

Using the Sperm Quality Analyzer (SQA IIc) to evaluate dog ejaculates

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ABSTRACT: 38 fresh ejaculates were examined, of which 23 were cryopreserved in three diluents differing in concentration of glycerol and examined after thawing. For each ejaculate we established the concentration, motility, speed of sperm, percentage of live sperm and the morphological image of the ejaculate using routine laboratory diagnosis and SQA parameters at the initial value and after a 120-minute survival test. The values of the parameters found through routine diagnosis and using the SQA device were statistically confronted. From the results it emerges that the device functions on the basis of the turbulence effect of the semen, and therefore on the intensity and character of the moving mass of sperm. From a research perspective, the SQA device is less usable, since the values it provides are not absolutely comparable with the values found through routine laboratory diagnosis. For the practitioner, however, the device may be useful, since the value of the sperm motility index (SMI) indicates the quality of the semen through the close connection of two parameters, concentration and motility. For a more precise assessment of the ejaculate, however, it is necessary to also analyse the remaining parameters of routine laboratory sperm analysis. To be useful in clinical practice for the evaluation of semen, it would be necessary to establish SMI limit values for ejaculates of varying quality.

Keywords: dog; ejaculate; sperm analysis; Sperm Quality Analyzer (SQA)

The Sperm Quality Analyzer IIc is a small, compact device that combines optical detection and computer analysis of the data acquired. The device was developed for evaluating the semen of men, but several studies have already been published demonstrating its usability for evaluating semen quality in certain animals, including, among others, dogs (Iguer-Ouada and Verstegen; 2001; Neuman et al., 2002; Rijsselaere et al., 2002; Fukui et al., 2004; Vyt et al., 2004).

According to the basic data, the principle for establishing indicators is based on the turbulence effect of semen. This phenomenon has been studied in some domestic animals, particularly ruminants, and brings together the evaluation of the density and the movement of sperm. While examining a semen sample using electro-optical methods for microscopy, this phenomenon appears as moving waves prompting a change in optical density. This turbulence occurs only in very dense ejaculates and

is caused by the collective movement of individual sperm. The wave frequency depends on the concentration of motile sperm and on the intensity of their movement; the sperm motility index (SMI) was established to characterise them. In order that this phenomenon might also be used in the evaluation of human semen, equipment was developed that was capable of measuring the SMI in human semen, which has a low density and does not manifest the characteristic signs of collective movement (Bartoov et al., 1991). The SMI is defined as the frequency of changes in optical density caused by sperm movement in the optical field under examination. The SMI cannot be established if it is a matter of dead or immotile sperm, which do not cause changes in optical density (Bartoov et al., 1991; Iguer-Ouada and Verstegen, 2001). A number of studies have been devoted to the relationship of the SMI to motility parameters. The numerical value of the SMI is affected by both the number of

motile sperm and the quality of their movement, as indicated by Schieferstein et al. (1998) and Bartoov et al. (1991). The SMI value falls during functional tests together with motility, when the same concentration of sperm in the sample is maintained (Bartoov et al., 1991; Iguer-Ouada and Verstegen, 2001). Although immotile sperm do not provide an SMI value, a statistically significant correlation has been found between the SMI and the overall concentration and percentage of motility in human semen (Bartoov et al., 1991).

Another parameter for evaluating semen while using the SQA device is FSC (functional sperm concentration), the value of which brings together active sperm and evaluates their quantity. Both the SMI and FSC parameters significantly correlated with the CASA parameters and the SQA device tested by the cited authors was judged to be useful in the management of male infertility (Suzuki et al., 2002). A positive correlation was found by comparing the values of the percentage of morphologically normal sperm obtained by the SQA IIC device with the evaluation of the morphology according to strict criteria. Shibahara et al. (2002) stated that the SQA IIC may be used as an initial screening method for evaluating the morphology of male sperm, but for a strategy for treating infertility they nevertheless recommended evaluation by microscopy using strict criteria (Kruger et al., 1986). Johnston et al. (1995) also presents a high correlation of the SMI with the morphological image of human semen obtained using microscopy. Iguer-Ouada and Verstegen (2001) discovered a statistically significant correlation between the SMI and the concentration of dog sperm in values of sperm concentration up to 200×10^6 spermatozoa/ml. At semen concentrations higher than 200×10^6 spermatozoa/ml, the correlations were not statistically significant. A similar conclusion was also reached by Rijsselaere et al. (2002), who in their study demonstrated the dependence of the SMI values on sperm motility and the concentration of fresh ejaculates up to a value of 150×10^6 spermatozoa/ml.

