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Optimal analysis conditions for sperm motility parameters with a CASA system in a passerine bird, *Passer montanus*

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Abstract

Background: Sperm motility parameters, which can be measured objectively and repeatedly by a computer-assisted sperm analysis (CASA) system, are important indicators of sperm quality. However, the sperm motility parameters assessed by a CASA system can be affected by various factors, including instrument components and settings, sperm preparation or analysis procedures. To date, no standardized protocol is available that would permit to assess sperm kinetic characteristics in passerine birds and this lack precludes any comparison of sperm swimming ability and sperm quality across species.

Methods: In this study, we chose the Tree Sparrow (*Passer montanus*) as the object to evaluate sperm motility parameters, including sperm motility, sperm velocity and sperm movement trajectory, at different analysis time, temperatures and pH using the WLJY-9000 CASA system.

Results: Sperm motility parameters remained statistically unchanged at 1–9 min. Progressive motility was similar at 38 °C and 40 °C, but a greater percentage of slow progressive sperm was detected at 38 °C compared to 40 °C and 42 °C. Additionally, progressive motility was lower and immotility was higher at 42 °C than 38 °C and/or 40 °C (close to the body temperature of the Tree Sparrow). The percentages of rapid progressive sperm, progressive sperm and immotile sperm were statistically similar at pH 7.0, 7.5 and 8.0 with the exception of lower percentage of progressive sperm at pH 7.0 compared to pH 7.5. In addition, slower sperm velocity and worse sperm movement trajectory were found at pH 6.0 and 9.0 than those at pH 8.0, 7.5 or 7.0.

Conclusions: Our study indicates that the ideal conditions for sperm motility parameters assessment in Tree Sparrow are obtained between 1 and 9 min after dilution, an environment at body temperature (40 °C) and a pH around 7.5–8.0. The results of this study provide a reference for the evaluation of sperm characteristics and sperm quality using a CASA system in passerine birds.

Keywords: Analysis time, Passerine bird, Sperm motility parameters, Temperature, pH

Background

The main function of sperm is to ensure fertility and enhance paternity. Consequently strong selection acts on both sperm quantity and quality, which favors sperm traits that increase fertilization success (Birkhead and Møller 1998). Sperm motility parameters, the common indicators of sperm quality, are generally assessed to reflect the potential fertility of individuals (Froman et al. 1999; Gasparini et al. 2010).

A method allowing the capture of successive images of spermatozoa and the analysis of their individual movement was first reported by Dott and Foster (1979), and since then, the computer-assisted sperm analysis (CASA) has been presented and gradually developed as a method to objectively evaluate sperm motility parameters (Amann and Waberski 2014). The system has long been used to diagnose male reproductive system diseases

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and evaluate the quality of frozen semen used for artificial insemination in animal husbandry (Contri et al. 2010; Broekhuijse et al. 2011), which currently also plays an important role in pet breeding and animal experiments (Lüpold et al. 2009; Guo et al. 2018). However, various factors prevent sperm motility parameters obtained via a CASA system from being compared across studies. Firstly, CASA systems are based on similar principles (Dott and Foster 1979), but their instrument components (e.g. optics and hardware characteristics, as well as algorithms for sperm identification and trajectory reconstruction) and settings (e.g. frame rate and frames per field) highly influence sperm motility parameters (Boryshpolets et al. 2013; Amann and Waberski 2014). In sperm sample preparation, the media used for dilution, sperm sample concentration, temperature and pH should be considered, which can also impact sperm motion characteristics evaluated by a CASA system (Contri et al. 2010; Kathiravan et al. 2011; Humann-Guilleminot et al. 2018). In addition, some factors during analysis procedure such as the number of sperm captured per slide and the technical competence of user are vital for reflecting sperm kinetic characteristics (Broekhuijse et al. 2011; Mortimer and Mortimer 2013). Therefore, although a CASA system can provide an objective assessment of sperm motility parameters, a standardized protocol has now become necessary. With such a standardized protocol, sperm motion characteristics would be accurately reflected and would then permit comparison across systems and groups.

Passerine birds account for over half of avian species, whose sperm generally have a helical shape and a spiraling forward movement (Humphreys 1972) and remain within the sperm storage tubules for a period of days to weeks after copulation (Birkhead and Møller 1998). Postcopulatory sexual selection, including sperm competition and cryptic female choice, is considered to be an important driver of evolutionary change in sperm traits (Birkhead and Møller 1998; Birkhead and Pizzari 2002), however, the sperm characteristics that are closely related to fertilization success have been unclear in passerine birds. Yet there has been a long-term interest in sperm motion characteristics of passerine birds, because faster sperm are presumed to outcompete rival sperms in the "race" to the ovum and more ejaculated motile sperm are likely to increase the odds of sperm from a certain male fertilizing the ovum. In addition, some passerine sperm motility parameters can also be used as indicators of the impact of pollutants in a wide range of species (Møller et al. 2008, 2014; Leidens et al. 2018).

CASA systems have been repeatedly used to assess sperm swimming ability in passerine birds (Lüpold et al. 2009; Bennison et al. 2016), but the procedure conditions have not been standardized thus far. Concerning the temperature at which the sperm movements should be analyzed, some studies assessed sperm motility parameters of passerine birds around body temperature, i.e. 40 °C, such as in House Sparrows (*Passer domesticus*, Mora et al. 2017), Azores Bullfinches (*Pyrrhula murina*, Lifjeld et al. 2013) and Great Tits (*Parus major*, Losdat and Helfenstein 2018), while others analyzed sperm movement below the bird's body temperature, such as in Eurasian Bullfinches (*Pyrrhula pyrrhula*) (35 °C; Birkhead et al. 2006) and Zebra Finches (*Taeniopygia guttata*) (38 °C; Bennison et al. 2016). Unfortunately, non-standardized procedure conditions likely affect the reliability of motility parameters detected by a CASA system, which makes it difficult for researchers to compare sperm motion characteristics estimates across time, studies and species.

