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Identification of sperm motility subpopulations in Gyr falcon (*Falco rusticolus*) ejaculate: a tool for investigating between subject variation

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ABSTRACT Subgroups of sperm which share similar motility features documented in mammals indicate between-subject variations that might be related to fertilizing potential of the respective ejaculates. The objectives of this study were to define subpopulations of motile sperm in Gyr falcon semen using kinematic parameters driven by Computer Assisted Semen Analysis (CASA) and to investigate the subject-related variations in these subpopulations. A total of 24 fresh ejaculates from 6 falcons were used to assign each of the 20473 sperms into 3 subpopulations by a multivariate cluster analysis. The proportion of sperms in different sub-populations were compared among subjects by a generalized linear model and repeatability of sperm frequency in different subpopulations was investigated by corelation analysis.
The resulting 3 categories of sperm indicated significant differences in all kinematic parameters ($p < 0.05$). Subpopulation 1 (15.91%) contained sperms with the highest velocity and progressiveness of movement trajectory while subpopulation 3 (6.4%) included the least progressively motile sperms. Proportion of rapid and medium progressive sperm were consistently higher in the ejaculate of three falcons compared to the two other birds which also had the highest proportion of slow non-progressive sperms ($p < 0.05$). Respective proportion of sperms in each subpopulations indicated significant repeatability over multiple measurements ($p < 0.05$). In conclusion, subpopulations of motile sperm in Gyr falcon can be identified using kinematic parameters generated by CASA. Individual differences in the proportion of these sub-populations might have potential application for identifying the males with higher fertilizing capacity.

Keywords: CASA, falcon, sperm kinematic, subpopulation

INTRODUCTION

Artificial insemination technique (AI) forms the core of all falcon breeding programs which are intended to alleviate the pressure on wild populations since the adult birds of this species hardly copulate under captive situation (Bailey and Lierz, 2017). The outcome of falcon AI plans largely depends on semen characteristics in terms of quality and quantity (Parks and Hardaswick, 1987) which are often measured by routine laboratory techniques. Accurate assessment of avian semen achieved by Computer Assisted Sperm Analysis (CASA) as compared to conven-

tional methods, offers the advantage of being less time consuming and allows for objective assessment of motility by providing quantitative measurements and the details of sperm movement trajectory (Häder, 1988; Fischer et al., 2014; Jepson et al., 2019; Velverde et al., 2019).

CASA generates precise values for defined motion parameters (Mortimer, 2000; Mortimer et al., 2015) which facilitate the study of individual sperms and assigning them into different subpopulations based on their motion characteristics. Presence of sperm subpopulations with different motion characteristics is documented in a number of mammals including humans (Mortimer ST and Mortimer D, 1990; Garrett et al., 2003), dog (Núñez-Martínez et al., 2006; Dorado et al., 2011), boar (Flores et al., 2008; Ramió et al., 2008), gazelle (Abaigar et al., 2001), donkey (Flores et al., 2008; Gacem et al., 2021), bull (Muiño et al., 2008; Muiño et al., 2009; Ferraz et al., 2014), stallion (Quintero-Moreno et al., 2003; Ortega-Ferrusola et al., 2009; Gacem et al., 2021), deer (Martinez-Pastor et al., 2005), sheep (Santolaria et al., 2015) and goat (Dorado et al., 2010). The semen contains a heterogeneous population of sperms (Mortimer et al., 2015) not solely due to the presence of malformed or defective spermatozoa but to differences between otherwise normal spermatozoa (Martínez-Pastor, 2021) and therefore, assessment of changes in sperm subpopulations rather than in the whole ejaculate might deliver a more complete understanding about the effect of different treatments, processing and preservation methods, pathologies as well as interspecies or individual variations on sperm quality. Moreover, studies suggest that both *in-vitro* and *in-vivo* fertility potential of mammalian ejaculate is related to the proportion of sperm in a specific sub-population with regards to motility (Garrett et al., 2003; Ferraz et al., 2014; Santolaria et al., 2015).