Apart from indices, the device provides parameters given by the WHO for the qualitative evaluation of semen, that is to say values for concentration, motility and the percentage of normal sperm. According to Comodo et al. (1997), the SMI values are converted into sperm concentration, total motility and morphologically normal sperm using specific algorithms.

The aim of this study was to establish whether the SQA device would be useful for assessing dog semen quality, given the biological differences between ejaculates from dogs and humans. The values obtained by the SQA device and the values obtained using routine methods for sperm assessment were compared in an attempt to determine the usability of each individual parameter and the device itself for assessment of the quality of ejaculates from dogs in clinical practice.

MATERIAL AND METHODS

Semen obtained from dogs was assessed on the one hand using the SQA device and on the other using routinely applied laboratory methods for sperm analysis (Veznik et al., 2005).

Animals and method of collection

Twenty dogs of different breeds, ranging in age from 1 to 11 years old (mean age 4.5 years) were included in the study. The breeds were: an Afghan Hound, Bassett Hound, Beagle, Bull Terrier, Czech Short-haired Terrier, Doberman, Yorkshire Terrier, Cocker Spaniel, German Shepherd dog, Labrador Retriever, Leonberger, Rottweiler, Shetland Sheepdog and Irish Wolfhound. In all, 38 ejaculates were evaluated, and 23 of those were cryopreserved in three diluents differing in concentration of glycerol. The semen was collected by manual manipulation into plastic test-tubes with a funnel. Clenching the collector in the palm was sufficient to minimize thermal shock. Mainly the second sperm rich fraction of each ejaculate, was collected. In all, 226 prepared samples of ejaculate were evaluated.

Method of work with the SQA

The semen was drawn into single-use plastic capillaries for measuring in the SQA and placed in the device so that the results could be read from the display. The device works on the principle of detecting changes in a beam of light after it has passed through the ejaculate in the capillary. The SQA measures changes in light impulses caused by sperm movement in time periods. The shape and frequency of the waves of the electrical signal is

analysed in 10-second periods. This test is repeated 4 times over a 45-second evaluation period and the values are processed to produce the final result, which appears on the display.

The SQA IIC evaluates the following six parameters designed to estimate the fertility of the sample:

SMI – sperm motility index. Value generated by computer on the basis of detected changes in a beam of light. This parameter is the basis for the other parameters. It is an item of numerical data internal to the device which determines the quality of the ejaculate.

FSC – functional sperm concentration. Defines the concentration of live, functional sperm in millions per ml (sperm which are motile and also morphological normal).

MSC – motile sperm concentration. Defines the concentration of progressively motile sperm in millions per ml.

Total Cell Concentration – one of the World Health Organization (WHO) parameters, expressing the total number of cells, living or dead, per ml.

Percent Normal Motility – another WHO parameter, expressing the percentage of cells with normal motility.

Percent Normal Morphology – a third WHO parameter, the percentage of cells with a normal morphology. A normal morphology is defined by “strict criteria” in the WHO manual (strict criteria – Kruger et al., 1986).

Overall qualitative assessment of ejaculates using routine sperm analysis and SQA

Plan of laboratory examination of semen. After collection, the test-tube with semen was placed on a laboratory thermal desk heated to 35°C and the following parameters were examined: semen volume, sperm concentration, motility and speed of sperm movement, identification of live and dead sperm and assessment of the morphology.

The concentration was examined hemocytometrically in a Bürker Chamber. The motility was established using a subjective method in a microscope 200–400× magnification. A drop of semen was placed on a microscope slide and covered with a coverslip, at 35°C. The speed of movement was established using the propulsivity method according to Baker et al. (1957). Eosin-nigrosin dye was used to identify live and dead sperm. The morphological assessment was carried out by examining a prepara-

tion dyed according to Karras (Gamcik et al., 1976) by microscope magnified 1 000× while using immersion, and evaluated using a multiparametric method, the SASMO programme (Veznik et al., 2001).

The survival test was carried out at laboratory temperature (22°C) in ejaculates diluted with physiological saline buffered with a phosphate buffer at pH 7.2. The ejaculate was diluted to a concentration of 100×10^6 spermatozoa/ml. After 120 min the ejaculate was heated to 35°C and then evaluated by establishing functional indicators and the morphological assessment.

