



RESEARCH PAPER

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Survival and morphologic evaluation of bull sperm in Iranian paitle next of thawing with papaniculao and eosin-nigrosin method

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Abstract

The aim of this study was to evaluate two methods of Papanicolaou and Eosin–Nigrosin to test the viability and morphology of sperms of Iranian Payots Holstein cows. Thirty samples of Iranian payot were prepared from Sheikh Hasan Cattle Breeding Centre, from each Payot, 2 spread slide samples were stained, and 200 sperm were counted and the numbers of dead and live sperms were calculated. According to the results obtained by staining of Eosin–Nigrosin it was showed that 90 percent of sperms were alive. From the results obtained, disorders such as: multiple tails, 2 tails, none of the groups were observed without head and on the other hand the variables of kink tail, 90-degree curvature of the sperm tail and asymmetric input of tail to the head of sperm, it appropriated the largest amount of sperm defects for itself. it can be said with regard to the results, Iranian Payots of Holstein cows from the aspect of sperm evaluation and reproductive ability of them have good position and being non-reproductive in some cases may be relate to condition of female genitalia or the amount of skills of inoculation agent and wrong time of inoculation.

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Introduction

One of the factors that reduce fertility and quality of frozen semen is keeping and improper use of them. Major centers of artificial insemination for keeping of frozen semen use strict management system and this factor causes high quality of work and production. Melting of the frozen semen is very important and vital. This function depends heavily on the type of diluent. Some centers that provide frozen semen attach the way of melting of semen with a brochure to the semen Payot. Sperm morphology assessment is one of the necessary factors for analyzing sperm and samples of semen provide an assessment of the proper reproduction from one stallion. Also Theriogenology society at first for morphology evaluation of sperm in stallion has recommended using wetmount slide and contrast phase microscope (Kenney *et al.*, 1983).

Comparison between various methods shows that, Eosin nigrosin is staining that is recommended for morphology assessment of a cow's sperm (Chenoweth *et al.*, 1992).

Some centers have recommended for using 30 Celsius degrees water for thawing the semen. When semen was thawed, we should not put them inside the very cool pipette because it makes a double shock for sperm. Thawed semen should not be cooled again because severe damages happen to the sperm. Damages that happen to sperm during freezing and thawing of sperm are as follows: Structural changes in specific organs of sperm such as Acrosome, Acrosome rupture, degenerative changes in the Acrosome, rupture of the plasma membrane which is on Acrosome that this condition causes swelling of the Acrosome and loss of components of the Acrosome. Since method Papanicolaou and Eosin – Nigrosin are proper methods for scrutiny of sperm so in this research we decided to use two methods of Papanicolaou and Eosin– Nigrosin to test the viability and morphology of sperms of Iranian Payots Holstein cows after thawing and evaluating their quality from this aspect.

Materials and methods

30 samples of Iranian payot were prepared from Sheikh Hasan Cattle Breeding Centre and were transported by liquid nitrogen tanks to the laboratory and in water with the temperature of 35 Celsius degrees for was thawed for 3 minutes in laboratory that finally, 30 samples of Payot were prepared. Each of these samples was used to evaluate (morphology, survival, number of sperms). From each Payot, 2 spread slide samples were prepared for staining Papanicolaou and Eosin nigrosin and after spreading were fixed in 96 alcohol.

Staining of Papanicolaou

This method widely were used in most of Andrology laboratories. It gives the color for Spermatozoides and other cells as well. The acrosomal region and behind the acrosomal of head, small cytoplasmic pieces and tail give permission to be colored that is a good method for evaluating the sperm morphology.

Staining of Eosin Nigrosin

This staining method is based on the principle that Eosin color pass through cell membrane of dead sperms and make them pink or red color, while live sperms don't permit color to pass through and remain colorless, and nigrosin or Aniline blue creates a dark blue background on the slide in which live and dead sperms are seen clearly.

Damaged and dead sperm with suction of Eosin become pink while live sperm remained white. Nigrosin with creating a dark background in slide makes evaluation easier. In this study 200 sperm were counted and the numbers of dead and live sperms were calculated. In this staining, live sperms were observed with white or light pink heads and dead sperms were observed with red or dark pink heads. If only the neck of sperm took the color and the head of sperm remains colorless so it is said leakyneck membrane. The membrane of these cells was not completely disappeared.

Results

According to the observations of sperm computation

that was performed by Neobar slide in each of the samples, the numbers that were obtained were compared statistically and a significant difference was found (Table 1). In the observations based on sperm morphology parameters was performed by specific Papanicolaou staining.

From the results obtained, disorders such as: multiple tails, 2 tails, none of the groups were observed without head and on the other hand the variables of kink tail, 90-degree curvature of the sperm tail and asymmetric input of tail to the head of sperm, it appropriated the largest amount of sperm defects for itself.

Table 1. Comparative evaluation of the average amount of sperm in the study groups.

