İstanbul Üniversitesi
Veteriner Fakültesi Dergisi

Journal of the Faculty of
Veterinary Medicine Istanbul University

İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ., 42 (1), 20-25, 2016
doi: 10.16988/iuvfd.2016.74833

Araştırma Makalesi ***Research Article***

Evaluation of Spermatological Parameters of Frozen Bull Semen with Conventional and CASA (Computer-Aided Sperm Analysis) Method

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Geliş Tarihi / Received:
25 December 2014

Kabul Tarihi / Accepted:
24 February 2015

Key Words:
Bull, CASA, frozen semen, conventional examination, semen evaluation

Anahtar Kelimeler:
Boğa, CASA, dondurulmuş sperma, konvansiyonel muayene, spermanın değerlendirilmesi

Abstract

The aim of this study was to compare the assessments made by the CASA system and subjective method (by using phase-contrast microscope) for spermatological examinations of imported and locally produced semen. Frozen semen (imported and local production) belonging to 20 different bulls was examined by phase contrast light microscope method (conventional method) and method supported by computer (CASA method) for evaluating the principle semen characteristics. For imported semen, considering the average values of samples examined by the two methods, significant differences ($P<0.05$) were observed in the values of motility and concentration as well as the rates of abnormal and dead spermatozoa. For domestic semen, significant differences ($P<0.05$) were observed for the concentration of samples by the conventional evaluations and for the assessment of motility and concentration by the CASA method. It was observed that there were significantly ($P<0.05$) higher data only for the concentration assessed by both methods, while no such differences between the values of motility as well as the rates of abnormal and dead spermatozoa were found when considering the general average rates. By using the two methods, findings from the examination of pre-determined parameters were compared and their reliabilities were displayed herein.

Özet

Dondurulmuş Boğa Spermalarında Spermatolojik Parametrelerin Konvansiyonel ve CASA (Bilgisayar Destekli Sperm Analiz) Yöntemiyle Değerlendirilmesi

Bu araştırmada, ithal ve yerli üretim spermaların ülkemizde geleneksel yöntemlerle faz-kontrast ışık mikroskobu kullanılarak subjektif olarak yapılan spermatolojik muayenelerinin, CASA sistemiyle yapılan değerlendirmelerle karşılaştırılması amaçlandı. Farklı 20 boğaya ait dondurulmuş spermalar (ithal ve yerli üretim) faz-kontrast ışık mikroskobu yöntemi (konvansiyonel yöntem) ve bilgisayar destekli sperm analiz yöntemi (CASA yöntemi) ile muayene edildi ve başlıca spermatolojik özellikler yönüyle değerlendirildi. İthal spermalarda muayene edilen örneklerin ortalama değerleri arasında spermatozoa motilitesi, spermatozoa yoğunluğu, anormal spermatozoa ve ölü spermatozoa oranları arasında gözlenen farklılıklar istatistiksel açıdan önemli oldu ($P<0,05$). Yerli spermalarda ise konvansiyonel yöntemle numuneler arasında saptanan spermatozoa yoğunluğu yönüyle gözlenen farklılıklar ve CASA yöntemiyle elde edilen spermatozoa motilitesi ve spermatozoa yoğunluğu bakımından gözlenen farklılıklar istatistiksel açıdan önemli bulundu ($P<0,05$). Genel ortalama değerlere bakıldığında konvansiyonel ve CASA yöntemiyle elde edilen sonuçlara göre yalnızca spermatozoa yoğunluğunda konvansiyonel yöntemle önemli ölçüde daha yüksek veriler alındığı, spermatozoa motilitesi, anormal spermatozoa oranı ve ölü spermatozoa değerlerindeki farklılıkların istatistiksel açıdan önemsiz olduğu belirlendi ($P<0,05$). Her iki yöntemle, belirlenen muayene parametrelerine ilişkin bulgular karşılaştırıldı ve yöntemlerin güvenilirlikleri ortaya konuldu.