The aim of this study was to determine the optimal CASA conditions for assessment of sperm kinetic characteristics in Tree Sparrows (*Passer montanus*) by comparing sperm motility, sperm velocity and sperm movement trajectory at different analysis time, temperatures and pH values. We wish to make recommendations for the standardized estimation of passerine sperm motility parameters using a CASA system that would allow evaluating sperm motion characteristics more accurately and repeatedly.

Methods

Model species

A total of 63 adult Tree Sparrows (11 females and 12 males in 2017, 40 males in 2018) captured with mist nets in May of 2017 and 2018 were used for this study. The sample area, Liujiaxia (35°56′N, 103°15′E) in a northwestern part of China, is a relatively unpolluted village and provides a suitable habitat for Tree Sparrows. Each bird captured was weighed and measured for their wing length and tarsus length, and also sexed based on the presence of a brood patch (Selander and Yang 1966). The body temperature of the adult captured in 2017 was also estimated. On completion, the females were ringed with a uniquely numbered metal band and then released. And males were protected from light and brought back alive to the laboratory, 48 (8 in 2017 and 40 in 2018) of them were used for evaluating sperm motility parameters under different conditions.

The male Tree Sparrows captured in this study were also used for the analysis of several physiological and biochemical indexes and for histology observations. Therefore, these birds were euthanized according to the "Animal Experimental Ethical Inspection Form" (see Additional file 1: Fig. S1), and their seminal glomera were collected conveniently. However, it is to be noted that a

non-invasive method to obtain sperm by gently massaging the bird's cloaca works well in passerine birds (Wolfson 1952), and it should be advocated whenever euthanasia is not absolutely necessary.

CASA system

Sperm motility parameters were assessed using the WLJY-9000 (WEI-LI New Century Technical Development, China) device with the standardized 10 μm-depth slide chambers. This system, based on the *WHO laboratory manual for the examination and processing of human semen*, has a thermostat that can maintain the temperature from room temperature up to 70 °C. In addition, the maximum velocity of sperm movement detected by WLJY-9000 is 180 μm/s, up to 20 fields can be captured in each analysis. In each field, 4–20 frames (20 frames in the present study) were tracked for sperm motility parameters assessment, and no more than 1000 spermatozoa can be identified.

Estimating body temperature

The body temperature of the captured 12 males and 11 females was immediately detected with a UT325 thermometer (Uni-Trend, China). A clean probe was slightly inserted into the cloaca approximately 1 cm deep and removed when the reading was stable.

The test results showed the body temperatures of adult Tree Sparrows were 40.13 ± 1.56 °C for male, and 40.38 ± 1.36 °C for female. We used these values as bases for exploring the optimal conditions of sperm motility parameters evaluation using the CASA system.

Sperm motility parameters detected by the CASA system

Male Tree Sparrows were acclimatized to the laboratory for 3 h before they were euthanized. Then, they were immediately dissected for a series of experiments, and their right seminal glomus was extracted for obtaining sperm suspension in preheated Hank's Balanced Salt Solution (HBSS, an isotonic solution that can buffer pH and preserve the sample's osmotic pressure). Sperm swam out from the seminal glomus into the surrounding media and diffused, forming a "cloud", and in about 10 s the "cloud" diffused completely in the medium. The sperm suspension was then kept warm (at the corresponding analysis temperature) in a water bath until analysis. Next, 10 μL of diluted semen were dropped on the center of the pre-warmed slide chamber and a coverslip was placed over the sample. And the slide was put in the thermostat and sperm motility parameters were assessed by the WLJY-9000 CASA system at 100× magnification. For each analysis, sperm motility parameters were collected and recorded by the capture of at least 5 nonconsecutive fields (a total of at least 500 spermatozoa) within 30 s. The sperm in each field were selected by adjusting the grayscale threshold, and the selected debris and round cells were manually deleted prior to analysis. The following sperm motility parameters were determined: (1) Sperm motility: rapid progressive motility (the percentage of rapid progressive sperm with a linear velocity ≥ 25 μm/s), slow progressive motility (the percentage of slow progressive sperm with a linear velocity < 25 μm/s), non-progressive motility (all other patterns of motility with an absence of progression), immotility (the percentage of immotile sperm). In addition, progressive motility (the sum of rapid and slow progressive motility) is a vital indicator of ejaculated sperm to evaluate their swimming ability (Kathiravan et al. 2011), which was assessed in this study; (2) Sperm velocity: the curvilinear velocity (VCL), straight-line velocity (VSL) and average path velocity (VAP) can be evaluated by the CASA system, and these three sperm velocity parameters were strongly associated with each other (Pearson's $r > 0.96$, $p < 0.001$), thus the present study chose VCL, the velocity over the actual sperm trajectory, as a measure of sperm swimming speed (hereafter referred to as sperm velocity); (3) Sperm movement trajectory: path linearity (the linearity of actual sperm track, LIN = VSL/VCL), path wobble (departure of actual sperm track from average path, WOB = VAP/VCL), and path straightness (linearity of the average path, STR = VSL/VAP).

