answered in order to continue. Comment sections were made available for justification of answers if necessary. The survey was designed to take no more than 10 min to complete, and all responses were collected anonymously.

2.2. Respondents. A total of 108 responses were recorded. Any clinics or laboratories which are not part of the United Kingdom were excluded ($n = 1$) as this survey was intended to represent the current local coverage of fertility clinics and laboratories. One response was excluded as it was not answered by a trained laboratory member of staff. Some laboratories would have been contacted more than once if they were registered with ARCS, HFEA, and/or NEQAS. Individual survey answers were analysed for overlaps and repeats within the answers to determine whether there were any persons answering more than once and none were found.

2.3. Statistical Analysis. The data were analysed using the Jisc Online Survey Analyse tool. Statistical analyses were performed using a two-tailed χ^2 test at significance level of $p < 0.05$ for both within group, out of group and between category analyses. A Yate's correction for continuity was used to compensate for deviations for results less than $n = 5$.

3. Results

3.1. Section 1: Distribution of Laboratories. Q1: Description of the laboratory.

Table 1: Distribution of laboratories performing semen analysis in the UK.

Laboratory	Group 1 ($n=38$)	Group 2 ($n=17$)	Group 3 ($n=42$)	Group 4 ($n=9$)
NHS pathology	63%	18%	0%	22%
NHS fertility	16%	18%	24%	33%
Private pathology	3%	0%	0%	0%
Private fertility	5%	35%	52%	11%
Combination of above	13%	29%	24%	33%

Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation.

Table 2: The purpose of laboratory semen analysis.

Purpose of semen analysis	Group 1 ($n=38$)	Group 2 ($n=17$)	Group 3 ($n=42$)	Group 4 ($n=9$)
Diagnostic test of male infertility	100%	100%	76% $^{*}p=0.006$	100%
To select which assisted conception procedure to use	18%	65% $^{*}p=0.0007$	86% $^{*}p<0.00001$	44%
Sperm donor work-up	16%	59% $^{*}p=0.001$	50% $^{*}p=0.001$	11%
Other (e.g., post-vasectomy analysis)	13%	12%	5%	11%

Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation. *Significantly different from Group 1.

A total of 106 laboratories were included in the study. Laboratories taking part in the survey were either pathology laboratories ($n=30$), fertility clinic laboratories ($n=53$), or a combination of both ($n=23$). The majority of pathology laboratories was National Health Services (NHS) government funded organisations ($n=27$) while most fertility clinics were either NHS ($n=22$) or privately ($n=31$) funded. The remainder were jointly funded from both the NHS and private sector.

Q3: Is your laboratory UKAS accredited and/or HFEA licenced?

Table 1 shows the distribution of laboratories that took part in the survey and their accreditation status. There was a fairly even distribution of UKAS ($n=55$) vs. non-UKAS ($n=51$) accredited laboratories. The majority of respondents from the public sector (NHS) was UKAS accredited, while private fertility laboratories contributed the majority of respondents from HFEA licenced clinics. For the purposes of this study, responses were divided into four categories based on accreditation status: Group 1, UKAS only ($n=38$); Group 2, UKAS and HFEA ($n=17$); Group 3, HFEA only ($n=42$); and lastly, Group 4, neither UKAS nor HFEA ($n=9$). All responses were individually processed to verify they were in the correct categories. Two laboratories were in the process of UKAS application and were therefore included in Group 1. One laboratory was Clinical Pathology Accreditation (CPA) accredited (to WHO 2010 recommendations), as well as HFEA licenced, and therefore was included in Group 2. One laboratory was UKAS accredited for microbiology, but not andrology, and was therefore included in Group 4.

Q4: What is the purpose of your laboratory semen analysis?

Laboratories were asked to state the purpose of their semen analysis (Table 2). All Group 1 laboratories (100%) conduct semen analyses as a diagnostic test for male infertility with 18% also indicating the test is used for determining which assisted conception treatment to use. In contrast, significantly less Group 3 clinics compared to Group 1 use the test for diagnostic purposes (76%, $p<0.001$) with the majority using semen analyses primarily to select which assisted conception procedure to use (86%, $p<0.00001$). Clinics that have both UKAS accreditation and an HFEA licence (Group 2) are more likely to use the test to determine ART procedure than laboratories with UKAS accreditation alone (Group 1) (65% vs. 18%, respectively, $p=0.0007$). Several laboratories in almost all groups also provide semen analysis post-vasectomy and/or prior to sperm cryopreservation.