Preparing the semen for long-term conservation. The individual ejaculates were divided into 3 aliquots and centrifuged at 500 G for 10 min, the seminal plasma was separated and then frozen and stored at –18°C. Each aliquot was diluted with a Tris-fructose-citric acid-egg yolk extender (modified according to Andersen, 1975) of three different glycerol concentrations (4%, 6%, 8%, respectively) to the final sperm concentration of around 500×10^6 spermatozoa/ml. The diluted semen was filled into labelled 0.5 ml straws, which were sealed with polyvinylalcohol. The straws were equilibrated at 4°C for 2 hours and then frozen in liquid nitrogen vapour in a metal straw-holder frozen to –100°C and were after 10 minutes plunged into the liquid nitrogen.

Thawing and incubation. The straws were thawed in a water bath at 65°C for 6 seconds and then emptied into a 35°C physiological saline buffered to pH 7.2 that contain 20% seminal plasma, to obtain a final concentration of 100×10^6 spermatozoa/ml. In the sample thus obtained, the SQA parameters and the parameters according to routine laboratory examination by microscopy were again established as an initial value (0 min) and after a 120-minute survival test (120 min).

To compare the results obtained by evaluating the measurements using the SQA IIC and methods of routine sperm analysis, an investigation was conducted based on the statistical processing of the data obtained and the indices constructed.

The study of factors influencing SQA results

The dog semen was prepared for evaluation by centrifuging the sperm, decanting and resuspending in Hayem’s solution for various concentrations of immotile sperm. A descending order of concentrations was prepared. The concentration of sperm

from each dilution was established on the SQA and hemocytometrically in a Bürker chamber.

To analyse the effects of motility and concentration of sperm, the fresh dog semen was diluted with buffered saline at ratios of 2 : 1, 1 : 1 and 1 : 2. The concentration for the resulting groups of ejaculates was established using a Bürker chamber and the Sperm Quality Analyser and the subjective motility using a microscope and the SQA.

The assertion of Comodo et al. (1997) that all the SQA parameters derived mathematically from the SMI values was tested by a statistical comparison of the individual SQA and SMI parameters.

Statistical analysis

The statistical analysis was carried out using the STATplus programme (Matouskova et al., 1992). The level of significance was set at $P < 0.05$.

RESULTS

Overall qualitative assessment of ejaculates using routine sperm analysis and SQA

The average initial values for individual parameters of routine sperm analysis and values after the 120-minute survival test are shown at Table 1. The average initial values of SQA parameters and values after the 120-minute survival test are shown in Table 2.

At the initial value, the average concentrations differed from the concentrations established by the SQA and with the SMI value. Both the average initial motility of the fresh semen and the motility after 120 min demonstrated a high correlation with the SQA values ($P < 0.01$). There was also a high correlation for the SMI values, both initially and after 120 min, with the motility values ($P < 0.01$). The initial value for the concentration of motile

Table 1. Average values for sperm analysis of fresh semen ($n = 38$)

	0 min				120 min			
	Average	SD	Max.	Min.	Average	SD	Max.	Min.
Motility (%)	77.4	13.19	95.0	35.0	52.5	17.31	90.0	10.0
Sp. speed ($\mu\text{m/s}$)	58.4	33.65	144.8	11.9	34.6	29.55	114.3	0
Live sperm (%)	83.7	12.15	97.0	39.0	80.4	12.64	93.1	38.0
Normospermia (%)	68.6	20.80	92.0	7.5	61.4	23.00	87.0	2.5
Concentration (mil./ml)	261.032	224.3951	1 225.600	17.066				
c(M) (mil./ml)	200.887	189.6417	1 103.040	9.386				
c(M + N) (mil./ml)	144.058	151.0130	843.826	1.558				

0 min = initial value; 120 min = value after 120-minute survival test; SD = standard deviation; Max. = maximum value; Min. = minimum value; c(M) = concentration of motile sperm; c(M+N) = concentration of motile and morphologically normal sperm

Table 2. Average values for SQA parameters, fresh semen ($n = 38$)

	0 min				120 min			
	Average	SD	Max.	Min.	Average	SD	Max.	Min.
Motility (%)	83.2	10.92	91.0	27.0	63.5	19.16	89.0	17.0
Concentration (mil./ml)	198.500	36.8795	225.000	22.000	127.711	66.9755	220.000	12.000
Normospermia (%)	52.6	6.28	57.0	19.0	41.9	11.65	56.0	17.0
FSC (mil./ml)	120.7	26.20	138.0	2.2	65.9	46.30	135.0	1.2
MSC (mil./ml)	168.5	38.56	204.0	5.9	91.8	60.65	195.0	2.0
SMI (mil./ml)	507.0	94.44	582.0	66.0	327.1	156.22	567.0	45.0