Effect of time since dilution and until analysis on sperm motility parameters

The right seminal glomus of 8 males brought back to the laboratory and euthanized in 2017 was removed and cut in half in 0.5 mL of HBSS, which could keep the sperm concentration between 2 and 50×10^6 sperm/mL (the recommended concentration for sperm motility parameters assessment in *WHO laboratory manual for the examination and processing of human semen*). The preheated temperature of HBSS was set to 40 °C (the body temperature of Tree Sparrows), and the pH was set to 7.5 (according to the semen pH of poultry) (Orunmuyi et al. 2013) and because weak alkaline pH has shown increased sperm movement (Holm and Wishart 1998). Next, a 10-μL sperm suspension was used for the assessment of the sperm motility parameters by the WLJY-9000 CASA system at 3, 5, 15, 20, and 30 min after dilution.

The initial experiment results showed that the percentage of progressive sperm decreased with time, which largely dropped after 15 min. Based on the results of this first experiment, in 2018, sperm samples of 8 other males were used to assess sperm motility parameters at 1 (the time sperm were suspended fully in medium and load the sperm suspension onto the microscope slides), 3, 5, 7, 9,

11, 13, and 15 min after dilution in HBSS with pH 7.5 at 40 °C.

Effect of temperature on sperm motility parameters

We evaluated the effect of the temperature on sperm motion characteristics using sperm samples from 8 males, whose right seminal glomus was divided into 3 equal portions with ophthalmic scissors in 2018. Then, the three pieces were put immediately into different Eppendorf tubes containing 0.2 mL of HBSS with pH 7.5 and placed in a water bath at either 38, 40 or 42 °C. Approximately 4 min after dilution, motility parameters of sperm from different Eppendorf tubes were analyzed by the WLJY-9000 CASA system at 38, 40 and 42 °C.

Effect of pH on sperm motility parameters

Based on the semen pH of poultry (Orunmuyi et al. 2013) and previous results in other species (Holm and Wishart 1998), the pH values of HBSS in this experiment were set to three groups (7.0, 7.5 and 8.0 in group 1, 6.0, 7.0 and 7.5 in group 2, and 7.5, 8.0 and 9.0 in group 3) to control the impact of individual variation on the comparison of sperm motility parameters between different pH levels. A total of 24 birds (7 in group 1, 8 in group 2 and 9 in group 3) were used in this experiment, the right seminal glomus of which was divided into 3 equal portions with ophthalmic scissors. Then, the 3 sections were immediately put into Eppendorf tubes containing 0.2 mL of 40 °C pre-warmed HBSS with different pH values. Approximately 4 min after dilution, sperm motility parameters were assessed by the WLJY-9000 CASA system at 40 °C.

Statistical analysis

Experimental data are expressed as mean values ± standard deviation (SD) or ratio. Statistical analyses were performed using SPSS 20.0 statistical software (IBM SPSS Inc., USA). Two-tailed Pearson correlation analysis was used to check for relationships between sperm velocity parameters. All proportion data including sperm motility and sperm movement trajectory were logit transformed prior to analysis.

The change of sperm motility parameters at different analysis time was investigated using the repeated measures analysis of general linear model (GLM). In order to validate the univariate *F*-test, the "Epsilon" values (when the Greenhouse–Geisser was > 0.7, the Huynh–Feldt correction should be used, and if not, the Greenhouse–Geisser correction should be used) would be used to calculate an appropriate adjustment to the degrees of freedom when the assumption of sphericity was not met ($p < 0.05$). Besides, curve fittings were performed using SigmaPlot 14.0 software (Systat Software Inc., USA) to simulate the association between analysis time (independent variable) and sperm motility parameters (dependent variables), and the equations that most closely fit the actual data were found (large coefficient of determination, and $p < 0.05$).

To investigate the effect of temperature and pH on sperm motility parameters, the univariate analysis of GLM was conducted. The sperm motility parameters entered as dependent variable, while temperature or pH entered as a fixed factor. Besides, male identity was entered as a random factor to control for individual variation, and the time since dilution and until analysis was a covariate.

The model assumptions were validated by testing the residuals' normality, and pairwise comparisons were corrected by the Bonferroni method. Besides, SigmaPlot 14.0 software was also used for bar graphs, line charts and scatter plot.

Results

Effect of time since dilution and until analysis on sperm motility parameters

We found that the amount of time elapsed since dilution until analysis affected the rapid progressive motility, immotility and progressive motility (Table 1), and high correlation coefficients ($R^2 > 0.93$) were observed when sigmoid model and polynomial model were used to calculate the change of these three parameters with time (Fig. 1a, d, e). The lower rapid progressive motility and progressive motility at 13 min (rapid progressive motility: $p = 0.02$, progressive motility: $p = 0.02$) and 15 min (rapid progressive motility: $p = 0.009$, progressive motility: $p = 0.009$), as well as higher immotility at 15 min ($p = 0.02$) were detected compared to 1 min (Fig. 1a, d, e). However, the percentages of slow progressive sperm and non-progressive sperm remained statistically unchanged within 15 min after dilution (Fig. 1b, c).

The sperm velocity was also influenced by the analysis time (Table 1), which changing pattern followed a sigmoid model ($R^2 = 0.96$). Until 9 min after dilution, the sperm velocity remained significantly unchanged ($p > 0.05$), which was significantly decreased at 11 min ($p = 0.04$), 13 min ($p = 0.04$) and 15 min ($p = 0.004$) compared to 1 min (Fig. 2a). The study also indicated no significant difference in sperm LIN, WOB and STR across time (Fig. 2b).