Introduction

Nowadays, use of frozen semen in cattle breeding emerges as an inevitable process. Protection of animal health and sustainability, to guide and increase in the reproductive performance, artificial insemination (AI) with frozen semen are seen as indispensable.

Although AI with frozen semen in cattle is still inadequate, it is rapidly developing and expanding in

Turkey. As of the Ministry of Food, Agriculture and Livestock data, over three and a half million cattle were inseminated by using frozen semen in 2014. In the same year, over one million domestic semen productions were made, and well over three and a half million frozen bull semen were imported (Ministry of Food, Agriculture and Livestock, 2014).

Especially in case of imported and domestic production of frozen bull semen, some spermatological parameters are very important and directly affect the fertilization rate. For these reasons, the principle spermatological parameters of thawed semen should be detected, certain degrees of values are required, and then it is allowed to use.

So far, examinations with a light microscope and subjective assessments were carried out in Turkey. But in recent years, semen evaluations and selection of stud bulls have being conducted by an objective method, called CASA systems. Indeed, the system is defined as an objective method in the world. It allows making a correct decision in semen export, import and production, and thus an increasing reproductive performance is expected in animal breeding. In addition, the efficiency of evaluation and selection of stud bulls will be increased by using objective methods. By using CASA systems in Turkey, well avoiding different detections and assessments as being faced in currently phase contrast light microscope examinations used depending on the choice of evaluating person, the associations will be in the harmony with current practices of developed countries.

Frozen-thawed bulls' semen evaluation was made by subjective methods for many years (Rodriguez Martinez, 2000; Uysal et al., 2006; Uysal et al., 2007). In this aspect, several tests and examination methods have been used. However, even one *in vitro* examination method was not enough to obtain an accurate estimate of fertilization potential of spermatozoa (Januskauskas and Zilinskas, 2002). To some extend development of computer-aided sperm analysis system could eliminate the individual factors. Therefore, in recent years more objective semen evaluations are possible (Hoflack et al., 2007; Sundararaman et al., 2007; Verstegen et al., 2002). In many animal species, examinations of principle spermatological parameters have been carried out by using CASA system (Budworth et al., 1988; Davis et al., 1963; Davis and Katz, 1992; Davis and Katz, 1993), but a few independent laboratory assessments of spermatological parameters are combined to confirm the accuracy of the CASA system (Januskauskas and Zilinskas, 2002).

The purpose of this study is to compare objective computer-aided sperm analyze' system and subjective conventional method for the evaluation of frozen bull semen.

Materials and Methods

Research materials consisted of frozen bull semen. Examinations and evaluations of samples were performed at the Department of Reproduction and AI laboratory. Frozen bull semen (totally 100 straws) were provided from private companies which import semen from different countries and public and private associations which produce semen in Turkey.

Half of 20 different bull's semen were provided domestically while the other half from other countries. Five straws from each bull were thawed and then were examined and evaluated by using CASA and conventional method (phase-contrast microscope with heating stage). Semen thawing and the assessment of spermatological parameters were based on the routine laboratory process at Ankara University Faculty of Veterinary Medicine, Department of Reproduction and AI (Tekin, 1994). Accordingly, principal spermatological parameters (spermatozoa motility, semen concentration and the ratios of abnormal and dead spermatozoa) for each bull were determined. The conventional method and CASA assessments were compared and the results were presented.

Descriptive statistics of all data were calculated and shown as mean ± standard error of mean'. One way analysis of variance (ANOVA) was used to evaluate the significant differences of means among the groups. In conjunction with the ANOVA, Duncan test was used as multiple comparison test procedure. In order to evaluate the difference of means among the type of sperms and methods, Student t test was used. For all comparisons, differences were considered with a minimum of 5% significance level. All statistical analysis were analyzed using SPSS 14.0 for Windows.

Results

In this study, a total of 100 samples from 20 different bulls (5 samples of imported and 10 domestic bulls each) were evaluated. Results of average and overall average of spermatological parameters were obtained and given in Tables 1, 2 and 3.