0 min = initial value; 120 min = value after 120-minute survival test; SD = standard deviation; Max. = maximum value; Min. = minimum value; FSC = functional sperm concentration; MSC = motile sperm concentration; SMI = sperm motility index

Table 3. Average values of sperm analysis for frozen/thawed semen (cryopreserved in three diluents differing in concentration of glycerol, $n = 69$)

	0 min				120 min			
	Average	SD	Max.	Min.	Average	SD	Max.	Min.
Motility (%)	41.0	12.9	60.0	5.0	31.3	15.04	70.0	1.0
Sp. speed ($\mu\text{m/s}$)	59.6	33.55	195.3	0	45.3	28.11	102.3	0
Live sperm (%)	58.0	11.78	88.2	33.0	53.1	12.93	82.5	12.0
Normospermia (%)	40.0	13.91	77.0	16.5	24.9	10.17	59.5	5.0
Concentration (mil./ml)	92.305	30.430	168.0	44.8	92.305	30.430	168.0	44.8
c(M) (mil./ml)	38.496	20.818	141.100	6.400	28.447	15.893	67.200	2.592
c(M + N) (mil./ml)	14.686	8.186	37.600	1.750	7.142	5.508	26.343	0.492

0 min = initial value; 120 min = value after 120-minute survival test; SD = standard deviation; Max. = maximum value; Min. = minimum value; c(M) = concentration of motile sperm; c(M+N) = concentration of motile and morphologically normal sperm

sperm did not show any correlation with the SQA concentration. A significant correlation was, however, found with the MSC ($P < 0.05$). There was a correlation of the concentration of motile and morphologically normal sperm with the value of the FSC index ($P < 0.05$). The values for normospermia established by microscopy showed a correlation ($P < 0.01$) with the SQA values for normospermia, given the highly significant difference in the averages ($P < 0.01$); after 120 min no correlation was found. The same dependence was obtained when comparing the values for normospermia and the SMI at both the initial value and after 120 min.

Averages of 23 thawed ejaculates in three different concentrations of glycerol are shown in Tables 3

and 4. Correlations between the values obtained by routine analysis and SQA analyzer and statistical significance of differences between the averages of these values are shown in Table 5. The average concentration did not demonstrate a significant concordance with the concentration established by the SQA or with the SMI value. Similar results were obtained after the 120-minute test, when the correlation between the concentration and the SQA concentration and between the SMI and concentration also showed to be non-significant. The average initial motility of the frozen/thawed semen showed a correlation ($P < 0.01$) with the SQA value and the same high correlation was found after 120 min. In both cases, the difference between the averages was

Table 4. Average values of SQA parameters, frozen/thawed semen (cryopreserved in three diluents differing in concentration of glycerol, $n = 69$)

	0 min				120 min			
	Average	SD	Max.	Min.	Average	SD	Max.	Min.
Motility (%)	56.2	16.58	79.0	0	48.3	14.40	81.0	14.0
Concentration (mil./ml)	99.400	45.6990	183.000	0	72.884	37.9930	190.000	9.000
Normospermia (%)	37.9	10.71	51.0	0	33.1	9.24	51.0	16.0
FSC (mil./ml)	44.2	28.73	111.0	0.4	26.1	22.59	117.0	0.9
MSC (mil./ml)	63.0	36.49	144.0	0.5	40.8	29.16	153.0	1.2
SMI (mil./ml)	261.7	111.13	458.0	26.0	199.3	95.72	478.0	37.0

0 min = initial value; 120 min = value after 120-minute survival test; SD = standard deviation; Max. = maximum value; Min. = minimum value; FSC = functional sperm concentration; MSC = motile sperm concentration; SMI = sperm motility index

Table 6. Comparison of parameter values for routine sperm analysis and SQA parameters with SMI values for fresh semen ($n = 76$ examination summarized from the initial values and values after the 120 min survival test)

SMI	M (%)	M(SQA) (%)	N (%)	N(SQA) (%)	C (mil./ml)	c(SQA (mil./ml)	L (%)	S ($\mu\text{m/s}$)
Over 400	75.7	84.7	71.3	53.5	217.254	203.796	84.7	55.2
251 to 400	52.9	65.2	53.8	44.4	129.352	127.500	81.7	30.3
100 to 250	41.4	48.7	60.7	33.0	84.509	68.714	82.1	34.4
up to 100	32.5	28.2	44.4	19.2	119.644	24.000	60.9	27.8

M = motility; M(SQA) = SQA motility; N = normospermia; N(SQA) = SQA normospermia; C = sperm concentration; c(SQA) = SQA sperm concentration; L = live sperm; S = sperm speed

highly significant ($P < 0.01$). There was a high correlation ($P < 0.01$) for the SMI values with the motility values both at initial values and after 120 min.