Studies on sperm characteristics and sub-populations in avian semen are very scarce. Sperm subpopulations with regards to only head morphometry have been identified in birds and described to show between-species variation (Garcia-Herreros, 2016; Villaverde-Morcillo et al., 2017). Sperm mobility is proposed to be the main determinant of fertility in birds (Froman et al., 1999) but inherent inaccuracy of subjective measurement in conventional methods (Fischer et al., 2014) necessitates application of objective computer assisted approaches. The problem with such systems is the multiplicity of generated parameters which further complicate the interpretation of results. The study of motility subpopulations which reflect the motility characteristics of the sample can help as a practical tool for subjective evaluation and comparison of motility across different birds and provide grounds to investigate the association of motility with fertility.

This article aimed at: 1) identifying motile sperm sub populations in Gyr falcon semen using kinematic parameters derived from CASA; 2) investigating the within and between-subject variations in motility subpopulations of sperm in this species.

MATERIALS AND METHODS

Experimental design

All animal procedures were carried out with regards to ARRIVE code of conduct and were in compliance to UAE animal rights regulations. Four ejaculates from 6 birds were used to carry out a forward observational study to identify the motility subpopulations of sperm in Gyr falcon ejaculate. Upon collection, semen was subjected to conventional evaluations and samples were analyzed by CASA. The kinematic parameters in these samples were measured and used to separate populations of motile sperms by multivariate cluster analysis. It was hypothesized that sperms within ejaculate of falcons can be categorized into distinct sub-groups with similar motion characteristics. The objectives were to define motility characteristics of different groups of sperm and investigate the within-subject and between subject variations in the proportion of sperms in each of those sub-groups.

Experimental location and birds

The study was conducted at Marghum Falconry, Dubai, UAE. Six male adult Gyr falcons (Falco rusticolus) with a sound history of health and fertility, were used for bi-weekly semen collection during the peak of the breeding season (January to March). Male and female falcons were handled and raised as described by Bailey and Lierz (2017). They were individually housed in open facilities with *ad libitum* access to water. Full experimental procedures were carried out in compliance with guidelines from Government of the United Arab Emirates Animal Care and Use.

Semen collection and processing

All falcons were behaviorally imprinted to humans and semen was collected Collection has done by forced abdominal massage, adapted to this species (Bailey and Lierz. 2017). Collected semen was recovered by glass capillaries (75 μL, ID: 0.90 mm / OD:1.60 mm, Interhatch, chesterfield, UK) and placed in 0.5 mL centrifuge tubes (Axygen®, Corning, USA) and transferred to laboratory to be extended in 1:1 (v:v) Raptor extender (Interhatch, chesterfield, UK) before downstream procedures. A total of 24 ejaculates with mean concentration of 56.61 ± 9.77 × 10^3 sperm/μL were used for this study. All samples with concentration less than 30 × 10^3 sperm/μL were excluded from the assays and insemination (Bailey and Lierz, 2017).

Semen analysis

1) Sperm viability

Viability of sperms were analysed using EOSIN B 2% which stains dead cells in pink as described elsewhere (Fischer et al., 2020). In brief, staining solution and the diluted sample were mixed for 30 seconds on a slide and a thin smear was prepared at room temperature. All smears were evaluated under 400-fold magnification by a light microscope and the proportion of live cells to all cells were reported.