Effect of temperature on sperm motility parameters

The rapid progressive motility and non-progressive motility remained statistically unaffected by different temperatures (Table 1). However, temperature affected the percentages of slow progressive sperm, immotile

Table 1 Degrees of freedom, *F*-values and significance levels denoting the effect of time since dilution and until analysis, temperature and pH on the sperm motility parameters

Dependent variables	Independent variables					
	Time since dilution and until analysis		Temperature		pH levels	
	F_{df}	*p* value	F_{df}	*p* value	F_{df}	*p* value
Rapid progressive motility	$23.90_{2.16, 15.10}$	*< 0.001*	$2.24_{2, 13}$	0.15	$12.38_{4, 39}$	*< 0.001*
Slow progressive motility	$2.06_{1.85, 12.95}$	0.17	$4.56_{2, 13}$	*0.03*	$0.58_{4, 39}$	0.68
Non-progressive motility	$1.01_{7, 49}$	0.44	$1.46_{2, 13}$	0.27	$0.46_{4, 39}$	0.76
Immotility	$10.85_{7, 49}$	*< 0.001*	$6.84_{2, 13}$	*0.01*	$6.27_{4, 39}$	*0.001*
Progressive motility	$20.19_{2.05, 14.32}$	*< 0.001*	$4.28_{2, 13}$	*0.04*	$15.26_{4, 39}$	*< 0.001*
Sperm velocity	$11.65_{7, 49}$	*< 0.001*	$0.97_{2, 13}$	0.41	$11.22_{4, 39}$	*< 0.001*
LIN	$2.39_{2.19, 15.35}$	0.12	$0.23_{2, 13}$	0.80	$10.35_{4, 39}$	*< 0.001*
WOB	$3.08_{2.34, 16.40}$	0.07	$0.21_{2, 13}$	0.81	$10.49_{4, 39}$	*< 0.001*
STR	$0.39_{2.82, 19.75}$	0.75	$0.47_{2, 13}$	0.64	$7.29_{4, 39}$	*< 0.001*

The details of the building of general linear models are shown in Additional file 2: Tables S1–S3

LIN linearity, *WOB* wobble, *STR* straightness

Italic values are significant in the analysis

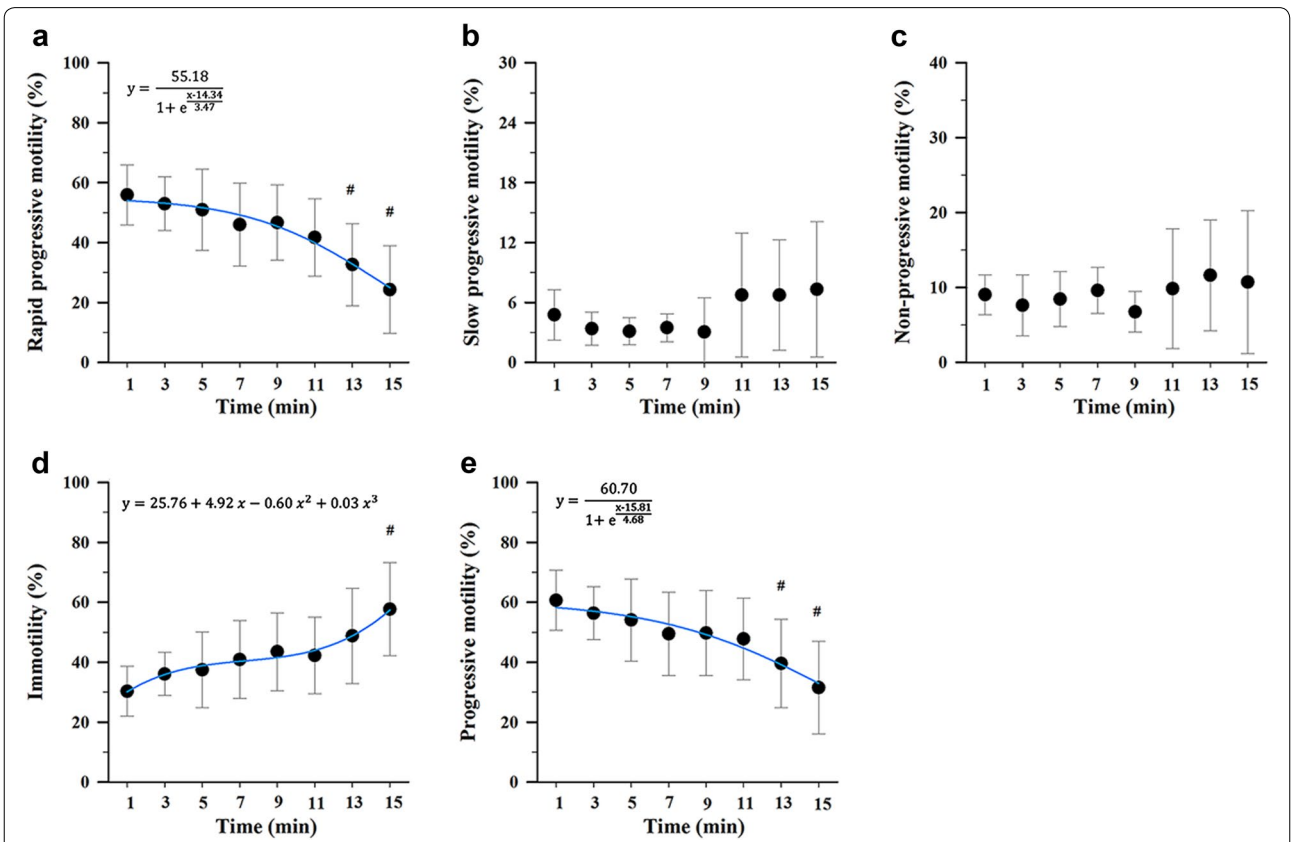

Fig. 1 The change of **a** rapid progressive motility, **b** slow progressive motility, **c** non-progressive motility, **d** immotility and **e** progressive motility with the time since dilution and until analysis. The blue curves are the regression lines ($R^2 > 0.93$). $^{\#}p < 0.05$ compared to 1 min

sperm and progressive sperm (Table 1). More slow progressive sperm was found at 38 °C compared to 40 °C ($p = 0.04$) and 42 °C ($p = 0.01$) (Fig. 3b). And at 42 °C, immotility was higher than 40 °C ($p = 0.003$) and progressive motility was lower than 40 °C ($p = 0.02$) and 38 °C ($p = 0.03$) (Fig. 3d, e).