3.2. Section 2: Laboratory Compliance. Q5: Does your laboratory carry out semen analysis AND report ALL parameters strictly according to WHO 2010 guidelines?

A total of 95% UKAS only accredited laboratories state they perform and report semen analyses strictly according to WHO criteria while only 71% of HFEA licenced clinics claim to follow these recommendations ($p=0.006$), unless they were also UKAS accredited (88%) (Table 3). All UKAS accredited laboratories (Group 1) and 98% of HFEA licenced clinics (Group 3) use WHO 2010 reference limits with one HFEA laboratory using WHO 1999 for non-diagnostic purposes. In-house reference limits are used in one laboratory with no accreditation (Group 4). However, when asked whether their laboratories adhere to ISO 15189:2012 and WHO 2010 criteria, 97% Group 1 follow these specifications, yet only 74% of Group 3 and 78% of Group 4 laboratories confirmed they do ($p=0.003$ and 0.031, respectively, vs. Group 1). There is no significant difference between Groups 1 and 2, with regards to adherence to ISO 15189:2012 and WHO criteria.

Table 3: Laboratory compliance to World Health Organization (WHO) criteria.

Laboratory compliance	Group 1 ($n=38$)	Group 2 ($n=17$)	Group 3 ($n=42$)	Group 4 ($n=9$)
Q5. Does your laboratory carry out semen analysis AND report ALL parameters strictly according to WHO 2010 guidelines?	95%	88%	71% $^{*}p=0.006$	89%
Q6. Do you use WHO 2010 reference values on your semen analysis report?	100%	100%	98%	89%
Q15. Does your laboratory adhere to best practice guidelines, i.e., ISO 15189 and WHO 2010 criteria?	97%	88%	74% $^{*}p=0.003$	78% $^{*}p=0.031$

ISO: International Organization for Standardization. Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation. ISO: International Organization for Standardization. *Significantly different from Group 1.

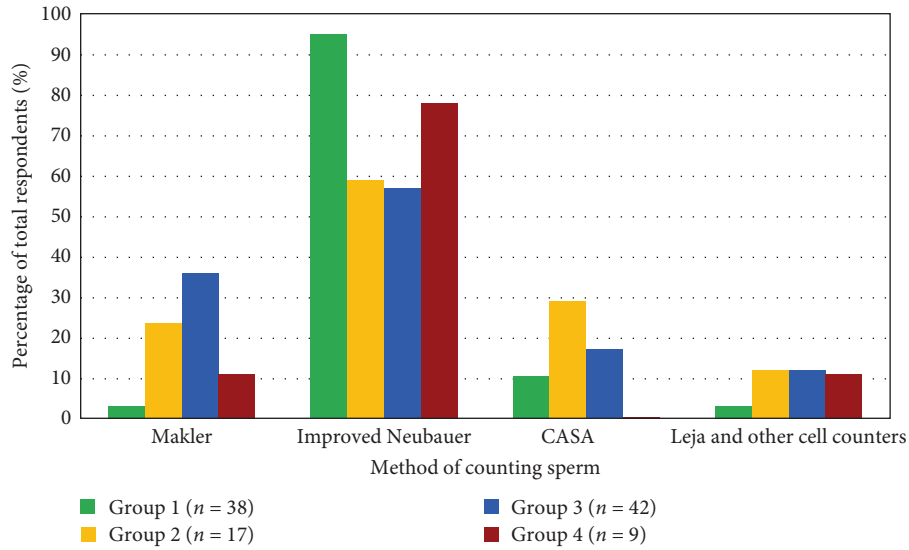

Figure 1: Methods of counting sperm. Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation. CASA: computer assisted semen analysis.

3.3. Section 3: Performance of Semen Analysis

3.3.1. Motility. Q7 and Q8: Does your laboratory use manual, CASA or other methods to assess motility? How does your laboratory report motility?