For imported semen, considering the average values of samples examined by the two methods, significant differences ($P<0.05$) were observed in the values of motility and concentration as well as the rates of abnormal and dead spermatozoa. Also, significant differences ($P<0.05$) were found in the 7^{th} sample for motility, in the 2^{nd}, 3^{rd} and 9^{th} samples for concentration, and in the 4^{th}, 5^{th}, 7^{th} and 9^{th} samples for abnormal spermatozoa rate.

Table 1. Average values of principle spermatological parameters in imported frozen bull semen (n=50).
Tablo 1. İthal dondurulmuş boğa spermalarında başlıca spermatolojik parametrelerin ortalama değerleri (n=50).

Evaluation Method	Bull No	SpermatozoaMotility (%) X±Sd	Spermatozoa Concentration (x10^9/ml) X±Sd	Abnormal Spermatozoa rate (%) X±Sd	Dead Spermatozoa rate (%) X±Sd
Conventional	1	59±4.84^{b}	17.86±0.92^{a}	34.4±1.88^{ab}	31.2±3.87^{ab}
	2	34±2.91^{a}	59.9±1.93^{ac}	46.6±1.72^{b}	38.8±1.98^{ab}
	3	55±2.23^{b}	46.9±2.18^{a}	30.4±2.71^{a}	32.8±2.59^{ab}
	4	53±1.22^{b}	40.6±9.03^{a}	23.8±1.68^{a}	34.6±1.60^{ab}
	5	59±1.00^{b}	47.4±7.97^{a}	29.6±3.14^{a}	29.8±2.45^{a}
	6	42±2.54^{a}	45.2±8.65^{a}	26.6±1.91^{a}	49±3.30^{b}
	7	52±1.22^{b}	66.2±2.76^{c}	25.2±1.95^{a}	36.8±1.06^{ab}
	8	58±2.54^{b}	50.92±2.90^{ac}	38±1.22^{ab}	35.2±1.65^{ab}
	9	36±3.67^{a}	66.45±3.60^{c}	22±1.34^{a}	42.2±1.01^{b}
	10	44±1.87^{ab}	29.25±1.85^{b}	36.4±2.99^{ab}	27.6±1.46^{a}
Average Value		**49.2±2.40**	**47.06±4.17**	**31.3±2.05**	**35.8±2.09**
CASA	1	58.1±3.50^{b}	18.36±1.26^{a}	38.6±1.86^{a}	32.2±2.63^{a}
	2	36.3±1.77^{a}	30.3±1.26^{b}	48.6±0.97^{b}	32.8±1.60^{a}
	3	55.18±2.34^{b}	28.1±1.06^{b}	36.4±1.36^{a}	30.5±2.24^{a}
	4	56.74±2.73^{b}	29.96±1.98^{b}	32.06±2.54^{a}	34.6±1.12^{ab}
	5	58.28±3.98^{b}	37.82±3.48^{bc}	33.6±4.22^{a}	31.3±3.21^{a}
	6	49.14±4.47^{b}	44.8±7.87^{c}	47.14±2.77^{ab}	43.8±2.89^{b}
	7	61.58±1.07^{b}	60.2±5.14^{d}	37.6±2.13^{a}	34±1.30^{ab}
	8	61±1.61^{b}	42.86±4.04^{c}	37.6±2.13^{a}	33.6±0.92^{a}
	9	37.88±5.31^{a}	47.6±5.87^{c}	31.2±1.64^{ab}	45.76±9.94^{b}
	10	54.6±5.19^{b}	25.62±1.06^{a}	36.2±0.86^{a}	27.4±2.48^{a}
Average Value		**52.8±3.19**	**36.56±3.30**	**38.34±2.13**	**34.59±2.83**

^{a,b,c}: The difference of group averages in the same column between different superscripts are significant (P<0.05)
^{a,b,c}: Aynı sütunda farklı harfleri taşıyan grup ortalamaları arası farklılıklar önemli (P<0,05)

For domestic semen, significant differences (P<0.05) were observed for the concentration of samples by the conventional evaluations and for the assessment of motility and concentration by the CASA method. Further, there were significant differences (P<0.05) between the two methods, in the 1st and 4th samples for concentration, and in the 1st sample for the abnormal sperm rate.