Group	Mean±SE	SD
TizHoush	15300000±682519.84 ^a	1182159.04
Arvan	16950000±525198.37 ^{ab}	909670.27
Takin	16800000±545482.75 ^{ab}	950000.00
Dushina	17633333.33±367801.27 ^{ab}	637050.49
Yashar	16216666.67±261937.22 ^{ab}	453688.58
Vafa	16916663.67±616666.66 ^{ab}	1068097.99
Khoshgam	17500000±115470.05 ^{ab}	200000.00
Mahbod	18266666.67±316666.67 ^b	548482.75
Barzmehr	17433333.33±753510.30 ^{ab}	1305118.13
Chilan	16050000±653834.84 ^{ab}	377491.72

* Different letters indicate significant statistic difference.

According to the results obtained by staining of Eosin–Nigrosin that were performed to know if sperms are alive or dead. Whatever was obtained from mean of data showed that 90 percent of sperms were alive.



Fig. 1. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), all sperm are normal.

Discussion

Assessment of sperm morphology is one of the essential components of sperm analysis and semen samples, provides a proper reproduction evaluation from a bull. Comparison between various methods shows that Eosin Nigrosin staining is recommended for evaluation of sperm morphology of bull. Because using of it is easy compared to Papanicolaou staining and it is used widespread and significantly and it was

recommended by the World Health Organization for human sperm morphology evaluation. Aims of the present study are at determining the effectiveness of these methods for the assessment of bull sperm morphology. Talbot and Chakson (1981) reported that sperm shows reaction in acrosome area that is white or without staining. The sperm acrosome shows reaction against Tripian blue and get one light color from Tripian blue of dead sperm or base mark of brown of alive sperm (Talbot and Chacon, 1981).



Fig. 2. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of defects of asymmetric input of tail to head of sperm.

A possible explanation in the study of human's sperm and bull's sperm is the acrosome reaction that shows

in the inner membrane of Acrosomal and related components with it that glycoprotein in the inner acrosome membrane changes happen to it in the structure of the sugars (Holt, 1995).

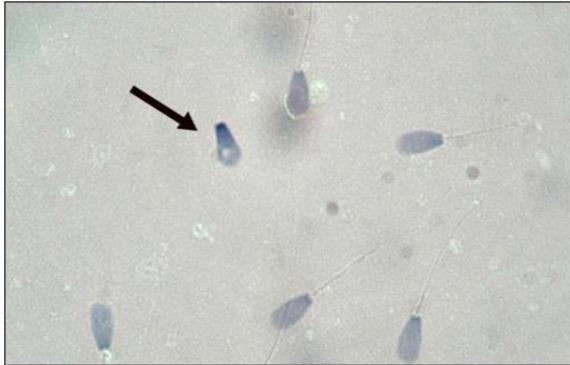


Fig. 3. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of absence of tail and presence of vacuoles in the head of sperm.

Sperm morphology evaluation is the biggest technical challenges for andrology laboratory (Alvarez *et al.*, 2005- Brazil, 2010).



Fig. 4. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of no entry of asymmetrical of tail to head and curved tail of sperm.

Differences in morphology results of sperm among various assessment methods can only represent the shape, weak structure of sperm and lack of proficiency in the person that evaluate in finding difference and detaching from the seeming shape of processed sperm to attribute it to a particular method. Comparative studies of sperm morphology evaluation methods are used only for normal sperm which in itself is not a reliability criterion that make conclusion, or little instances have been done for explaining the possible causes of differences in the

amount of defects in sperm (Alvarez *et al.*, 2005- Comhaire and Vermeulen, 1995).



Fig. 5. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of the curved tail with 90 grade.

Specific sperm defects were counted in the present study. Using of Papanicolaou method makes easy observation acrosome defects and cytoplasmic droplets.



Fig. 6. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing the presence of vacuoles in the head of sperm.

A common concern with Eosin- Nigrosin staining is the probable of introducing of defects of tail in the form of curved and circular tail. These types of false cases have been also observed in other studies (Comhaire and Vermeulen, 1995). But using of the warm slides with quick drying of sample impedes from any increase in the curvature and being convoluted of tails of sperm in the present study. Although using of samples that were stained by Papanicolaou are recommended way to assess human sperm (Arthur *et al.*, 1996).

Significant differences are among veterinarians for

classification that is based on sperm morphology. This diversity makes it difficult to compare results for Veterinarians and diagnose problems that may be related to specific defects of sperm. Moreover, it may also prevent semen reference values and guidelines to achieve the semen liquid with desired quality.

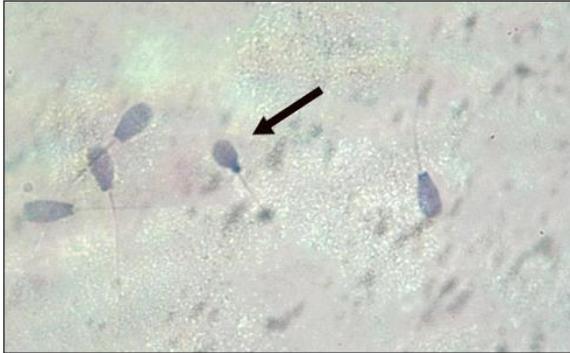


Fig. 7. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of defect in the head of sperm in a round form.