The method for assessing and reporting of motility is similar between laboratories, with the majority using the manual method (Group 1, 82%; Group 2, 71%; Group 3, 90%; Group 4, 100%). Both HFEA licenced and UKAS accredited laboratories primarily use the CASA system as an alternative method for assessing motility, dependent on sperm concentration (Group 1, 21%; Group 2, 29%; Group 3, 17%; and Group 4, 0%). There was no significant difference in the methods used between the groups. The majority of laboratories is reporting progressive (≥65%), non-progressive motility (≥64%), and immotile sperm (≥62%), however a smaller proportion of laboratories accredited by UKAS and licenced by HFEA (Group 2) are also reporting rapid and sluggish progressive motility (53% and 59%, respectively), as well as those laboratories without accreditation (Group 4; 44% and 33%, respectively). Previous (WHO 1999) and current (WHO 2021) WHO recommendations required laboratories to report and distinguish between rapid and sluggish motility, however the WHO 2010 recommendations did not. Interestingly, five of the HFEA licenced laboratories (Groups 2 and 3) are using an in-house reporting method for progressive motility, scoring out of 4, while none of the laboratories in Group 1 use this method, which is not recommended by WHO 2010.

3.3.2. Count. Q9 and Q10: Does your laboratory perform sperm counts on motile or immobilised sperm? What chamber does your laboratory use for counting sperm?

The standard WHO recommendation for counting sperm (WHO 2010 and 2021) is under fixed conditions, and by using an improved Neubauer counting chamber. Not all laboratories perform analysis for the individual parameters according to WHO 2010 criteria and there is a significant difference in methodology between laboratories. Only 59% of Group 2 and 57% of Group 3 laboratories perform the test on fixed samples, whereas 84% Group 1 laboratories fix the samples prior to counting (Group 1 vs. Group 2, $p=0.041$; Group 1 vs. Group 3, $p=0.008$). A total of 67% Group 4 laboratories count fixed sperm, and this was not significantly different to Group 1.

Figure 1 shows that almost all Group 1 laboratories (95%) use an improved Neubauer chamber for sperm counts which were significantly higher than the proportion of Group 2 (59%, $p=0.0009$) and Group 3 (57%, $p=0.0001$)

laboratories. Again, there was no significant difference between Group 1 and Group 4 laboratories. A considerable number of Group 2 and Group 3 laboratories additionally use Makler chambers (23.5% and 36.0%, respectively), CASA systems (29.0% and 17.0%, respectively), or Leja or Cell Vision chambers (12% in each case) which would be performed on motile sperm. Unlike assessment of fixed sperm on a Neubauer chamber, these methods for counting sperm are not endorsed by WHO 2010, WHO 2021, or ISO 23162:2021. Group 4 laboratories primarily use improved Neubauer chambers to assess sperm count (78%).

3.3.3. Morphology. Q11 and Q12: In your laboratory, is morphology performed on fixed or motile samples? Are samples stained for morphology in the lab and if so, with what stain?

Almost all UKAS accredited (Group 1) laboratories and those without any accreditation (Group 4) conduct morphology assessments using fixed (Group 1, 97%; Group 4, 89%) and stained (Group 1, 100%; Group 4, 89%) conditions as required by WHO criteria, whereas for Group 2, 76.5% fix and 82% stain sperm for morphology; and in Group 3, only 52% fix and 48% stain sperm prior to assessing sperm morphology (Figure 2(a)). Both the fixed and stained results for Groups 2 and 3 are significantly different to Group 1 (Group 2, fixed $p = 0.013$ and stained $p = 0.048$; Group 3, $p < 0.00001$, for both fixing and staining). Overall assessment of sperm morphology according to WHO 2010 criteria indicated Group 4 laboratories are not significantly different from Group 1. Laboratories that do not perform morphology on stained samples acknowledge that they would require specific training in interpretation of the slides. According to WHO 2010 recommendations and ISO 23162:2021, only Papanicolou, Diff-Quick, and Shorr staining are recommended for morphology analysis. Upon further investigation, 89.5% of Group 1; 52.5% of Group 2; 16% of Group 3; and 55.5% of Group 4 laboratories use these recommended staining methods. Groups 2 and 3 laboratory results were once again significantly different to Group 1 in this regard ($p = 0.002$ and $p = 0.0008$, respectively; Figure 2(b)). No laboratories reported use of Shorr staining.

Q13 Does your laboratory report specific types of sperm defects (e.g., globozoospermia, pyriform heads, etc.)?