The concentration of motile sperm showed a high correlation with the SQA concentration at the initial value and after 120 min ($P < 0.01$). The correlation between the concentration of motile sperm and the MSC was also highly significant ($P < 0.01$). The concentration of motile and morphologically normal sperm showed a highly significant correlation with the FSC index both at the initial value and after 120 min of survival test ($P < 0.01$). In both cases, the difference in the averages was highly significant ($P < 0.01$).

The values for normospermia established by microscopy at the initial value showed no significant correlation with the values for normospermia established using the SQA device, nor with the SMI values. In contrast, 120 min after thawing the values for normospermia did correlate with both the SQA values for normospermia and the SMI values ($P < 0.05$).

The comparison of the parameter values of routine sperm analysis and the SQA parameters for fresh semen arranged into 4 categories according to SMI values are shown in Table 6. Assessment is summarized from the initial values and values after the 120-minute survival test.

The study of selected factors influencing the SQA results

The study was focused on the effect of sperm motility on the SQA indicator. Figure 1 depicts the declining concentration of the killed semen, while the concentration evaluated using the SQA did not produce any values. In Figure 2 the descending series of concentrations of fresh semen, diluted with physiological saline, in comparison with the concentration found using the SQA is shown, and at the same time the motility established by microscopy and with the SQA and the SMI values.

The statistical evaluation of the dependences of individual SQA parameters on the SMI showed a high significance in all measurements (average $r = 0.98$, $P < 0.01$).

DISCUSSION

The concentrations obtained through routine laboratory diagnosis and the SQA were not statistically different. After the 120-minute survival test, the fall in values of the SQA concentration was evident, while the sperm concentration in the experimental samples of sperm remained unchanged. From

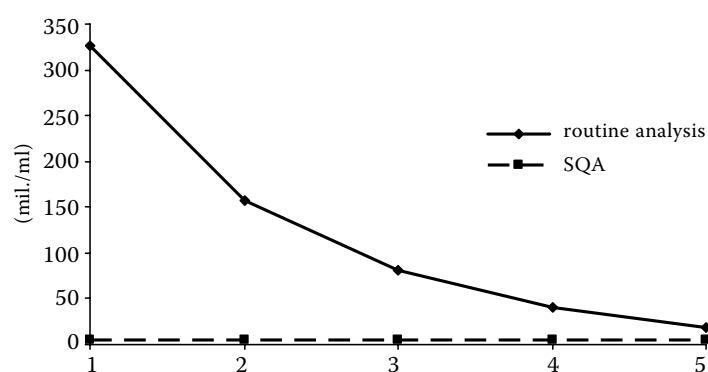


Figure 1. Sperm concentration and concentration established by SQA in killed semen

