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Studies on the Observation of Sperm Morphology and Sperm Concentration Using Unopette in Cattle and Dog

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소 및 개에 있어서 Unopette를 사용한 정자형태 및 정자농도의 검사

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초 록

소와 犬에 있어서 Unopette가 정자의 형태학적 검사 및 정자농도의 검사를 위하여 사용될 수 있는가를 알아보기 위하여 본 연구를 수행하였다.

소정액 및 犬精液을 Unopette에 희석한 후 3~5℃에 보존하면서 시간경과에 따라서 위상차현미경하에서 관찰하여 다음과 같은 결과를 얻었다.

1. Unopette를 사용하여 관찰한 정자는 48시간까지는 hematoxylin-eosin을 사용하여 관찰한 정자보다는 높은 정상정자율을 나타내었다.
2. Unopette를 사용한 정자는 정자농도에 있어서 대조군 정자에 비하여 24시간까지는 큰 차이를 나타내지 않았다.
3. 따라서 Unopette는 정자의 형태학적 검사 및 정자농도의 검사를 위하여 유용하게 사용될 수 있다는 사실을 알 수 있었다.

INTRODUCTION

It was reported that observation of sperm morphology is used to evaluate the male fertility potential in bovine (Rao *et al.*, 1980; Peet and Micke, 1976; Pursel *et al.*, 1972; Marshall and Saacke, 1967; Salisbury and Mercier, 1945), equine (Voss, 1981; Ball, 1971) and human (Mahadevan and Trounson, 1984), and decline of conception may be related with morphologic defects (Morrow, 1980; Hahn *et al.*, 1969; Hancock, 1959).

Morphological characteristics of sperm have been evaluated stained smears of sperm. But it was clarified that procedures for preparing stained smears were detrimental to sperm morphology.

Also, phase contrast and differential interference contrast microscopy make it possible to evaluate spermatozoal morphologic features in wet preparations of semen (Johnson *et al.*, 1976).

Phosphate-buffered saline solution with 0.2 g of glutaraldehyde / 100ml (Johnson *et al.*, 1976) or buffered-formol saline solution (Salisbury and Mercier, 1945) will preserve morphologic characteristics of semen.

Unopette is used as a *in vitro* diagnostic system for the enumeration of erythrocytes in whole blood, and can be used easily.

This study was carried out to determine whether Unopette can be used for the observation of sperm morphology and sperm concentration.

MATERIALS AND METHODS

Bovine sperm were used with raw semen which collected with artificial vagina from six sexually mature Holstein bull. Canine sperm were

obtained by masturbation following a minimum of 48 hours of sexual abstinence from eight cross-bred male dogs. Sperm were diluted with Unopette solution at the ratio of 1:200 as 10㎕ of sperm was mixed to 1.99ml of Unopette solution.

Sperm morphology and concentration were examined under the phase contrast microscope every 24 hours during preservation in 3~5℃ after dilution. Control sperm which did'nt use Unopette was examined under 1000X microscope using hematoxylin-eosin staining. Differention of abnormal sperm followed Hafez (Hafez, 1980).

The significance of differences between two experimental group was dealt with t-test and the significance of differences between three experiment or more was performed by F-test.

RESULTS

Table 1 shows morphology and concentration of bovine sperm using Unopette that were examined under the phase contrast microscope. Percent of normal sperm revealed the highest value ($P<0.05$) at 0 hour immediately after dilution. Thereafter the more time was passed, the lower percent of normal sperm was showed. Percent of normal sperm at control sperm showed lower value than even preservation of 48 hours. Sperm concentration revealed similar value between 0 hour and 24 hours. After that time, the more time was passed, the lower value was showed.

Table 2 shows morphology and concentration of canine sperm using Unopette that were examined under the phase contrast microscope. Until preservation of 48 hours, sperm using Unopette showed higher percent of normal sperm than control sperm. Thereafter sperm using Unopette revealed lower percent of normal sperm than control sperm. Sperm concentration revealed similar value until 24 hours as compared with control sperm. After that time, the more time was passed, the lower value was showed.

Table 1. Morphology and concentration of bovine sperm using Unopette (n=12:mean±S.D.)

	Control[a]	Duration of preservation (hr)						
		0	24	48	72	96	120	144
Normal sperm(%)	78.3 ±4.05	85.1* ±3.16	82.4 ±6.59	79.9 ±7.02	76.8 ±6.53	74.7 ±6.61	71.6 ±8.96	69.5 ±9.47
Sperm concentration ($\times10^8$/ml)	13.7 ±1.54	13.5 ±1.45	13.4 ±1.86	11.8 ±1.73	11.4 ±1.98	10.5 ±2.01	10.3 ±1.87	9.8 ±1.50

[a]: Hematoxylin-eosin staining
* $P<0.05$

Table 2. Morphology and concentration of canine sperm using Unopette (n=12:mean±S.D.)

	Control[a]	Duration of preservation (hr)						
		0	24	48	72	96	120	144
Normal sperm(%)	81.8 ±4.37	88.5* ±3.12	85.9 ±5.83	83.6 ±7.56	77.0 ±7.08	76.4 ±6.81	73.7 ±5.93	70.3 ±9.74
Sperm concentration ($\times10^6$/ml)	132.7 ±15.1	130.4 ±16.4	128.7 ±21.9	116.2 ±19.0	105.9 ±25.4	102.5 ±20.8	97.5 ±17.3	92.8 ±15.9

[a]: Hematoxylin-eosin staining
* $P<0.05$

Table 3 shows the data of abnormalities of bovine sperm using Unopette that were examined under the phase contrast microscope. In percent of abnormal sperm, the more time was passed, the higher value was showed ($P<0.05$). Percent of abnormal heads revealed 8.2% at 0 hour, thereafter percent of abnormal heads tended to increase in proportion to the time lapse.