Although, the percentage of abnormal forms are reported by all laboratories that conduct morphology assessment, individual sperm defect reporting is more likely to be reported by laboratories with HFEA accreditation (Group 2, 76.5%; Group 3, 71%) than UKAS only accredited laboratories (Group 1, 58%), however this is not significantly different. Most Group 4 laboratories report individual defects (89%). HFEA accredited (Group 3) laboratories report on specific types of abnormality such as tapered or pyriform heads and globozoospermia. UKAS accredited laboratories (Groups 1 and 2), report abnormal forms but tend not to report what type of abnormality is seen, citing reasons including that it is not a WHO requirement or that it may not be useful to users. Two Group 2 laboratories do not assess individual sperm defects as treatment recommendations would not be affected by this. Another stated that all patients with abnormal morphology are always advised ICSI treatment regardless of type of sperm defect. One Group 4 laboratory commented that they only provide a first line screening service and considered that any significant abnormality should be investigated in a dedicated andrology laboratory, while another provides a service for doctors (GPs) who they considered did not require the details of particular sperm defects.

3.3.4. Other Parameters. Q14 What other seminal fluid parameters does your lab report?

Volume and viscosity were assessed in almost every laboratory irrespective of whether they were HFEA licenced, or UKAS accredited (Figure 3). The majority of UKAS accredited labs (Group 1) reported appearance, pH and vitality (94%, 97%, and 71%, respectively), while significantly less HFEA licenced clinics reported these parameters. Laboratories with no accreditation (Group 4) were also less likely to report these parameters. Although, round cells are reported in most laboratories across the board, relatively few laboratories delineate between leucocytes and immature sperm cells. Surprisingly, Groups 2 and 3 laboratories are more likely to determine leucocytes (29% and 31%, respectively) compared to Group 1 laboratories, where only 8% identify leucocytes in semen ($p < 0.05$). A similar observation was found with regard to antisperm antibody testing where this was only offered in 37% Group 1 laboratories compared to 71% in Group 2 and 69% in Group 3. Although Group 1 laboratories are accredited for semen analysis against ISO 15189:2012, not all these laboratories see the benefit in testing for seminal fluid constitution outside of the standard sperm parameters.

3.4. Section 4: Quality Control. Almost all laboratories take part in NEQAS irrespective of whether they have UKAS accreditation or an HFEA licence (Table 4). Significantly fewer laboratories with no accreditation or licence take part in NEQAS (Group 1 vs. Group 4, $p = 0.038$). Interestingly, in all groups, not all the laboratories that take part in NEQAS actually perform the semen analysis for their patients using the same method. Based on responses from laboratories which do not implement the same method as NEQAS, it is clear that CASA systems for semen analysis are used in the majority of these laboratories and they are not designed to be used with fixed NEQAS samples. Furthermore, laboratories differ in the implementation of quality control. While all laboratories with UKAS accreditation (Groups 1 and 2) implement IQC, significantly less Group 3 (90.5%, $p = 0.01$ vs Group 1) and particularly Group 4 (56.0%, $p < 0.001$ vs Group 1; $p = 0.003$ vs Group 2; $p = 0.009$ vs Group 3) laboratories perform IQC. For these laboratories, many cite lack of time, resources and/or staff to conduct IQC for semen analysis and resort to using the NEQAS samples for their quality control instead.

3.5. Section 5: Reporting Results. When semen parameters are outside of the reference range, the majority of HFEA (Group 3) laboratories (64%) comment that the sample is suitable for ICSI treatment and 36% recommend referral to a fertility clinic. In contrast, none of the UKAS (Group 1) laboratories comment on suitability for fertility treatment, with 39.5% not passing any comment, 34% simply stating the results are