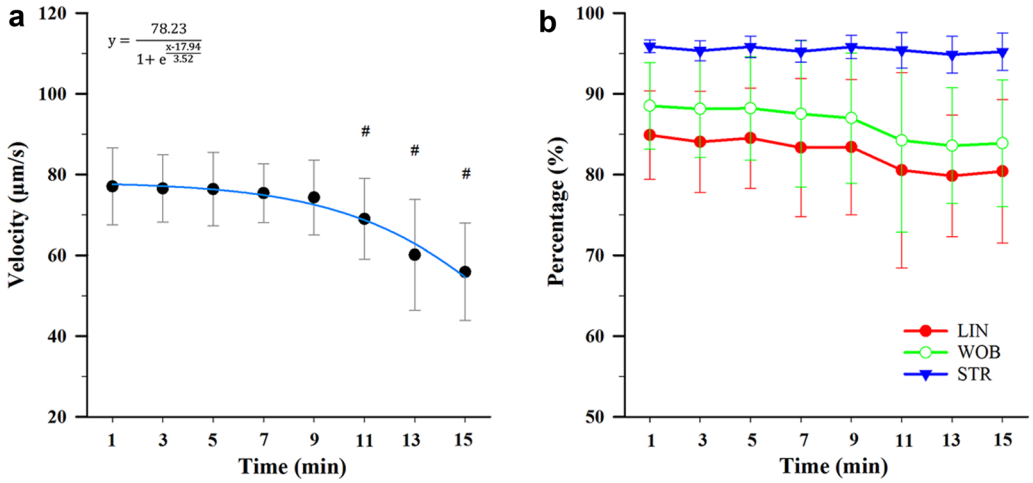

Fig. 2 The change of **a** sperm velocity (blue curve is the regression line, $R^2 = 0.96$) and **b** sperm movement trajectory with the time since dilution and until analysis. LIN, WOB and STR are the abbreviations of linearity, wobble and straightness, respectively. $^{\#}p < 0.05$ compared to 1 min

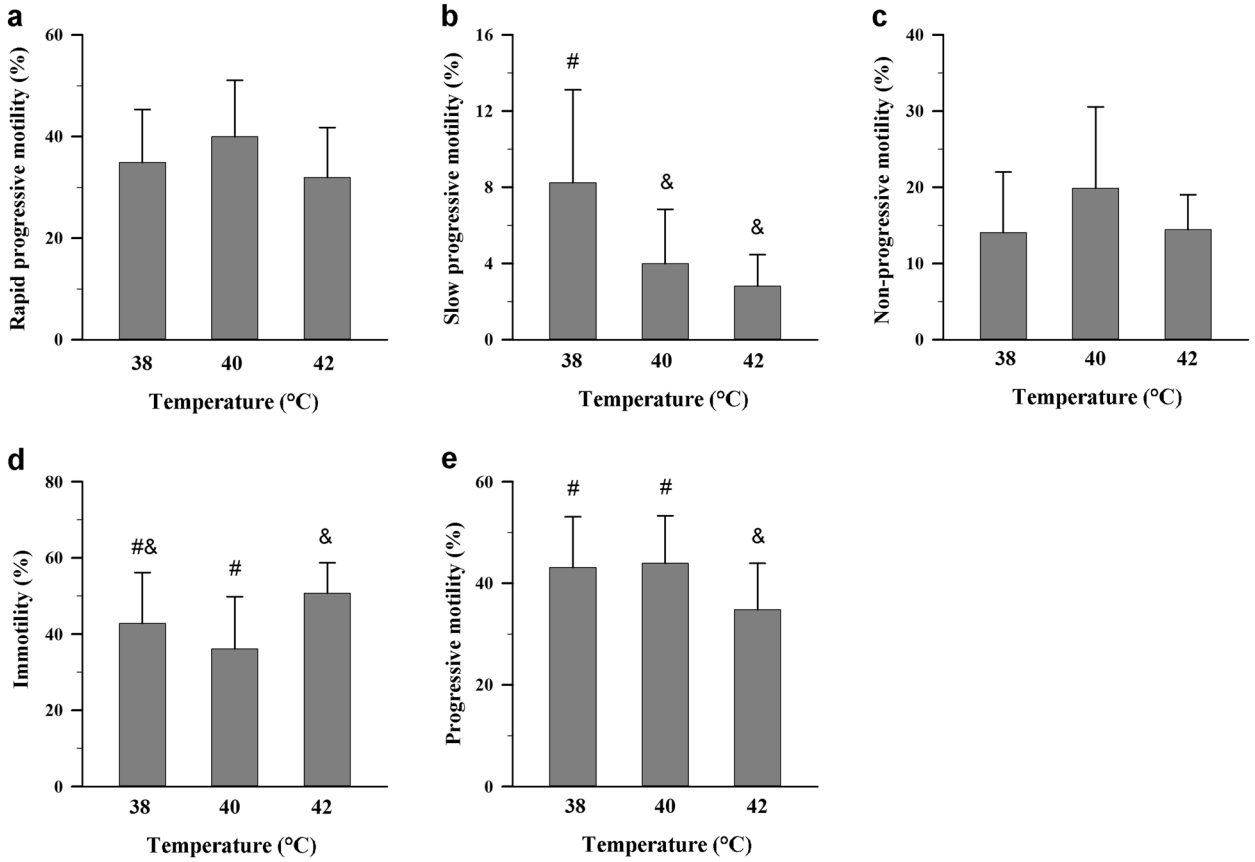

Fig. 3 Effect of temperature on **a** rapid progressive motility, **b** slow progressive motility, **c** non-progressive motility, **d** immotility and **e** progressive motility. Different symbols in each column graph denote significant differences between temperature groups ($p < 0.05$)

In contrast, no significant differences in sperm velocity and sperm movement trajectory were detected across temperatures groups (Fig. 4).

