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Original Article

A survey on the status of semen analysis in 118 laboratories in China

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Abstract

Collecting baseline information on how laboratories perform testing is a reasonable first step towards establishing intra- and inter-laboratory standardization and quality control for semen analysis. We carried out a survey of the laboratories performing the testing in Mainland China. A questionnaire, composed of 36 questions covering all aspects of semen analysis, was designed, and a copy was distributed to each of the 145 laboratories. Of these, 118 laboratories completed the questionnaires. The survey results showed that semen volume was measured visually in 53.6% (59/110) of the responding laboratories, and 70.9% (73/103) of laboratories analysed incompletely liquefied semen without any treatment. In addition, both manual-microscopic and computer-assisted semen-analysis systems were applied to analyse sperm concentration, motility and morphology. However, more than five methods were employed in routine sperm staining. An enzyme-linked immunosorbent assay was commonly used for determining whether antisperm antibodies were present. Several seminal biochemical markers were analysed in only 27.1% (32/118) of the responding laboratories. Generally, there was a lack of intra- and inter-laboratory quality control measures for semen analysis in all laboratories responding to this survey. In conclusion, the methods of semen analysis and the interpretation of test results in the surveyed laboratories differed markedly. In particular, many laboratories employed methods other than those recommended by the World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction (1999). These findings suggest an urgent need for the standardization of semen analysis with acceptable quality controls for each parameter to make the results repeatable and meaningful.

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1 Introduction

Semen analysis is performed as a routine clinical laboratory test in hospital laboratories, with more sophisticated testing in assisted reproductive technology (ART) and andrology laboratories. The volume of semen analyses carried out in ART laboratories is typically much higher than that in hospital laboratories. In general, patients are asked to abstain from ejaculation for 3–7 days before sampling and testing. They submit a semen sample collected by masturbation, and a brief history is taken to determine whether a patient has experienced events (for example, use of certain medications or recent fever) that could affect the results and a routine analysis of the collected specimen. Essential testing consists of assessment of liquefaction, volume, viscosity, pH, motility, vitality, concentration (including, in some laboratories, total sperm count) and stained morphology. Other testing involves examination of antisperm antibodies, leukocytes and seminal biochemi-

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stry markers; identification of spermatogenic cells; and sperm function tests, microbiology cultures and other tests. It is important that testing be accurate because clinical diagnosis and treatment are based directly on those results. Moreover, consistency in semen-analysis methods among laboratories is necessary to ensure comparable results and to avoid expensive retesting for patients. However, several studies suggest that results of semen analysis often vary both within the same laboratory and among laboratories because of a lack of standardization, quality control measures, proficiency testing, updated training and continuing education [1–4].

An urgent need for the standardization and quality control of semen analysis has been considered by the staff of these laboratories [4–6]. Collecting baseline information on how laboratories perform testing is a reasonable first step towards establishing intra- and inter-laboratory standardization and quality control for semen analysis [3]. As information on the current status of semen analysis in laboratories in Mainland China was not available, we carried out a survey of the laboratories performing the testing. We used information and contacts provided by the Chinese Andrology Congress and training programs in andrology diagnostic techniques from 2005 to 2007.

2 Materials and methods

2.1 Design of the questionnaire

A questionnaire was designed to survey semen analysis laboratories in Mainland China. Questions regarding testing were based on procedures outlined in the WHO Manual (1999) [7]. The questionnaire was composed of 36 questions regarding method of specimen collection, required abstinence time, liquefaction, semen volume, pH, sperm concentration, motility, vitality and morphology. Additional questions focused on the presence of non-sperm components of the semen, including other cell components such as leukocytes, red blood cells and epithelial cells; antisperm antibodies; biochemical assays for accessory sex organ functions such as seminal fructose, α-glucosidase, acid phosphatase and carnitine; and sperm function tests. In addition, respondents were asked to give their opinions about their respective laboratory's methods of semen analysis and quality control. They were also asked to provide the location and level of their hospital.

2.2 Distribution and response to the questionnaire

A copy of the questionnaire was distributed to each of the investigated laboratories, and a cover letter was included to explain the questionnaire and to guarantee the reliability of the data collected. A total of 145 copies of the questionnaire were distributed, and 118 laboratories completed and returned them.

2.3 Analysis of questionnaires

The questionnaire responses were evaluated, and percentages were calculated. The results were analysed using the descriptive statistics method.