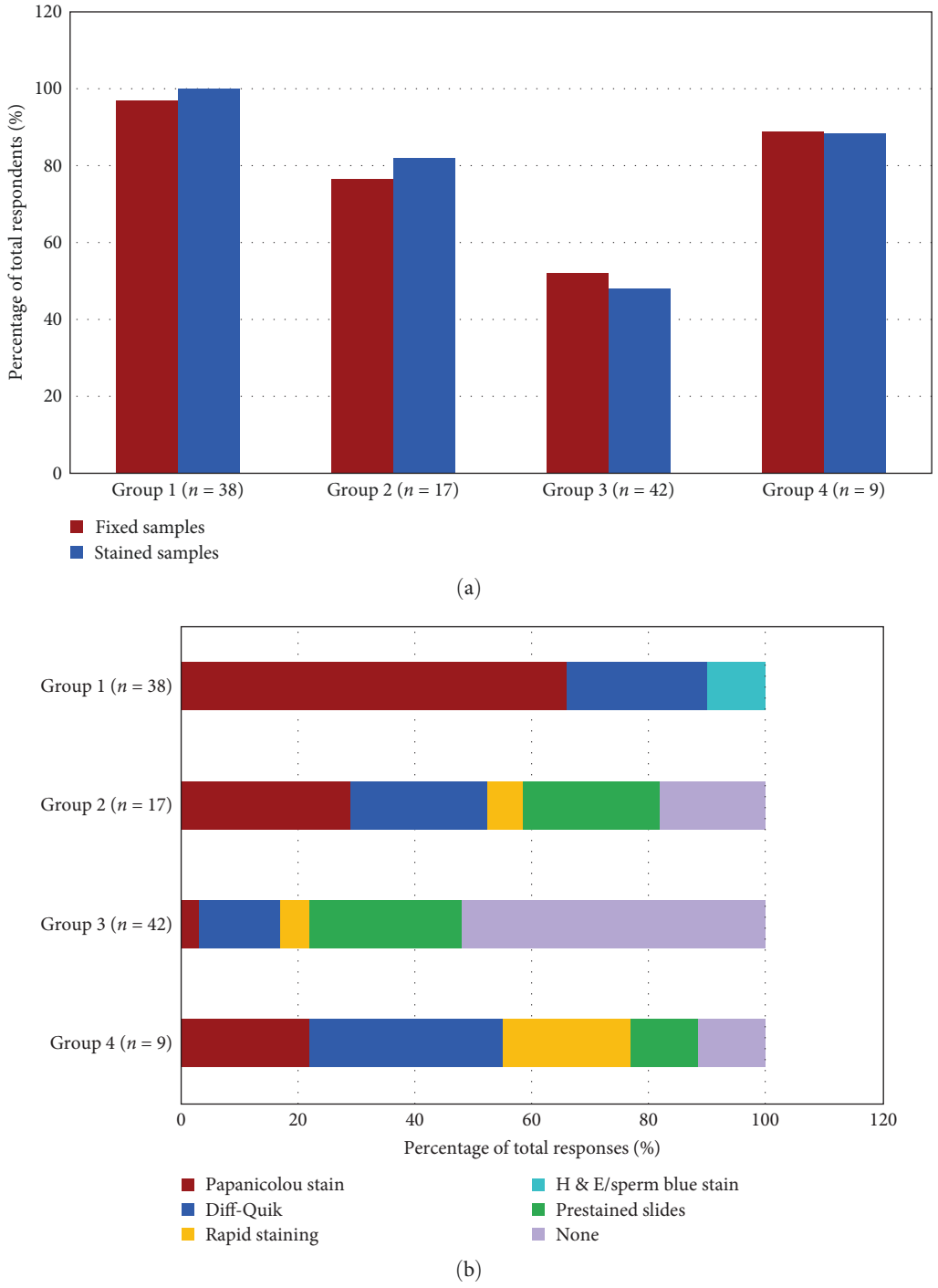

Figure 2: Methods in which different laboratories stain and/or fix their samples. Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation.

below range according to WHO 2010 criteria and 10.5% suggesting a repeat sample. A significant number of Groups 2 and 3 clinics also suggest referral to a urologist and/or to repeat the test (Table 5).

4. Discussion

It is clear from the results of this survey that there is a difference in approach to semen analysis in UKAS accredited laboratories compared to those laboratories which hold an HFEA licence or no accreditation at all [21]. UKAS accredited laboratories are almost always performing semen analysis according to ISO 15189:2012, whereas HFEA licenced and non-accredited laboratories are significantly less likely to. A limitation of this survey is that based on the number of laboratories that were contacted, only a proportion of them responded. In addition, laboratories performing semen analysis without an HFEA licence, and where the staff are not