Effect of pH on sperm motility parameters

The pH significantly affected sperm motility recorded by the WLJY-9000 CASA system, including rapid

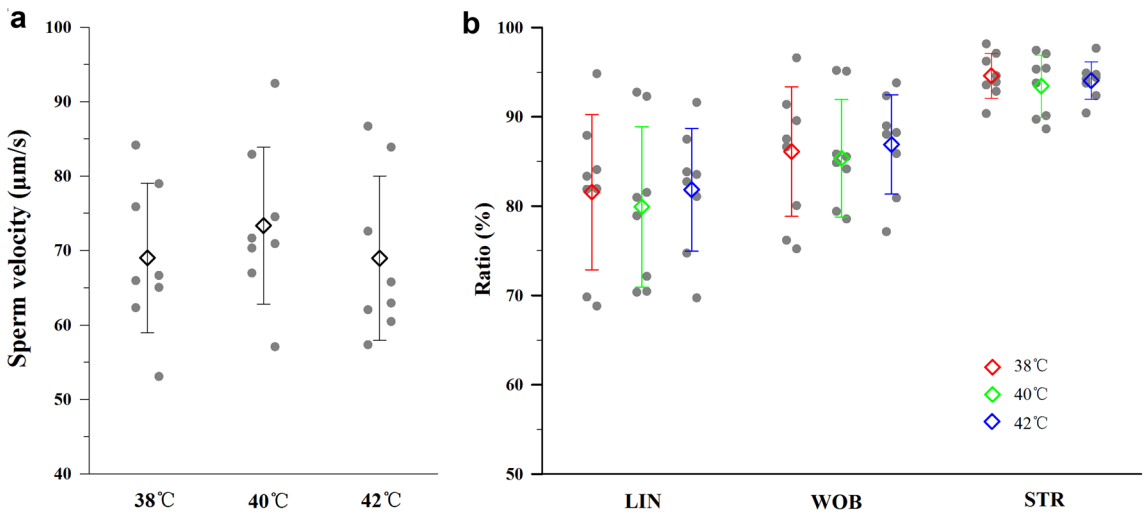

Fig. 4 Effect of temperature on **a** sperm velocity and **b** sperm movement trajectory. The gray dots represent samples, and the hollow diamonds represent average values. Abbreviations are the same as those in Fig. 2

progressive motility, immotility and progressive motility (Table 1). And these parameters were statistically similar at pH 7.0, 7.5 and 8.0 ($p > 0.05$) with the exception of lower percentage of progressive sperm at pH 7.0 compared to pH 7.5 ($p = 0.04$) (Fig. 5a, d, e). In addition, a significant increase in immotility and a significant decrease in rapid progressive motility and progressive motility were observed at pH 6.0 (all $p < 0.001$ compared

