Özkök, A O. International Journal of Science Letters (IJSL). 2022. 4(2): 269-276
https://doi.org/10.38058/ijsl.1141372

Research Article

Evaluation of different methods used in morphological examination of canary sperm

Arda Onur Özkök

Department of Veterinary, Suluova Vocational School, Amasya University, Amasya/Turkey

Abstract

By determining the species-specific structure by morphological examination of the sperm, it is possible to improve some of the disadvantages related to short and long-term storage and use in artificial insemination applications. When the morphology of the canary spermatozoon is evaluated, it is seen that it has a long indented acrosome as well as a rather long flagellum. To determine the morphological structure of canary semen, morphological examination methods used in different poultry can be applied and visually evaluated with different shapes. This study aims to provide information about the comparison and usability of various staining methods used in the morphological examination of canary semen from songbirds. In this study, semen from 12 male Gloster canaries was collected to determine morphological parameters in semen. Collected semen with different morphological evaluation methods; Fixation with 5% formaldehyde, Formalin fixation and Giemsa staining, Giemsa staining, Formalin fixation, and SpermBlue® staining and SpermBlue® staining were evaluated. In the results of the study, while the nucleus was more prominent in Giemsa staining compared to other staining methods used for morphological evaluation, acrosome was observed in SpermBlue® and Giemsa staining. On the other hand, when the sperm fixed with 5% formaldehyde solution were evaluated, it was seen that the acrosome and nucleus were indistinguishable, while the changes in the flagellum were determined much more clearly. As a result of the study, it was reported that the morphological structure of canary semen could be evaluated with all morphological examination methods used.

Article History

Received 06.07.2022
Accepted 24.07.2022

Keywords

Canary,
Birds,
Sperm,
Morphology,
Stain

1. Introduction

Studies related to infertility problems, reproductive performance, and artificial insemination practices in poultry are generally seen in various exotic songbirds such as sparrows and finches. Studies on canary semen are very limited. Examination of the morphological structure of the sperm and obtaining information about it provide important information about

Correspondence: arda.ozkok@amasya.edu.tr

the fertile ability and quality of the sperm. Lupold et al. (2019) reported that there is a relationship between the morphological structure and motility of spermatozoa in songbirds. It has been determined that in poultry, the spermatozoon can provide more energy due to the longer middle part and therefore the motility value may be higher. In addition, the morphological features of the winged spermatozoa such as the head, middle part, and the structure of the flagellum and the fertility of the poultry are highly correlated with the success of artificial insemination. (Lupold et al., 2019).

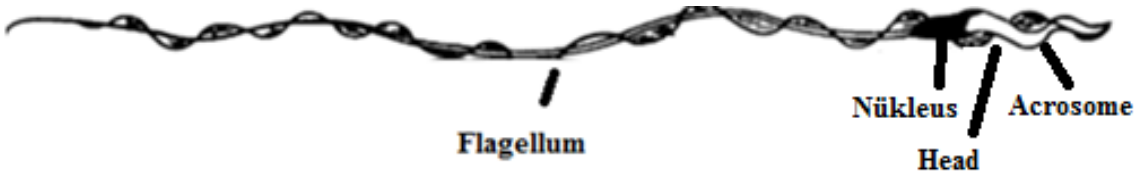

Figure 1. Morphological appearance of the canary spermatozoon (Humphreys, 1972).

The canary spermatozoon has a length of about 300 µm. In addition, the head of winged spermatozoa is considerably larger than that of mammalian spermatozoa (Humphreys, 1972). Thus, the winged spermatozoon has an extremely long flagellum as well as a large acrosome. Due to this morphological structure, poultry semen has some advantages and disadvantages. In addition, this situation directly affects the fertility ability of spermatozoa. In a study, it was emphasized that the long spermatozoon length in birds has a positive effect on the fertilization ability. It has also been reported that there may be a relationship between age and the length of spermatozoa in male birds (Cramer et al., 2020). It is thought that there is a relatively direct ratio between the total length of the spermatozoa and the motility of the sperm. It has been stated that the reason for this situation is that the length of the flagellum affects the pushing ability. In addition, studies have emphasized the importance of mitochondria in the middle part of the spermatozoon. However, it has been reported that the relationship between sperm motility and morphology may be selective in songbirds, so more studies are needed on the subject (Cramer et al., 2021). Detection of defects in spermatozoa morphology in poultry, as in mammals, is very necessary to evaluate reproductive performance. In a study conducted for this purpose, head, middle part, and tail anomalies were examined to determine the abnormal spermatozoa ratio (Scheneider et al., 2018).