Seminal fluid parameters	Group 1 ($n = 38$)	Group 2 ($n = 17$)	Group 3 ($n = 42$)	Group 4 ($n = 9$)
Volume	100%	100%	100%	100%
pH	97%	71% $^{*}p = 0.003$	64% $^{*}p = 0.0002$	56% $^{*}p = 0.0003$
Appearance	92%	82%	71% $^{*}p = 0.018$	56% $^{*}p = 0.006$
Liquefaction	74%	88%	88%	56% $^{**}p = 0.02$
Viscosity	97%	100%	90.5%	100%
Vitality	71%	53%	40.5% $^{*}p = 0.006$	67%
Round cells/other cells	89.5%	100%	95%	78% $^{*}p = 0.043$
Peroxidase positive cells or leukocytes	8%	29% $^{*}p = 0.036$	31% $^{*}p = 0.01$	11%
Antisperm antibodies	37%	71% $^{*}p = 0.021$	69% $^{*}p = 0.004$	44%

Figure 3: Proportion of laboratories reporting additional seminal fluid parameters. Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation. Dark green (80%–100%), light green (60%–79%), yellow (40%–59%), orange (20%–39%), red (0%–19%). *Significantly different from Group 1. **Significantly different from Group 3.

Table 4: Quality control in the laboratory.

Quality control	Group 1 ($n = 38$)	Group 2 ($n = 17$)	Group 3 ($n = 42$)	Group 4 ($n = 9$)
Q16. Does your laboratory take part in the United Kingdom NEQAS assessment?	100%	94%	98%	89%
Q16a. Does your laboratory implement the exact same method of assessment for the patient samples, as they do for the United Kingdom NEQAS samples?	95%	87.5%	88%	87.5%
Q17. Does your laboratory have internal quality controls in place?	100%	100%	90.5%	56%

Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: No accreditation.

Table 5: Comments included on report if values are outside of normal range.

Report comments	Group 1 ($n = 38$)	Group 2 ($n = 17$)	Group 3 ($n = 42$)	Group 4 ($n = 9$)
This sample suitable for ICSI treatment	0%	29% $^{*}p = 0.003$	64% $^{*}p < 0.00001$ $^{**}p = 0.03$	22% $^{*}p = 0.04$
Referral to a fertility clinic	13%	23.5%	36% $^{*}p = 0.04$	11%
Referral to a urologist	8%	35% $^{*}p = 0.016$	31% $^{*}p = 0.022$	11%
Below/out of parameter range	34%	12%	29%	33%
Repeat analysis	10.5%	23.5%	24%	22%
None	39.5%	18%	7% $^{*}p = 0.001$	22%

Group 1: UKAS only; Group 2: UKAS and HFEA; Group 3: HFEA only; Group 4: no accreditation. *Significantly different from Group 1. **Significantly different from Group 2.

members of ARCS or who do not partake in NEQAS would not have been contacted at all. Such laboratories are by default less likely to follow WHO recommendations which could have introduced bias into the survey results, favouring those who perform analyses as per WHO guidelines. The discrepancies between laboratories performing semen analysis have been of major concern to scientists and clinicians in the field for many years. Various studies around the globe have reported lack of standardisation in performance, quality control and reporting of semen analysis between laboratories, showing no sign of improvement over the years (USA [22]; Spain [23]; China [24]; Italy [25]; Poland [26]; Belgium [27]; Iran [28]; India [29]).

It is also concerning that while almost all HFEA licenced clinics and non-accredited laboratories state they are performing semen analysis according to standard WHO criteria, only some of them are [21], since a considerable number of these laboratories are performing manual sperm counts and morphology assessment on motile samples, and morphology is not always performed on WHO recommended stained samples. This indicates the laboratories are either unfamiliar with the requirements of WHO recommendations or they are misinterpreting them. It is not physically possible to perform an accurate assessment of sperm count or morphology on a motile sample [12]. Furthermore, the lack of detail in an unstained sample which is usually examined at lower magnification, could lead to significant inaccuracies in morphological assessment. Riddell et al. [30] conducted a survey on performance of sperm morphology in 35 UK laboratories indicating whether they were adhering to the WHO standards. They found only two laboratories (5%) were conforming to the WHO recommendations and complying with the quality control measures. This lack of adherence to best practice is not unique to UK laboratories, as a survey of 122 studies from the international scientific community, showed that 70% laboratories from all over the world who reported data for semen analysis, claimed they were following WHO criteria, yet overall agreement with WHO recommendations was weak [31].