Table 4 shows the date of abnormalities of canine sperm using Unopette that were examined under the phase contrast microscope. Percent of abnormal sperm revealed the lowest value ($P<0.05$) at 0 hour. Thereafter the more time was passed, the higher value was showed. Proximal droplets and bent / coiled midpiece and tails were not seen after preservation of 72 hours.

Table 3. Abnormalities of the bovine sperm using Unopette (n=12:mean±S.D.)

	Control[a]	Duration of preservation (hr)						
		0	24	48	72	96	120	144
Abnormal sperm	21.7 ±4.05	14.9 ±3.11	17.6 ±6.59	20.1 ±7.02	23.2 ±6.53	25.3 ±6.81	28.4 ±8.96	30.5* ±9.47
Abnormal heads(%)	5.4 ±4.92	8.2 ±1.82	9.9 ±6.90	11.9 ±8.38	13.9 ±7.17	16.6 ±5.09	20.4 ±9.31	20.7 ±9.75
Detached heads(%)	3.1 ±2.57	2.3 ±1.27	3.0 ±3.51	1.9 ±2.08	1.5 ±1.86	2.1 ±2.38	2.5 ±1.90	2.2 ±2.05
Proximal droplets(%)	0.8 ±0.84	0.2 ±0.21	0.3 ±0.36	0.3 ±0.32	0.1 ±0.18	–	–	–
Distal droplets(%)	0.4 ±0.56	0.2 ±0.37	0.3 ±0.62	0.3 ±0.41	–	–	–	–
Bent / coiled midpieces and tails(%)	12.0 ±6.93	4.0 ±2.62	4.1 ±2.65	5.7 ±3.84	7.7 ±4.20	6.6 ±4.09	5.5 ±3.83	7.6 ±4.76

[a]: Hematoxylin-eosin staining

* $P<0.05$

DISCUSSION

It is the quality of the sperm rather than the quantity that is important in defining male fertility potential, and a complete semen analysis consists of five basic determinants : volume, density, motility, qualitative sperm movement (forward progression), and morphologic characteristics (Lipshultz, 1982). Also, *in vitro* fertilization rate by sperm was used to predict the male fertility potential (Lacham *et al.*, 1989: Berger and Horton, 1988: Clarker and Johnson, 1988: Comhaire *et al.*, 1988, Mahadevan *et al.*, 1988, Tanphaichitr *et al.*, 1988: Wheeler and Seidel, 1987).

Pedersen and Koefoed-Johnsen (1979) indicated that wet unstained preparations gave an abnormality rate of 18.65%, eosin-nigrosin 15.67 %, and Indian ink 14.81% when the sperm from Jersey bulls were examined. Also, Harasymowycz *et al.* (1976) indicated that abnormality revealed 20.83%, 22.20%, 20.33% and 20.37%, repectively, for Hancock's stain, Blom's stain, glutaraldehyde fixed and formol saline fixed. As shown in Table 3 using sperm from Holstein bull, control sperm

Table 4. Abnormalities of the canine sperm using Unopette (n=12:mean±S.D.)

	Control[a]	Duration of preservation (hr)						
		0	24	48	72	96	120	144
Abnormal sperm(%)	18.2 ±4.37	11.5 ±3.12	14.1 ±5.83	16.4 ±7.56	23.0 ±7.08	23.6 ±6.81	26.3 ±5.93	29.7* ±9.74
Abnormal heads(%)	5.8 ±5.42	5.3 ±1.35	6.8 ±4.26	9.8 ±6.58	13.4 ±5.75	15.2 ±5.01	17.9 ±8.76	20.9 ±10.24
Detached heads(%)	2.8 ±2.47	2.4 ±2.51	3.0 ±2.47	1.5 ±2.10	1.6 ±1.92	1.9 ±2.31	2.2 ±1.73	1.9 ±2.09
Proximal droplets(%)	0.7 ±0.82	0.3 ±0.47	0.3 ±0.36	0.2 ±0.25	–	–	–	–
Distal droplets(%)	0.3 ±0.53	0.1 ±0.46	0.2 ±0.39	0.3 ±0.38	–	–	–	–
Bent / coiled midpieces and tails(%)	8.6 ±8.04	3.4 ±2.89	3.8 ±2.83	4.6 ±4.06	8.0 ±5.82	6.5 ±4.75	6.2 ±4.02	6.9 ±4.06

[a]: Hematoxylin-eosin staining

* $P<0.05$

using hematoxylin-eosin staining revealed 21.7% of abnormality. It is considered that these differences in abnormalities resulted from difference at breeds and staining methods.

As shown in this work, control sperm using hematoxylin-eosin showed higher percent of abnormal sperm than sperm using Unopette at pres-ervaion of 0 hour (Table 3, 4). It is considered that the difference resulted from the difference of mechanical damage between two methods.

According to the above-mentioned result, until preservation of 48 hours, sperm using Unopette showed higher percent of normal sperm than control sperm and sperm concentration revealed similar value until 24 hours. So, it is suggested that Unopette can be used as appropriate solution for preservation in terms of morphological observation and sperm concentration.

ABSTRACT

This study was conducted to determine whether Unopette can be used for the observation of sperm morphology and sperm concentration.

Bovine sperm and canine sperm were observed with phase contrast microscope after dilution with Unopette according to duration of preservation at 3~5℃.

The results obtained in this study were as follows :

1. Sperm using Unopette showed high normal sperm (%) than sperm using hematoxylin-eosin until 48 hours.

2. Sperm using Unopette revealed no difference in sperm concentration until 24 hours, as compared with control sperm.

3. As a result, Unopette was assessed as appropriate solution for preservation in terms of morphological observation and sperm concentration.

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