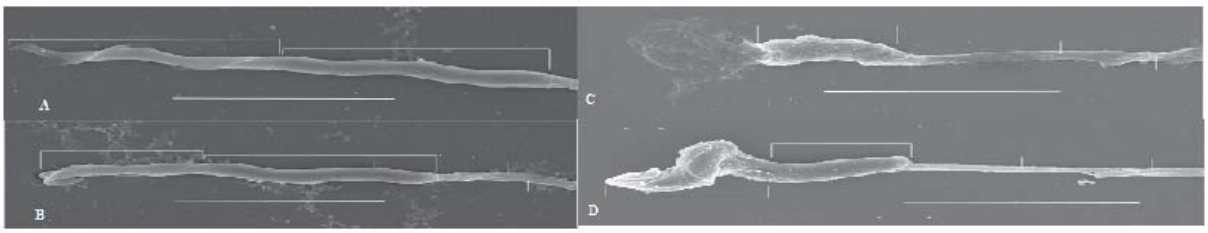

Figure 2. Head-related morphological disorders in winged semen (A: normal acrosome, B: acrosome damage, C: plasma membrane and acrosome deficiency, D: degenerated acrosome) (Hermosell et al., 2013).

2. Materials and Methods

Many methods are used to determine morphological parameters in songbirds. There are very limited resources on the morphology of canary semen. In the study, various staining methods used in the morphological examination of canary sperm, which is one of the songbirds, were evaluated. For this purpose, 12 Gloster male canaries were used in the study. The canaries were selected from young males 1-3 years old. The canaries were kept in production cages measuring 60x50x40 cm against negative effects such as lubrication and stress. Before the semen was collected, the canaries were prepared for semen collection by encouraging sufficient heat generation at the optimum temperature (16-20°C) and with a photoperiodic light application for 15 hours daily.

2.1. Collection of Semen

Cloacal massage method was used to collect semen from canaries. In order to obtain semen from small birds, semen is seen to come out when gently stimulated from the abdomen to the cloacal protuberance located dorsal to the cloaca in male birds. With the application of pressure between the thumb and forefinger of the cloacal protuberance, the semen comes out of the cloaca (Gee et al., 2004). Sperm taken from the cloacal region were collected with the help of a hematocrit capillary tube. Care was taken to avoid contamination while semen was collected. Semen samples contaminated with feces during semen collection were not used in the study.

2.2. Morphological Examination Methods

2.2.1. Detection with 5% Formaldehyde

After the semen sample taken from the canary was fixed with 1 drop of 5% formalin solution, smear was taken on the slide. The slides were left to dry. After drying, it was examined under a light microscope at 40x magnification (Kleven et al., 2009). While the acrosome and nucleus were indistinguishable in the head, it was determined that the flagellum was determined quite clearly.