Because the use of it in comparison with staining by Papanicolaou method is easy is used in widespread and is recommended by the World Health Organization for the assessment of human sperm morphology. In Several evaluations of the laboratories, to evaluate changes of human sperm morphology using of this staining is recommended that making conclusion about it depends on technicians (Comhaire and Vermeulen, 1995).

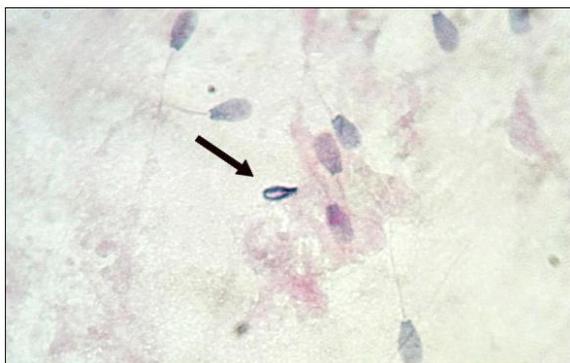


Fig. 8. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of defect because of small head of sperm

These differences may show species differences in resilience of sperm, or may be related to different methods of preparation of sample, Perhaps the separation method may eliminate the separation

heads of sperm from tails or minimize it (Organization, 2010).



Fig. 9. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of defect of sperm tail in being short and asymmetric of entry of tail to head of sperm.

Comparative studies of sperm morphology assessment methods are used only for normal sperm, which in itself is not a reliable criterion to make conclusion or little instances have been done for explaining the possible causes of differences in the amounts various sperm defects (Freneau *et al.*, 2010- Kruger *et al.*, 1987).



Fig. 10. View of a smear prepared from cattle sperm of native breeds (Papanicolaou staining * 100), Fletcher: Observing of defect of short tail, complex tail and sperm having two heads.

Although some authors have suggested that it may increase the amount of sperm defects in wet Wetmount preparations, resulting in seeming shape (Meschede *et al.*, 1993).

This can lead to decrease the centric part of circularcurved and simultaneously with increasing of separated heads in stained samples.

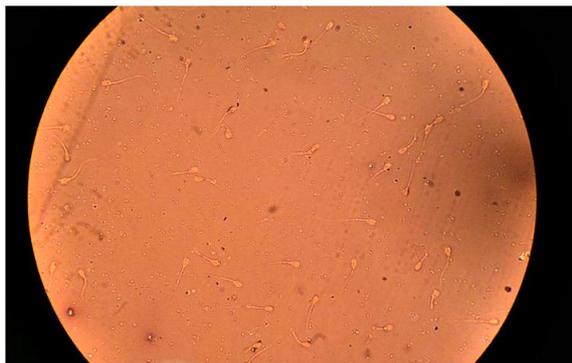


Fig. 11. View of a smear prepared from cattle sperm of native breeds (Eosin-Nigrosin staining * 40), Observing of normal sperm without staining.

Sperm morphology assessment is available and can be done and the results largely depend on the skill and experience of the appraiser. Unfortunately, appraisers were trained sporadically because of these people have not been updated (Brazil, 2010). A possible explanation in the study of human's and bull's sperm, acrosome reaction in the inner membrane acrosomal and related components to it demonstrates that the glycoprotein in inner membrane of acrosome changes in the structure of the sugars. One of the reasons for the various staining patterns in sperm of bull, because of the proximity of the plasma membrane to the nucleus membrane in the form of longitudinally that allow dye penetration at that part of the head.

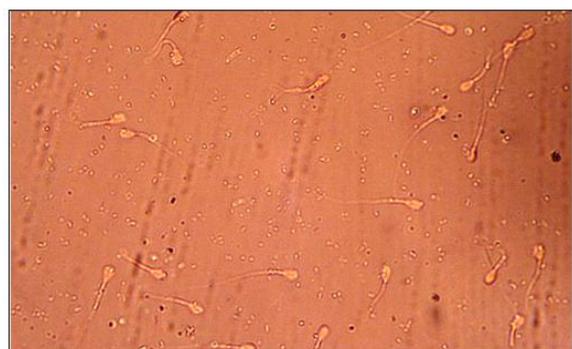


Fig. 12. View of a smear prepared from cattle sperm of native breeds (Eosin-Nigrosin staining * 100), Observing of normal sperm without staining.

Results related to the number of sperm in Iranian Payots due to the fact that the study is performed after thawing of the frozen Payots, showed that Payots have adequate levels for securing of the fertility.

Conclusions

In conclusion it can be said with regard to the results, Iranian Payots of Holstein cows from the aspect of sperm evaluation and reproductive ability of them have good position and being non-reproductive in some cases may be relate to condition of female genitalia or the amount of skills of inoculation agent and wrong time of inoculation.

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