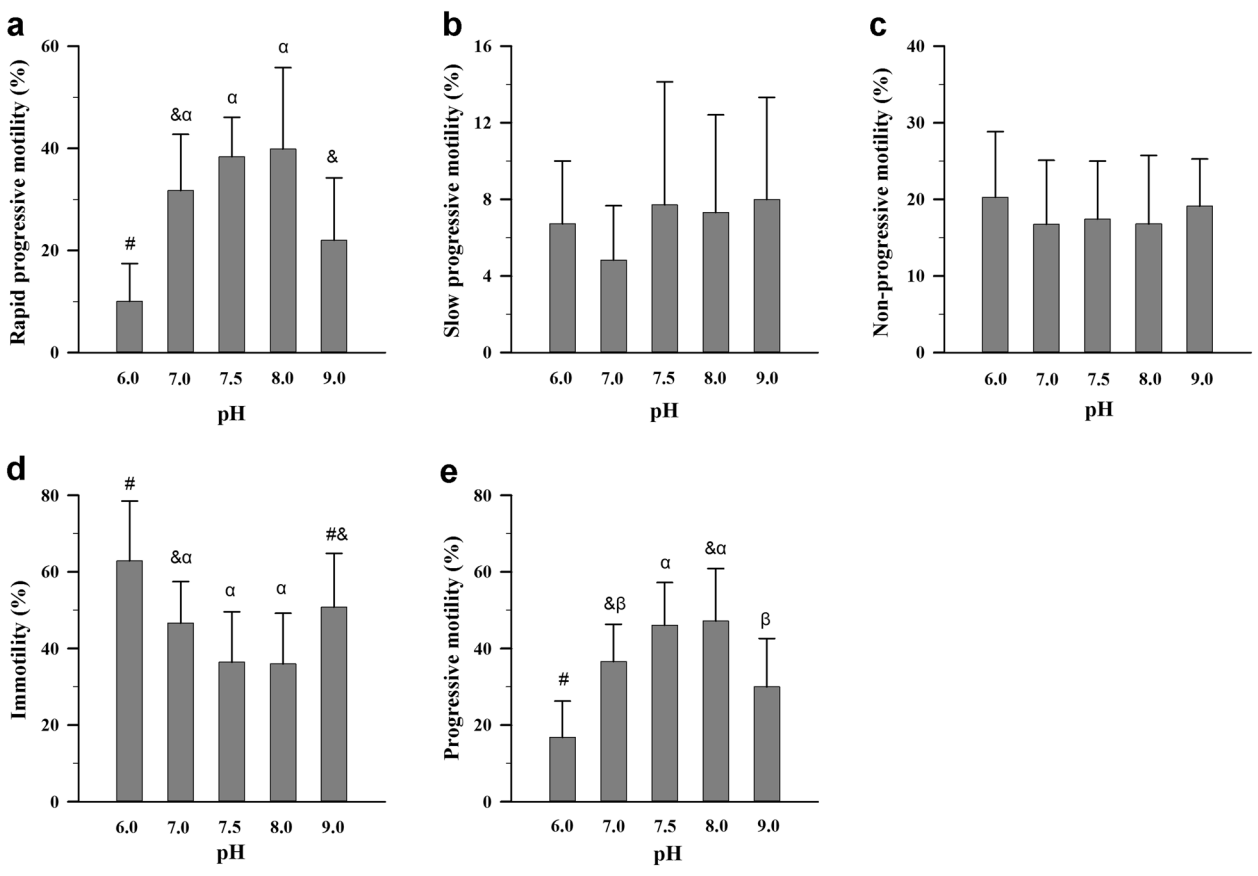

Fig. 5 Effect of pH on **a** rapid progressive motility, **b** slow progressive motility, **c** non-progressive motility, **d** immotility and **e** progressive motility. Different symbols in each column graph denote significant differences between pH values ($p < 0.05$)

to pH 7.5 and pH 8.0) and 9.0 (rapid progressive motility: $p=0.04$ compared to pH 7.5 and pH 8.0; progressive motility: $p=0.002$ compared to pH 7.5 and $p=0.001$ compared to pH 8.0) (Fig. 5a, d, e).

Besides, pH also impacted sperm velocity and sperm movement trajectory (Table 1). Sperm velocity in media with pH 7.0, 7.5, 8.0 and 9.0 was statistically similar ($p>0.05$), which was statistically higher compared to pH 6.0 (all $p<0.001$) (Fig. 6a). Moreover, lower sperm LIN, WOB and STR were observed at pH 6.0 compared to pH 7.0, 7.5, 8.0 and 9.0 ($p<0.047$) (Fig. 6b).

Discussion

CASA systems have been repeatedly used to assess sperm motility parameters of passerine birds in order to explore the evolution of sperm structure and energetics (Rowe et al. 2013; Bennison et al. 2016) or sperm morphology and velocity (Bennison et al. 2014; Rowe et al. 2015; Mora et al. 2017) in relation to sperm competition, or to assess the toxicity of environmental pollutants on sperm quality (Møller et al. 2014). However, inconsistent CASA operations are performed across these studies, which precludes any accurate and repeatable assessment of sperm swimming ability and prevents any comparison within and across species. In this study, three separate experiments were carried out to compare the sperm motility parameters of Tree Sparrow in different conditions, which allowed us to identify optimal analysis time, temperatures and pH for CASA.

Reliable assessments of sperm motility parameters can be performed provided that the extender does not significantly alter the sperm kinetic characteristics over the time period of the analysis (Davis and Katz 1993). In our study, the progressive motility and sperm velocity decreased with time followed a sigmoid model, but all sperm motility parameters remained statistically unchanged within 9 min after dilution in a balanced salt solution, and this period (from 1 min to 9 min after dilution) provides a possibility for obtaining stable assessment of sperm kinetic characteristics in Tree Sparrow. However, other studies on Great Tits, Blue Tits (*Cyanistes caeruleus*) and Pied Flycatchers (*Ficedula hypoleuca*) also show the sperm motile performance declines over time in vitro within about 5 min when sperm stock solution was prepared by a neutral medium, phosphate-buffered saline (PBS) (Cramer et al. 2016a, b), which differs from the findings in our study. Decreased sperm motile performance in birds has been found under the neutral pH conditions (Fig. 5e) or in PBS (Humann-Guilleminot et al. 2018); therefore, the neutral PBS is likely to lead to the short survival of sperm in these studies. Besides, different selection mechanisms of sperm competiton are shown across animal taxa (Birkhead and Møller 1998), and the sperm longevity may be more important for increasing the fertilizing ability of Tree Sparrow compared to the Great Tits, Blue Tits or Pied Flycatchers, so their sperm motility parameters remained longer.

The body temperature of Tree Sparrows that we measured is somewhat on the lower side of the expected mean body temperature of passerine birds (Riley 1937; Binkley et al. 1971; Møller 2010; Skold-Chiriac et al. 2015). It