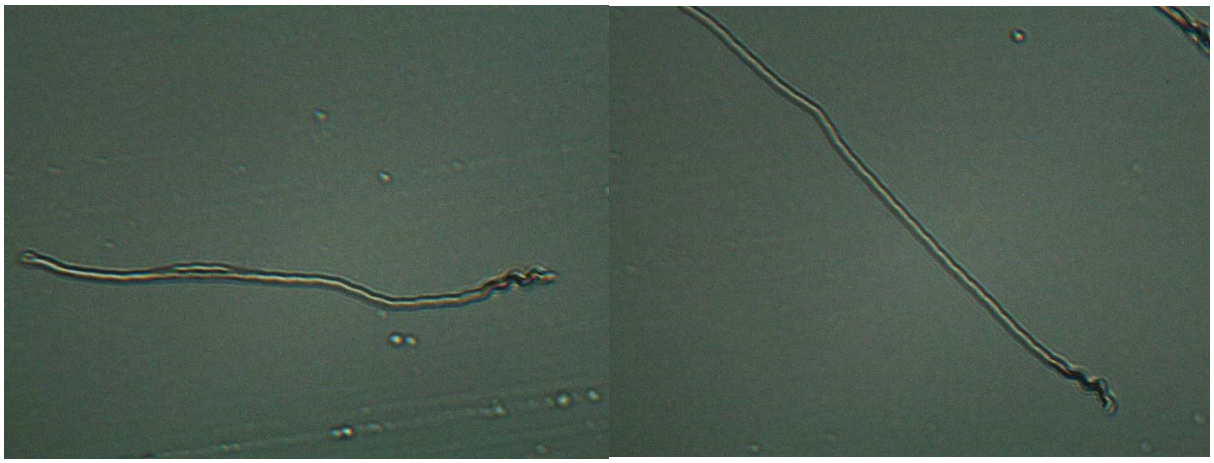

Figure 3. Morphological evaluation of canary spermatozoa with 5% formaldehyde fixation

2.2.2. Formalin Fixation and Giemsa Staining

10 µl of semen sample from the collected semen was fixed with 5% formalin solution. After drying by smearing on the slide, it was painted with Giemsa paint (Camer et al., 2020). After fixation with 5% formolin solution, it was painted with Giemsa dye for 15 minutes. It was evaluated under a light microscope at 40x magnification. evaluated. After the fixation, acrosome and nucleus structures could be distinguished in the head region, while the flagellum was observed to be clearly defined.

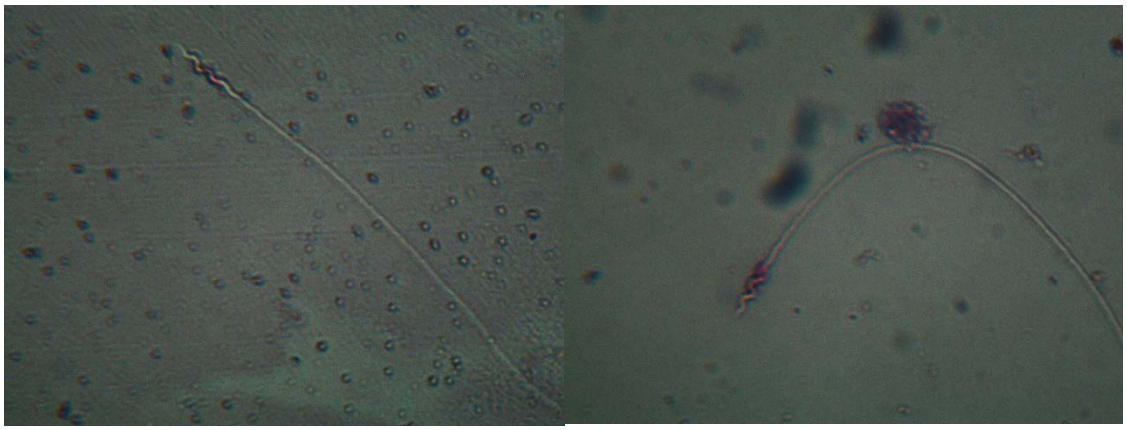

Figure 4. Morphological evaluation of canary spermatozoa by Giemsa staining after 5% formaldehyde fixation

2.2.3. Giemsa Staining

A drop (10 µl) of semen taken by cloacal massage was taken, smeared and dried, and then stained with Giemsa dye for 15 minutes. Afterward, spermatozoa were evaluated under a light microscope at 40x magnification. It was observed that spermatozoa nucleus and acrosome could be distinguished more clearly with this method compared to the Giemsa staining method after fixation with 5% formaldehyde, which is another method. It was determined that the only disadvantage in Giemsa staining was that the paint residue partially blocked the clear vision.