3 Results

3.1 General information regarding respondents

The respondents were from 29 provinces, autonomous regions and municipal cities in Mainland China; Tibet and Shanxi were not represented. In Mainland China, general hospitals are usually ranked as primary (< 100 beds), secondary (100–500 beds) or tertiary (> 500 beds) hospitals. Of the respondents, 27.9% (24/86) were from secondary and tertiary hospitals. Interestingly, 27.9% (24/86) of the respondents were from primary hospitals. The remaining 44.2% (38/86) were from family-planning institutes or guidance centres, special hospitals or university hospitals.

3.2 Abstinence duration and collection of semen samples

Of the respondents, 81.4% (96/118) required an abstinence time of 3–7 days, 7.6% (9/118) required 1–2 days and 9.3% (11/118) did not have the requirements for abstinence before testing. The majority (88.1% [104/118]) required masturbation for semen sample collection, whereas 2.5% (3/118) required patients to use a semen-collection device. Some laboratories (4.2% [5/118]) permitted either masturbation or use of a semen collector, and 5.1% (6/118) allowed coitus interruptus or other methods for collection.

3.3 Physical chemistry analysis of semen samples

Semen samples were treated and analysed using different methods for physical chemistry parameters. The results are shown in Table 1.

3.4 Analysis of sperm concentration, motility, vitality and morphology

The methods to analyse, and the expression of results for, sperm concentration, motility, vitality and morphology are listed in Table 2. The majority of respondents reported that they performed these tests manually, but in some cases automation was used as well. Experience with the computer-assisted semen analysis (CASA) system ranged from 6 months to 10 years. The majority (55.9% [33/59]) of CASA users had purchased the system from Beijing Weili Corporation (Beijing, China) and 23.7% (14/59) had purchased it from Qinghua Tongfang Corporation (Beijing, China). The remaining 20.3% (12/59) of laboratories used brands of CASA systems that were manufactured outside China. The users of automated semen analysers had varying degrees of satisfaction with CASA performance (Table 3). The majority (70.8% [34/48]) of CASA users performed manual interventions to reduce the deviation of

results.

3.5 Staining methods for examining sperm morphology, seminal leukocytes and spermatogenic cells

The analysis of sperm morphology, seminal leukocytes and spermatogenic cells was closely related to staining techniques. The most common methods of sperm staining included Wright–Giemsa staining (46.2% [30/65]), Wright staining (33.8% [22/65]) and modified Papanicolaou staining (15.4% [10/65]). Among the others (10.8% [7/65]) were Shorr, Diff-Quik and haematoxylin–eosin (HE) staining. For the detection of seminal leukocytes, 77.6% (83/107) of laboratories relied on microscopic assessment of unstained specimens, with only 22.4% (24/107) of respondents making the interpretation from stained specimens. Only 13.9% (15/108) of laboratories detected seminal spermatogenic cells. Staining for precursors included the use of the Wright–Giemsa's stain (54.1% [20/37]), the modified Papanicolaou's stain (16.2% [6/37]) and other staining methods (29.7% [11/37]). However, these stains are not specific for precursors. The majority of laboratories (93.3% [97/104]) observed leukocytes when performing the basic analysis. Epithelial cells in semen were reported by 51.0% (53/104) of laboratories, and 16.3% (17/104)reported observing spermatogenic cells in semen samples.

Table 1. Semen samples treated and analysed by different methods for physical chemistry parameters. The numbers of centres to the percentages are given in parentheses.

Semen parameters	Methods	Laboratories (%)
Volume	Graduated cylinder	46.4 (51/110)
	Visual examination	53.6 (59/110)
	Weight analysis	0 (0/110)
Viscosity	No detection	28.4 (31/109)
	Pipette method	29.4 (32/109)
	Glass-stick method	37.6 (41/109)
	Other methods	4.6 (5/109)
Incomplete liquefaction	Untreated	70.9 (73/103)
	Chymotrypsin treatment	15.5 (16/103)
	Filter paper to filtrate	4.9 (5/103)
	Other methods, such as trypsin treatment	8.7 (9/103)
pH value	pH indicator paper	81.4 (92/113)
	Unspecified methods	1.8 (2/113)
	No detection	16.8 (19/113)

3.6 Determination of autoantibodies

Antisperm antibodies may make spermatozoa agglutinate. Most of the laboratories (59.3% [64/108]) responding to this survey observed sperm agglutination, but the remaining laboratories (40.7% [44/108]) did not check for the agglutination phenomenon. Only 55.1% (65/118) of reporting laboratories detected antisperm antibodies (Table 4). Besides the detection of antisperm antibodies, 44.1% (52/118) of laboratories also tested for other reproduction-associated autoantibodies, such as antibodies produced against the endometrium (Table 4).