It was observed that there were significantly (P<0.05) higher data only for the concentration assessed by both methods, while no such differences between the values of motility as well as the rates of abnormal and dead spermatozoa were found when considering the general average rates.

Discussion

In this study, the results pointed out that, from conventional and CASA methods, significantly higher values were taken in only concentration values. Differences between motility, abnormal and dead spermatozoa rates are statistically insignificant when general average rates are examined.

Routine practices of semen are usually based on the conventional semen evaluation criteria for determining the quality of imported semen in Turkey. However, developed countries have benefited more from the technology, and certain devices work with computed-aided software programs to reveal the spermatological parameters, in order to eliminate the human factor and subjective errors in individual assessment.

Farrell et al. (1998) investigated the relationship between spermatological traits and fertility by using the CASA system, and notified that the breeding potential of bulls could be evaluated easily and accurately. Didion (2008), examined spermatozoa motility, morphology and concentration in boar semen, and noticed that this system was able to provide a reliable analysis for fertility. Further, Tardiff et al. (1997), analyzed native and short-term stored (at 5°C) bull semen by CASA and notified that this method was the most effective system to select a bull for breeding and use its semen for AI.

Kastelic and Thundathil (2008), analyzed semen to state the fertility in bulls with *in vitro* data by conventional andrological examinations and CASA, and reflected that only subjective assessments may be

performed by conventional methods. They also noted that the spermatological traits can be affected by a number of factors and so CASA analysis were determined to be much more objective. Indeed, Gravance et al. (1999) concluded that there were some substantial differences in the results of technicians performing the evaluations by conventional methods and hence these routine methods are subjective. In contrast, in their study, the detection of head morphometric by CASA was reliable and objective and further, no differences were observed in subsequent re-assessments with this technique.

Table 2. Average values of principle spermatological parameters in local frozen bull semen (n=50).
Tablo 2. Yerli dondurulmuş boğa spermalarında başlıca spermatolojik parametrelerin ortalama değerleri (n=50).

Evaluation Method	Bull No	Spermatozoa Motility (%) X±Sd	Spermatozoa Concentration ($x10^9$/ml) X±Sd	Abnormal Spermatozoa rate (%) X±Sd	Dead Spermatozoa rate (%) X±Sd
Conventional	1	47±6.04	50.61±3.13^{a}	33.6±2.24	40.4±4.93
	2	47±2.54	78.25±6.61^{c}	40.8±1.49	44.6±2.24
	3	48±3.74	79.75±5.38^{c}	30.4±2.13	41±3.63
	4	49±3.67	72.88±1.63^{c}	41±1.84	40±3.43
	5	51±2.91	55.4±1.26^{a}	37±2.54	37.6±2.26
	6	47±4.06	38.06±2.42^{b}	31.2±1.35	39.8±5.02
	7	47±3.39	61.2±3.20^{d}	40.8±1.65	40.6±2.83
	8	47±3.39	74.4±3.61^{c}	30.8±1.42	39±2.50
	9	50±2.73	65.96±2.56^{ac}	40.8±1.20	39±2.91
	10	49±3.67	54.62±1.31^{a}	38.4±1.43	35.8±1.88
Average Value		**48.2±3.58**	**63.11±3.11**	**36.48±1.72**	**39.7±3.16**
CASA	1	46.06±7.90^{ab}	27.7±2.27^{a}	41±1.51	38.8±5.8
	2	43.8±4.82^{a}	58.22±7.67^{b}	41.64±1.59	42.4±3.23
	3	40.1±2.95^{a}	60.16±8.17^{b}	37.4±1.93	45±2.86
	4	46.9±1.50^{ab}	51.78±3.35^{b}	42.6±3.02	41.4±1.36
	5	53.4±4.14^{b}	53.7±4.00^{b}	37.04±2.13	37.6±2.03
	6	48.04±5.18^{ab}	33.06±1.98^{a}	34.4±1.07	39±5.29
	7	46.42±1.78^{ab}	56.18±3.66^{b}	40.34±2.21	39±2.64
	8	45.66±1.63^{ab}	57.1±5.48^{b}	34.8±2.51	39±2.42
	9	49.52±2.37^{ab}	59.62±2.37^{b}	43.4±0.60	39.6±1.96
	10	51.6±1.96^{ab}	52.42±2.28^{b}	36.34±1.65	36±2.21
Average Value		**47.1±3.42**	**50.99±4.12**	**35.26±1.82**	**39.78±2.98**