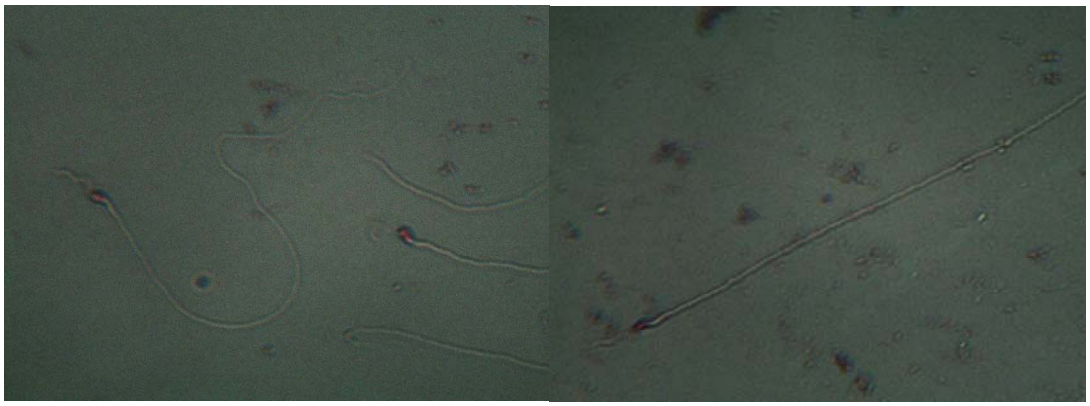

Figure 5. Morphological evaluation of canary spermatozoa by Giemsa staining

2.2.4. SpermBlue® Staining

One drop (10 µl) of the semen taken from the canary was taken, smeared and dried, and then stained with SpermBlue® for 15 minutes. Then, spermatozoa were evaluated under the light microscope at 40x magnification. No fixation was applied before staining. While the

spermatozoon nucleus and acrosome could not be clearly distinguished with this method, it was determined that it was sufficient for the determination of morphological anomalies.

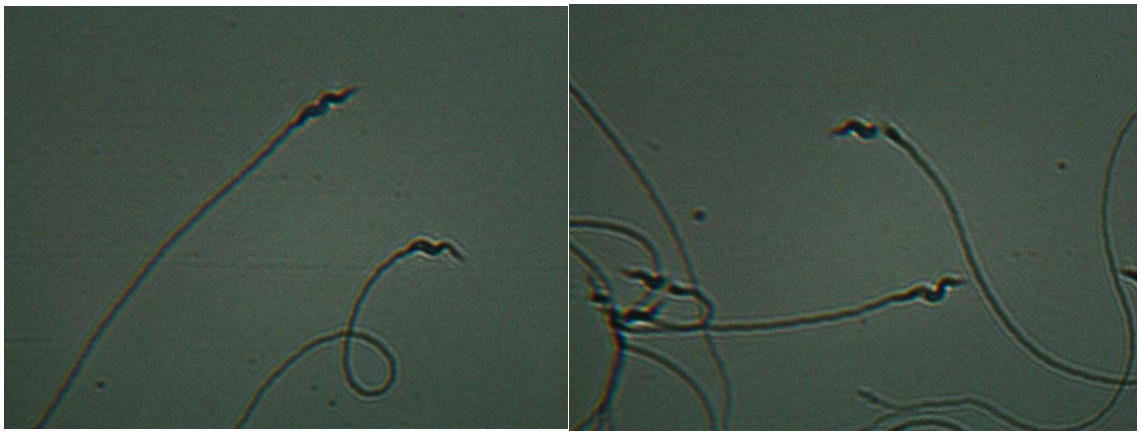

Figure 6. Morphological evaluation of canary spermatozoa by *SpermBlue®* staining

2.2.5. Formalin Fixation and SpermBlue® Staining

The collected semen was fixed with 5% formalin solution and dried by drawing a smear on the slide. Then 15 minutes with SpermBlue® dye. stained (Camer et al., 2020). Then, spermatozoa were evaluated under the light microscope at 40x magnification. After the fixation process, the acrosome and nucleus could be distinguished in the head of the spermatozoon, while the flagellum was determined more clearly and distinctly by SpermBlue® staining without fixation compared to the evaluation method.

Figure 7. Morphological evaluation of canary spermatozoa by *SpermBlue®* staining after 5% formaldehyde detection

3. Result and Discussion

Studies on preserving the qualities of semen in songbirds, improving storage conditions, and evaluating them are increasing. In this sense, it is very important to understand the morphological features of semen and to understand the relationship between semen performance. In this study, besides the different morphological detection methods of canary semen that are frequently used in the field, the effects, evaluation, and advantages and disadvantages of some semen dyes are presented. The current study contributes to this issue since studies on canary semen are very limited and there is not enough data in studies that visually examine the morphological structure of canary semen and semen of close species. It is predicted that the study will be decisive for the use of the appropriate method in the evaluation of spermatozoa morphology in songbird species that differ from mammalian semen and most bird species.