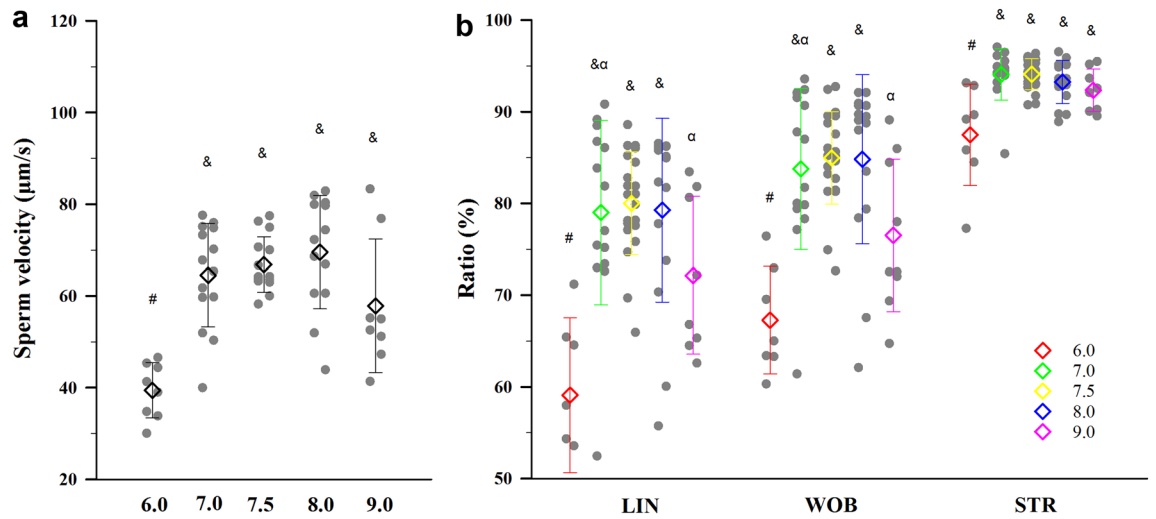

Fig. 6 Effect of pH on **a** sperm velocity and **b** sperm movement trajectory. Different symbols in each parameter indicate significant differences between pH values ($p<0.05$). The gray dots represent samples, and the hollow diamonds represent average values. Abbreviations are the same as those in Fig. 2

has been reported that the stress caused by capture and detection declined the body temperature of Barn Swallow (*Hirundo rustica*) and Great Tit (Møller 2010; Andreasson et al. 2019), which may lead to the slightly lower body temperature in Tree Sparrow. After insemination, avian sperm are held in the sperm storage tubules for a period of days to weeks before fertilizing the ova competitively (Birkhead and Møller 1998). Thus, the female reproductive tract provides an optimal environment for sperm motion, and our study likewise showed high sperm kinetic values of Tree Sparrows at their body temperature (40 °C). Similar to the study about sperm movement of ostriches (Bonato et al. 2012), more slow progressive sperm were observed at low temperatures in the present study. However, the sperm viability of ostriches is unaffected at 20 °C, and the poor sperm movement at low temperatures is reversible (Bonato et al. 2012). In contrast, we found more immotile sperm at high temperature, which may be related to the damaging effects of high temperature on sperm membrane (Wechalekar et al. 2010) or Na^+/K^+-ATPase activity (Thundathil et al. 2012). It is also worth noting that sperm motility parameters are sensitive to temperature changes. Low magnitude temperature variations (±2 °C) significantly influenced the kinetic parameters in our study, and, as a consequence, strict temperature control during the assessment of sperm motility parameters of passerine birds is key to accurately estimate sperm quality.

Semen of broilers has been shown to be alkaline (Orunmuyi et al. 2013), and in this study, the best sperm movement status was observed at pH 7.5 and 8.0 by comprehensive consideration of sperm motility, sperm velocity and sperm movement trajectory. So, the weakly alkaline environment with a pH close to semen pH is suitable to assess the sperm motility parameters of Tree Sparrow. Similarly, existing research has also shown that sperm movement is stimulated at a certain alkali pH range; for instance, the velocity and total motility of sperm from chickens, turkeys and quails are significantly greater at pH 8.0 compared to pH 7.0, and the sperm velocity is further increased at pH 9.0 for quail and chicken sperm (Holm and Wishart 1998). Ashizawa et al. (1994) found that the increased pH may act directly on axonemal phosphoprotein, mediated by a Ca^{2+}-related substance, which is likely to be a reason why sperm swimming better in the weakly alkaline environment. By contrast, as our study confirms a range of acidic to neutral pH that lead to poor sperm motion (Holm and Wishart 1998; Bonato et al. 2012), which may be related to a reduction in sperm metabolism to conserve energetic resources and promote sperm lifespan (Pinto et al. 1984). In some studies, sperm of passerine birds are suspended by a commercial extender (e.g. Dulbecco's modified Eagle medium) (Lifjeld et al. 2013; Rowe et al. 2013; Mora et al. 2017; Losdat and Helfenstein 2018); however, these neutral media may lead to an underestimate of the sperm kinetic characteristics in passerine birds according to our study.

Conclusions

Our results indicate that the time elapsed since dilution and until analysis, the temperature and the sperm-extender pH all affect sperm motility parameters as computed by the WLJY-9000 CASA system, especially, low magnitude temperature variations (±2 °C) can significantly influence the kinetic parameters of Tree Sparrow. We recommend that sperm motility parameters analyses of passerine sperm using a CASA system be performed at 40 °C with a pH comprised around 7.5–8.0 and at 1–9 min after sperm were suspended in the extender. Under these conditions, the valid motility results will be obtained to reflect sperm swimming ability accurately.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40657-019-0174-5.

Additional file 1: Fig. S1. Animal experimental ethical inspection form.

Additional file 2: Table S1. Summary of general linear models examining the effect of time since dilution and until analysis on sperm motility parameters. **Table S2.** Summary of general linear models examining the effect of temperature on sperm motility parameters. **Table S3.** Summary of general linear models examining the effect of pH on sperm motility parameters.

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Authors' contributions
YZ and YY conceived and designed the study. YY, JD, SA, RG, XB and WY conducted the field work. YY and RG carried out the analyses. YY analyzed the data and wrote the manuscript, and YZ revised it. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions of this article are available in the figshare repository, https://doi.org/10.6084/m9.figshare.9685292.

Ethics approval and consent to participate
Tree sparrows were sampled and processed with the permission of Committee on the Ethics of Animal Experiments of School of Life Sciences, Lanzhou University, China.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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