^{a,b,c}:The difference of group averages in the same column between different superscripts are significant (P<0.05)
^{a,b,c}: Aynı sütunda farklı harfleri taşıyan grup ortalamaları arası farklılıklar önemli (P<0,05)

Table 3. General average values of principle spermatological parameters in imported and local frozen bull semen (n: 50).
Tablo 3. İthal ve yerli dondurulmuş boğa spermalarında başlıca spermatolojik parametrelerin genel ortalama değerleri (n: 50).

Evaluation method	Bull No	Spermatozoa Motility (%) X±Sd	Spermatozoa Concentration ($x10^9$/ml) X±Sd	Abnormal Spermatozoa rate (%) X±Sd	Dead Spermatozoa rate (%) X±Sd
Conventional	Import	49.2±2.40	47.06±4.17^{a}	31.3±2.05	35.8±2.09
	Local	48.2±3.58	63.11±3.11^{b}	36.48±1.72	39.7±3.16
CASA	Import	52.8±3.19	36.56±3.30^{a}	38.34±2.13	34.59±2.83
	Local	47.1±3.42	50.99±4.12^{b}	35.26±1.82	39.78±2.98

$^{a, b}$: The difference of group averages in the same column between different superscripts are significant (P<0.05)
$^{a, b}$: Aynı sütunda farklı harfleri taşıyan grup ortalamaları arası farklılıklar önemli (P<0,05)

Defoin et al. (2008) attempted to estimate the post-thawing quality of bull semen by CASA used prior to freezing, observed that the evaluations made by this equipment were more beneficial than the conventional method. Sundararaman et al. (2007) examined spermatozoa morphology in bulls by CASA to eliminate the subjectivity of conventional methods found that a bull which was accepted clinically normal by conventional methods had a high percentage of certain types of head abnormalities and these details could have been proven only by CASA.

Comparing the present data, more detailed and fractional values were obtained by the CASA while the conventional method yielded exact values for motility, concentration, as well as the rates of abnormal and dead spermatozoa. For imported semen, considering the general averages in examinations of semen motility made by the two techniques, superior results were recorded by conventional method (by phase-contrast microscope). The concentration results were found lower in CASA. For the ratio of abnormal spermatozoa, much higher values were obtained by the CASA method. However, in terms of dead spermatozoa rate, higher results were obtained from both methods; in some cases by CASA and in some cases by conventional method. For domestic semen, the motility and concentration values were much higher with the conventional method, while abnormal sperm rates were lower, with dead sperm rates remaining virtually unchanged.

Between the average values of examples examined in imported semens, differences observed in the rates of motility, concentration, abnormal spermatozoa rates and dead spermatozoa rates have been important ($P<0.05$). Significant differences have been detected in 7th example for motility, in 2^{nd}, 3^{rd} and 9^{th} examples for concentration, 4^{th}, 5^{th}, 7^{th} and 9^{th} examples for abnormal spermatozoa rate for comparison of conventional and CASA methods ($P<0.05$).

In domestic semen; differences, observed in that concentration between examples detected by conventional evaluations and observed in point of motility and concentration held by CASA method have been found statistically significant while differences between conventional and CASA methods, for 1st and 4th examples in concentration; for 1st example in abnormal rate, have been recorded as important ($P<0.05$).

In conclusion, it has been observed that CASA method was much more specific for semen evaluation, it yielded definite and detailed values and had lower margins of error than that of subjective evaluations with the conventional method.

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