Birkhead et al. (2006), in their study on bullfinch semen morphology, which is a songbird species, determined the semen collected in 5% formaldehyde solution and then stained them with Hoechst 33342 dye and examined them at 100x magnification under a light microscope. Although the morphological staining method overlaps with our study, it does not coincide with the fact that the studies on the subject are generally 40x magnification. In addition, the spermatozoon of some songbird species was handled only as of the head part in the study, and the images were shared as electron microscope images. In another study, the semen collected was fixed in 5% formaldehyde solution and then examined with a light microscope at 16x magnification. Since the morphological appearance was not shared, a comparison with the results of this study could not be made Girndt et al. (2017) observed the semen detected in formaldehyde solution at 40x magnification without staining in order to examine the morphological structure of the semen they received from domestic sparrows in their study. Although the morphological appearance was evaluated in the study, no marking was made to distinguish different regions of the spermatozoon. However, it is reported that the outlines of the spermatozoa are clear and overlap when compared with our current study Helfenshtein et al. (2009), in a study in which they examined the relationship between the morphological structure of semen, motility, and spermatozoon length in domestic sparrows, evaluated the semen detected in 5% formaldehyde solution under a light microscope at 40x magnification after drying it by drawing a smear. Although the results of the study coincide with our current study, a comparison could not be made because there was no visual sharing. The reason why the staining method was not used in the study was explained by the fact that there was no

need for a detailed examination of sparrow spermatozoa. As a result, it is seen that there is not enough data for comparison in the studies examined and the dyeing methods are not used in the studies conducted on songbirds. The results of the study can be evaluated as a preliminary study for researchers and studies related to this subject.

References

Birkhead, TR., Immler, S., Pellatt, EJ., Freckleton, R. 2006. Unusual sperm morphology in the Eurasian Bullfinch (Pyrrhula pyrrhula). The Auk, 123(2), 383-392.

Cramer, ER., Garcia-del-Rey, E., Johannessen, LE., Laskemoen, T., Marthinsen, G., Johnsen, A., Lifjeld, JT. 2021. Longer sperm swim more slowly in the Canary Islands chiffchaff. Cells, 10(6), 1358.

Cramer, E., Krauss, N., Rowlison, T., Comizzoli, P. 2020. Sperm morphology and male age in black-throated blue warblers, an ecological model system. Animals, 10(7), 1175.

Gee, GF., Bertschinger, H., Donoghue, AM., Blanco, J., Soley, J. 2004. Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. Avian and Poultry Biology Reviews, 15(2), 47-101.

Girndt, A., Cockburn, G., Sanchez-Tojar, A., Løvlie, H., Schroeder, J. 2017. Method matters: Experimental evidence for shorter avian sperm in faecal compared to abdominal massage samples. PLoS One, 12(8), e0182853.

Helfenstein, F., Podevin, M., Richner, H. 2010. Sperm morphology, swimming velocity, and longevity in the house sparrow Passer domesticus. Behavioral Ecology and Sociobiology, 64(4), 557-565.

Hermosell, IG., Laskemoen, T., Rowe, M., Møller, AP., Mousseau, TA., Albrecht, T., Lifjeld, JT. 2013. Patterns of sperm damage in Chernobyl passerine birds suggest a trade-off between sperm length and integrity. Biology letters, *9*(5), 20130530.

Humphreys, PN. 1972. Brief observations on the semen and spermatozoa of certain passerine and non-passerine birds. Reproduction, 29(3), 327-336.

Kleven, O., Fossøy, F., Laskemoen, T., Robertson, RJ., Rudolfsen, G., Lifjeld, JT. 2009. Comparative evidence for the evolution of sperm swimming speed by sperm competition and female sperm storage duration in passerine birds. Evolution: International Journal of Organic Evolution, 63(9), 2466-2473.

Lüpold, S., Calhim, S., Immler, S., Birkhead, TR. 2009. Sperm morphology and sperm velocity in passerine birds. Proceedings of the Royal Society B: Biological Sciences, 276(1659), 1175-1181.

Schneider, H., Fischer, D., Failing, K., Ehling, C., Meinecke-Tillmann, S., Wehrend, A., Lierz, M. 2018. Investigations on a cryopreservation protocol for long-term storage of psittacine spermatozoa using cockatiel semen as an example. Theriogenology, 110, 8-17.