Both the methodology and the type of antibodies sought (agglutinating *vs.* immobilizing) varied. Laboratories relying on kits purchased the materials from various manufac-

Table 2. Analytical methods and expression of results for sperm concentration, motility, vitality and morphology. The numbers of centres to the percentages are given in parentheses.

Sperm parameters	Analytical methods			Expression of results
	Manual	CASA	Both	
Concentration	49.2% (58/118)[a]	33.9% (40/118)	16.1% (19/118)	Value* $\times 10^6$ mL^{-1} (100%)
Motility	50.9% (57/112)[b]	49.1% (55/112)		Grades a, b, c and d (71.8% [84/117]); Well, general and poor (26.5% [31/117]); unspecified (1.7% [2/117]).
Morphology	74.1% (80/108)	25.9% (28/108)		Total percentage of normal morphological sperm (65.5% [72/110]); the percentages of abnormal sperm head, neck and tail (34.5% [38/110]).
Vitality	Visual estimate: 67.9% (72/106) Staining method: 15.1% (16/106) Hypotonic swelling test: 4.7% (5/106) Unspecified: 12.3% (13/106)			Value%* (100%)

Abbreviation: CASA, computer-assisted semen analysis. [a]Measured by haemocytometer (60.2% [59/98]), macro chamber (24.5% [24/98]), Makler chamber (7.1% [7/98]), Cell-VU chamber (1.0% [1/98]) and unspecified (7.1% [7/98]). [b]Measured by haemocytometer (47.5% [47/99]), macro chamber (28.3% [28/99]) and unspecified (24.2% [24/99]). *Detected value.

turers and distributors, with 11.1% (3/27) of laboratories purchasing kits from outside China. Some of the laboratories, however, did not provide information regarding the manufacturers of their kits.

3.7 Determination of seminal biochemical markers

Only 27.1% (32/118) of responding laboratories examined seminal biochemical markers, such as seminal fructose, α-glucosidase, acid phosphatase and zinc. The percentages of laboratories that performed tests for these biochemical markers and the methods used are shown in Table 5.

Table 3. CASA users' attitudes towards sperm analysis. The numbers of centres to the percentages are given in parentheses.

Parameters	Technicians' opinion (%)		
	Satisfactory	Neutral	Unsatisfactory
Sperm count	35.7 (20/56)	60.7 (34/56)	3.6 (2/56)
Sperm motility	41.2 (21/51)	54.9 (28/51)	3.9 (2/51)
Sperm morphology	17.4 (8/46)	63.0 (29/46)	19.6 (9/46)

Abbreviation: CASA, computer-assisted semen analysis.

3.8 Semen culture and sperm function tests

The survey showed that only 14.2% (15/106) of laboratories performed microbiology cultures on specimens submitted for semen analysis.

Sperm function testing was optional and was performed in only 15.3% (18/118) of the laboratories. Available testing methods included the zona-free hamster oocyte test (22.2% [4/18]), the human zona pellucida-binding test (16.7% [3/18]), the acrosome reaction test (50% [9/18]) and other unspecified tests (16.7% [3/18]).

3.9 Laboratory technologists' evaluations of semen analysis methods

Only 11.7% (13/111) of technicians who performed the testing were satisfied with their laboratory's methods of semen analysis, whereas 34.2% (38/111) were not satisfied. The remaining 54.1% (60/111) expressed neutral feelings.

None of the laboratories performed intra- and inter-

Table 4. Methods used to detect antisperm antibodies (some other autoantibodies were analysed in different laboratories). The numbers of centres to the percentages are given in parentheses.