This survey revealed that irrespective of accreditation or licencing, there are laboratories in all groups that are failing to perform a fully comprehensive semen analysis [21]. Some laboratories do not report individual morphological defects, although to their credit, HFEA licenced laboratories are more likely to report this. Those that do not, state that although this is described in the WHO guideline, there is no requirement to do so. Others do not see the relevance to their users or how it would alter treatment of the patients, however genetic syndromes such as globozoospermia, macrocephaly, primary ciliary dyskinesia (PCD), etc., are clear causes of infertility and can instantly point to the root cause of the problem [32–34]. Such conditions would have a significant detrimental impact on patient management if they are missed. For example, natural pregnancy is virtually impossible with globozoospermia, IVF would invariably result in failed fertilisation and even with ICSI, fertilisation rates are extremely poor. However, this can be circumvented using oocyte activation in conjunction with ICSI [35, 36]. Sperm vitality is not routinely performed and again, this provides useful information about whether the sperm may have a structural defect preventing motility or whether they are exposed to a toxic environment which could affect fertility [37, 38].

Apart from the sperm parameters, additional seminal fluid parameters are important markers for male accessory gland and testicular function as they can indicate infection, inflammation, obstruction, or presence of toxins [3, 39–41]. Clearly this is relevant for determining general reproductive health as well as infertility and is pertinent to a comprehensive diagnostic test. Although standardised methods for these parameters are described in WHO criteria [12], many laboratories in this survey are often failing to include them in their test, some citing the reason that there is no requirement to report them. This may well be an oversight by WHO given that assessment of additional parameters, including specific sperm morphological defects, provides more information, not only for identifying clinical causes of infertility [3], but may also provide insight into potentially pathological conditions of the genitourinary tract [12, 39, 40, 42]. By excluding any of these parameters, an incomplete result is reported, which may result in a misdiagnosis or a diagnosis of "unexplained infertility". Furthermore, it is important to point out that infertility is often a secondary effect of an underlying systemic illness [43] so a fully comprehensive semen analysis is essential to provide a proper investigation of the infertile male.

Another quality issue in this survey is the use of WHO reference limits by several laboratories who do not always comply with the WHO protocols. Differences in methodology can lead to significant variation in the results and hence the expected reference limits for that test [44, 45]. Reference limits for any test are determined for a particular methodology, so laboratories using a different methodology to WHO would have to establish their own reference limits for semen analysis which in turn would need to be verified and validated [24, 46]. There is no indication as to whether this is the case from this survey.

An essential part of a standardised test is quality control to determine consistent performance of staff, equipment, and reagents to ensure reliability of all stages of the semen analysis and obtain accurate and reliable results [8]. This survey did not address monitoring of equipment or batch testing of supplies, all of which can impact the measurement uncertainty of a result [46–48]. However, general questions about quality control in terms of staff performance revealed that implementation of internal quality controls (IQC) and external quality assurance (EQA) differs between accredited and non-accredited laboratories, with the latter not performing as well [21]. Almost all laboratories take part in EQA irrespective of their accreditation, and although both HFEA licenced and UKAS accredited laboratories have IQC in place, only 56% of non-UKAS accredited laboratories have implemented this. Another area of concern is that while almost all laboratories claim to take part in EQA, not all the laboratories actually use the same methods for semen analysis for their patients. Unfortunately, poor adherence to quality control within laboratories has created increased variation, human error, and subjectivity within measurements and creates considerable inter- and intra-laboratory variability in

results [8, 15, 16, 48, 49]. Unreliability and inaccuracy of the results may have considerable negative consequences not only on patient diagnosis and management, but also in interpreting scientific data, thereby impeding scientific progress towards our understanding of male infertility [14].