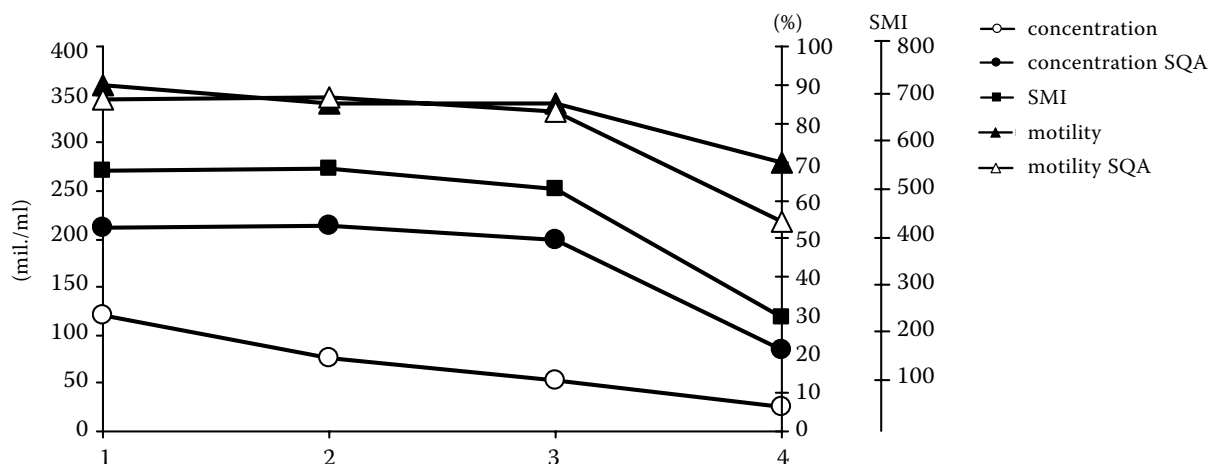


Figure 2. Artificial descending series of concentrations and motility values for fresh semen in comparison with SQA parameters

the results it was evident that the change in SQA concentration was influenced by a fall in sperm motility in the ejaculate. The fall in the sperm motility established was highly correlated with a fall in the SQA motility, and moreover both at the initial value and after the 120-minute survival test. The experiment with the concentration series of killed sperm proved convincingly the dependence of SQA measuring on sperm motility. Immotile sperm do not provide impulses permitting the establishment of an SMI value and, therefore, the prerequisites for the other SQA parameters, which were likewise zero. This finding concords with the contention of Bartoov et al. (1991) and also with the results of Iguer-Ouada and Verstegen (2001), who also assert the essential role of motility on the SMI.

The values for normospermia evaluated by the SQA attained a maximum of 57% in high quality ejaculates. In the experiment, the maximum value for normospermia obtained was 92% and the average value was 68.6%. The average value of normospermia is somewhat higher in comparison with the normospermia values (57.5%) presented by Veznik et al. (2003) in research into the qualitative evaluation of the ejaculates of a group of experimen-

tal dogs, and comes closer to the lower limit for the conventional value for normospermia of 70% (Veznik et al., 2000). The value for normospermia is closely derived from the SMI value, just like the other SQA parameters, which are merely a mathematical algorithm of the SMI.

Since the device is limited by maximum values which for sperm concentration do not exceed 200×10^6 spermatozoa/ml, even highly concentrated semen will induce in the device only the maximum possible SMI value, which in our research was 582. The SMI value is dependent on the fact that the sample will at the same time have a high motility. Iguer-Ouada and Verstegen (2001) showed that, at semen concentrations higher than 200×10^6 spermatozoa/ml, the correlation between the concentration of dog sperm and the SMI is statistically insignificant, probably because the system is saturated.

In the following case studies we demonstrated the significant influence on the SMI value of sperm motility and concentration. The results are presented in Table 7.

(A) A two-year old Labrador, brought in for a sperm examination for experimental purposes, was

Table 7. The two case studies

Dog	Analysis	Concentration (mil./ml)	Motility (%)	Normospermia (%)	SMI
A	routine	52.800	90.0	84.5	
	SQA	220.000	89.0	56.0	567.0
B	routine	536.000	35.0	7.5	
	SQA	107.000	62.0	43.0	293.0

found to produce semen of low concentration with good motility. A high SMI value was obtained as a result of a high percentage of motile spermatozoa even if sperm concentration/ml was lower.

(B) An ejaculate with a high concentration of sperm and at the same time a lower motility was taken from a four-year-old Czech short-haired terrier. The examination was carried out with the aim of discovering why bitches covered had not become pregnant. It follows from the present results that the SMI value was affected not only by a low motility of spermatozoa, but also by their high concentration. According to the SMI result, semen of this kind would be categorized as relatively good ejaculate.

The case studies demonstrate the fact that values of the SQA parameters are affected by both the motility of sperm and their concentration.

CONCLUSION

The SQA IIc device functions on the basis of the effect of semen turbulence, that is to say on the intensity and character of the moving mass of sperm. From a research perspective, the SQA device is less usable, since the values it gives are not absolutely comparable with the values obtained by routine laboratory diagnosis. For the practitioner, however, the device may be useful, since the SMI value shows the quality of semen by closely connecting two parameters, concentration and motility. For the precise examination of an ejaculate, it is, however, necessary to establish the other parameters of sperm analysis. This is in accordance with the contention of Rijsselaere et al. (2002), who consider the SQA IIc less usable in the sperm analysis of diluted, fresh semen, but on the contrary assert the possible suitability of the device for the objective examination of post-thaw motility.

For use in clinical practice for the purpose of evaluating semen, it would be necessary to establish the limit SMI values for groups of ejaculates of varying quality.

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