Detection items (number of laboratories)	Description of methods or autoantibodies	Percentage of laboratories (%)
Antisperm antibodies	ELISA	78.5 (51/65)
(n = 65)	Insolubilized enzyme method	3.1 (2/65)
	Immunobead test	1.5 (1/65)
	Sperm immobilization test	1.5 (1/65)
	Unspecified methods	12.3 (8/65)
	ELISA and immunobead tests	3.1 (2/65)
Other reproduction-associated autoantibodies	Anti-endometrium antibody	98.1 (51/52)
(n = 52)	Anti-phospholipid antibody	57.7 (30/52)
	Anti-ovarian antibody	53.8 (28/52)
	Anti-zona pellucida antibody	25.0 (13/52)
	Anti-nuclear antibody	13.5 (7/52)

Abbreviation: ELISA, enzyme-linked immunosorbent assay.

Table 5. Percentage of 32 responding laboratories detecting several seminal biochemical markers. The numbers of centres to the percentages are given in parentheses.

Markers	Total percentage of laboratories (%)	Description of methods	Percentage of applicable laboratories (%)
Fructose	96.9 (31/32)	Resorcinol method	89.3 (25/28)
		Indole coloration	7.1 (2/28)
		Other unspecified methods	3.6 (1/28)
α-Glucosidase	59.4 (19/32)	Glucose oxidase method	94.7 (18/19)
		Other unspecified methods	5.3 (1/19)
Acid phosphatase	46.9 (15/32)	Disodium phenyl phosphate method	93.3 (14/15)
		Other unclaimed methods	6.7 (1/15)
Carnitine	18.8 (6/32)	Elman's method	83.3 (5/6)
		Other undescribed methods	16.7 (1/6)
Zinc	21.9 (7/32)	Commercial kits	100 (7/7)

quality control of semen analysis. The investigation showed that 94.4% (102/108) of respondents felt that quality control for semen analysis is needed; the other 5.6% (6/108) did not recognize the importance of quality control. Some individuals suggested that the urgent need for quality control of semen analysis also entails the establishment of quality control centres for laboratories performing the semen analysis and associated testing, as well as routine distribution of quality control products for these laboratories.

4 Discussion

In this study, the respondents who completed the questionnaire were from most of the provinces in Mainland China, representing many types of hospital laboratories. Most of the respondents were technicians working in laboratories. Thus, the results should reliably reflect the status of the laboratories performing semen analysis in Mainland China. The results indicated that different methods were used to analyse semen and to express the results. Many of the methods were not in agreement with the recommendations given in the WHO Manual (1999) [7].

The survey showed that the required duration of abstinence in most of laboratories (81.4% [96/118]) was from 3 to 7 days, which is similar to that recommended by the WHO Manual (1999) [7]. However, the reported data showed that the abstinence time should be from 2 to 7 days for general semen parameters. For determining seminal α-glucosidase activity, the abstinence time should be from 4 to 7 days, because the α-glucosidase activity in semen samples from a patient who had abstained for only 2–3 days was significantly lower than that in those who had abstained for 4–5 days or 6–7 days [8, 9]. Although we did not include data on whether patient history was considered before semen samples were collected, it should be mentioned that such history (for example, fever) could affect the results of semen analysis.

Most of the semen samples (88.1% [104/118]) were collected by masturbation, as recommended by the WHO Manual (1999) [7]. The majority (81.4% [92/113]) of laboratories detected semen pH using a pH-indicator paper. In addition, more than half (53.6% [59/110]) of the laboratories measured semen volume with a visual estimate. For measuring semen, the WHO Manual (1999) [7] recommends using a graduated cylinder with a conical base or weighing standard containers with and without semen. Unfortunately, none of the laboratories detected semen volume with such analytical methods. In all, 71.6% (78/109) of laboratories examined semen for incomplete liquefaction and viscosity, and 70.9% (73/103) did so without treating such specimens. This experimental structure could lead to unreliable results, as the viscosity of semen samples obviously affects the accuracy of sampling [10]. Therefore, in structuring new guidelines in this area, the physical chemistry analysis of semen samples and the treatment of viscous or incomplete liquefaction should be emphasized.

Sperm concentration, motility, vitality and morphology are important parameters of sperm quality. Approximately 50% (59/118) of the laboratories reported that they analyse semen samples with a standard light microscope in the manual setting. CASA systems were also available in many of the laboratories. Most CASA systems used in the responding laboratories were manufactured in China. More than half of the CASA technicians were neutral regarding the use of the CASA system for determining sperm concentration, motility and morphology. When the CASA was used, most (70.8% [34/48]) laboratories also performed the analyses manually as a reference. Although it has been reported that semen can be analysed objectively and with high precision using CASA [11], accuracy requires standardization of all instruments. Determinations would also need to be made as to whether the various systems provide comparable results.