In the United Kingdom, medical practice is regulated by the National Institute of Clinical Excellence (NICE) guidelines which state that laboratories providing semen analysis should use methods and reference limits provided by the most recent WHO recommendations and that the reference limits used are only appropriate if using the WHO methodology [7]. NICE also state that the accuracy of the result is dependent on the sample being tested in a laboratory that adheres to accredited methods, where quality control is implemented and that procedures are routinely audited. Laboratories that do not perform semen analysis according to these principles are prone to unreliable results which may delay patient investigation and lead to inappropriate treatment [7]. So why is it that medical laboratories in the United Kingdom are failing to adhere to best practice guidelines, especially when the WHO protocols [12, 13] are very clear, are recommended by professional bodies as the gold standard for semen analysis [7, 50, 51] and are readily available across the globe? Many laboratories and clinics offer different services which often dictate their chosen methods of semen analysis. Some are purely diagnostic testing laboratories whereas others are embryology laboratories in the IVF clinics. Evidence shows that reliability of results is dependent upon rigorous staff training, strict compliance to WHO methods, implementation of IQC and use of EQA schemes [8, 16]. Lack of standardisation is due to a persistent failure to follow these principles, which has been recognised for many years [50]. However, manual semen analysis as advocated by WHO, is time-consuming and relatively costly and has been blamed for failure to implement good practice [50]. Indeed, in this survey, laboratories that do not implement IQC note time constraint, lack of resources, and staff as a factor [21]. This, along with the different skill sets of staff, the size and expertise of the clinic or laboratory, income, and management, may all contribute to the lack of compliance [15, 49]. Many fertility clinics may not have sufficient staff to perform semen analysis that is supported by the adequate external and internal quality assurances, as some of the comments in the survey have indicated, perhaps considering it unnecessary especially if their analyses are solely to determine whether the sample is suitable for a particular type of fertility treatment, rather than as a diagnostic tool for male reproductive health and infertility. Such a conclusion is supported by results in this survey which reveals the performance of clinics that have both HFEA and UKAS accreditation is mixed, probably depending on whether they are performing the analysis as a diagnostic test or simply to determine suitability for clinical use such as assisted conception treatment or cryopreservation. Other reasons for failing to adhere to WHO criteria may be because many diagnostic laboratories offering semen analyses are reluctantly performing andrology testing as part of a general pathology repertoire within an umbrella department such as microbiology or cytology, for example [17]. Such laboratories may not see the value in spending time and additional costs on a test that is not part of their main scope and may be lax in following best practice.

Semen analysis is the cornerstone to a diagnosis of male infertility and is the only routine test currently available [52–54]. It therefore must be performed according to the highest standards as so much is dependent on the result. Carrell and De Jonge [55] argue that the developments in the ART industry have led to a perceived decrease in the need for understanding the causes of male infertility, with many clinicians in the field mistakenly considering that male infertility does not need to be treated, as it can be circumvented with ICSI, thus undermining the value of a fully comprehensive and reliable semen analysis. This view is supported by the survey which shows that the main reason for semen analysis in an HFEA clinic is to determine which method of ART should be implemented, rather than using it as a diagnostic tool for male infertility [21]. Yet if a fully comprehensive standardised semen analysis can indicate an underlying reproductive health issue which can then be managed appropriately, then ICSI treatment and the emotional distress and cost associated with it may indeed be superfluous. Furthermore, semen analysis has long been known to be a marker of a man's general health [43, 56]. Therefore, it could be considered a moral obligation to perform a standardised semen analysis for men diagnosed with infertility to ensure the overall health of the patient as well as the most appropriate form of treatment.

Performance of semen analysis according to ISO 15189:2012 standards should be mandatory in all laboratories performing diagnostic semen analysis to secure an accurate and valid result. Failure to do so may have a detrimental effect on assisting men who may require further andrological or urological investigation. In no other area of medicine would we expect to provide results for a test that has not been properly validated or controlled, and where there may be risk of misdiagnosis. Of note, the HFEA states in its Code of Practice licence condition T50a [19] that the mandatory blood testing for virology for patients planning fertility treatment or storage of gametes and embryos must be carried out in a UKAS accredited laboratory. We would recommend that the same standards should be applied for semen analysis. By standardising semen analysis across different laboratory settings, results should be comparable thereby improving male fertility management. This would not only have significant implications regarding early diagnosis of male infertility but may progress research efforts into our understanding of male infertility.

5. Conclusions

There is a clear difference in semen assessment measures between UKAS vs. non-UKAS accredited laboratories both in terms of following best practice guidelines and application of QC. Additionally, there is a need for improved regulation regarding semen assessment within fertility clinics who tend not to be registered with UKAS and such regulation should be implemented in all laboratories performing diagnostic semen analysis to secure a valid result for an accurate diagnosis of male infertility.

Data Availability

All data generated or analysed during this study are included in this manuscript and its supplementary information files.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

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Supplementary Materials

Semen assessment in fertility and pathology laboratories and clinics. (*Supplementary Materials*)

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