Various counting chambers are used to analyse sperm concentration and motility. The haemocytometer has been considered the 'gold standard', even though it can be used only for the determination of sperm concentration. The survey showed that a haemocytometer manufactured in China was used as the first choice in 60.2% (59/98) of laboratories to analyse semen samples. However, several comparative studies [12–15] showed that a haemocytometer overestimates sperm concentration, which indicates that the chamber used for analyses of sperm concentration and motility should be standardized [16]. It is also noteworthy that some chambers, such as Makler, may not count enough spermatozoa per volume of semen used, when simultaneously analysing sperm concentration and motility.

Interestingly, the survey showed that the technicians who performed the semen analysis did not completely understand the general importance of correct determination of sperm vitality and morphology parameters for prediction of semen quality. Most of the laboratories analysed sperm motility (67.9% [72/106]) and sperm morphology (74.1% [80/108]) using microscopic visual estimates and did not perform the staining methods recommended by the WHO manual (1999) [7]. Moreover, 77.6% (83/107) of laboratories also relied on a microscope, in the manual setting, for examining leukocytes in the semen. Detection of spermatogenic cells was not emphasized in most laboratories, as only 13.9% (15/108) of laboratories investigated the presence of immature sperm precursors in semen. The staining methods available for cell analysis included Wright–Giemsa, Wright, modified Papanicolaou, Shorr,

Diff-Quik and HE staining. These data indicate that the determination of sperm morphology, leukocytes and spermatogenic cells need to be further emphasized and standardized. In addition, an acceptable method should be established to make the results comparable among laboratories.

Most of the laboratories carried out a determination of reproduction-associated antibodies. For example, antisperm antibodies were usually detected using an enzyme-linked immunosorbent assay with commercially available kits from more than 10 sources worldwide. Our preliminary data showed that results on detection of antisperm antibodies varied widely among four brands of kits, with up to 10-fold differences in positive rates (unpublished data). These data indicate an urgent need for standardization in detection of antisperm antibodies, especially in the preparation procedures for sperm antigen.

This study showed that only 27.1% (32/118) of laboratories carried out a determination of seminal biochemical markers. Among those laboratories, however, there were some identical methods—seminal α-glucosidase activity was determined using the glucose oxidase method (94.7% [18/19]) [8], fructose level with the resorcinol method (89.3% [25/28]) [17] and acid phosphatase activity with the disodium phenyl phosphate method (93.3% [14/15]) [18]. Unfortunately, these techniques were not completely in agreement with the methods recommended by the WHO Manual (1999) [7], probably because copies of WHO manuals are not available in most andrology laboratories in Mainland China, although the handbook Nan Ke Zhen Duan Xue (Diagnosis in Andrology), edited by Huang and Xu [19], has been available in most of China's andrology laboratories since 1999, when the first edition was published. This indicates that the availability of a professional handbook in a native language may play an important role in guiding semen analysis.

Only 15% (15/106; 18/118) of responding laboratories performed semen culture and sperm function tests, such as a penetration assay, a human zona pellucida-binding test and an acrosome reaction. This percentage is probably low because most of these assays are considered to be for scientific research purposes rather than for clinical diagnosis [20]. In addition, some people believe that the significance of these tests is unclear.

Finally, it should be emphasized that only 11.7% (13/111) of technicians who performed the testing were satisfied with their laboratory's methods of semen analysis. Moreover, none of the reporting laboratories performed intra- and inter-quality control. Therefore, in a previously published paper, we reported our investigation on inter-quality control for the analysis of sperm concentration, seminal fructose level, α-glucosidase activity and acid phosphatase activity in Nanjing city, Jiangsu province [21]. These data showed that there was large variation in the results, especially in the determination of seminal acid phosphatase activity.

In conclusion, this study focused on the current status of semen analysis, and the results indicated that standardization and quality control for each parameter of semen analysis are very much needed. To this end, an appropriate method and mode for expressing the results for each parameter of semen analysis should be optimized and standardized. In addition, regular training of technicians remains an important aspect of improving the quality of semen analyses. Moreover, administrative departments should think critically about semen analysis and adopt positive measures for monitoring and directing procedures for semen analysis, including a widely established intra- and inter-quality control system. Therefore, much work needs to be done.

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