2) Motility

The sperm motility assessment was carried out using CASA (ISAS® v1, Proiser R + D S.L., Paterna, Spain) based on the analysis of 100 consecutive, with the image capture speed every 20 ms. The camera used was Proiser 782m (Proiser R + D S.L., Paterna, Spain) attached to a microscope (Model: BX51; Olympus, Japan) with an eyepiece 1 × and a 10 × negative phase contrast objective and stage warmer (Thermo Plate, Tokai HIT, Olympus, Japan) maintained at a constant temperature of 38℃. The diluted sample (4 μL) was placed on special chamber (20 μ, Sperm Track, Proiser, Spain) and a minimum of 8 fields were captured in the centre of the slide. The movement of at least 400-700 cells including the immotile sperm were recorded for each sample from random fields. Range size of particles were defined between 11-72 and microns^2 in the CASA settings (Villaverde-Morcillo et al., 2017). The kinematic parameters recorded for each sperm, as described by elsewhere (Mortimer 2000, Mortimer et al., 2015): curvilinear velocity (VCL, μm/s): the average path velocity of the sperm head along its actual trajectory; straight-line velocity (VSL, μm/s): the average path velocity of the sperm head along a straight line from its first to its last position; average path velocity (VAP, μm/s): the average velocity of the sperm head along its average trajectory; percentage of linearity (LIN, %): the ratio between VSL and VCL; percentage of straightness (STR, %): the ratio between VSL and VAP; wobble coefficient (WOB, %): the ratio between VAP and VCL; mean amplitude of lateral head displacement (ALH, μm): the average value of the extreme side-to-side movement of the sperm head in each beat cycle; and beat cross frequency (BCF, Hz): the frequency with which the actual sperm trajectory crosses the average path trajectory.

Statistical analysis

The kinematic data obtained from CASA (VCL, VAP, VSL, LIN, STR, WOB, ALH and BCF) consisted of 20753 observations of individual sperms from fresh ejaculate of falcons. Using these data, a multivariate K-means cluster analysis based on computation of Euclidian distances was carried out to classify sperms into 3 distinct subpopulations as described elsewhere (Muiño et al., 2008), in such way that all sperms with similar kinematics were assigned to the same cluster while each observation belonged to a single cluster only. The specified number of clusters was inspired by studies on other animals and a preliminary analysis of hierarchical dendrograms constructed on individual observations using the Ward method (Holt, 1995). A generalized linear model procedure followed by LSD test was carried out to evaluate significant differences of kinematic parameters among clusters and between subject variation in the proportion of sperms in each subpopulation. The repeatability of the proportion of sperms in different clusters was assessed by regression analysis among values of each cluster over repeated measurements. A Chi square test of independence was used to investigate the association between falcons and relative fertility outcome in AI program in terms of fertilized egg and hatching where standardized residuals were used to identify cells with significant difference. Results were then compared with each other by chi-square test using 2 × 2 contingency tables. All statistical procedures were carried out by SPSS software package v22.

Table 1. Mean ± SEM of the sperm parameters in 6 individual Gyr falcon semen

	Subjects (n=6)						*p*-value
	Falcon A	Falcon B	Falcon C	Falcon D	Falcon E	Falcon F	
Live (%)	82.75 ± 1.36^{a}	86.75 ± 3.27^{ab}	86.37 ± 1.88^{ab}	85.75 ± 0.79^{ab}	89.00 ± 1.62^{b}	71.37 ± 2.79^{c}	< 0.027
Motility (%)	85.25 ± 1.43^{a}	87.75 ± 2.56^{ab}	89.00 ± 1.33^{ab}	86.62 ± 0.47^{ab}	89.50 ± 1.25^{b}	74.37 ± 1.90^{c}	< 0.014
VCL (μm/s)	40.02 ± 0.23^{a}	37.03 ± 0.20^{b}	38.79 ± 0.22^{c}	40.41 ± 0.27^{a}	33.08 ± 0.34^{d}	33.78 ± 0.32^{d}	< 0.000
VSL (μm/s)	25.77 ± 0.18^{a}	23.39 ± 0.17^{b}	25.39 ± 0.21^{a}	27.20 ± 0.25^{c}	19.93 ± 0.24^{d}	22.19 ± 0.30^{e}	< 0.001
VAP (μm/s)	28.50 ± 0.16^{a}	27.07 ± 0.14^{b}	28.65 ± 0.17^{a}	30.33 ± 0.21^{c}	23.31 ± 0.22^{d}	25.07 ± 0.25^{e}	< 0.000
LIN (%)	65.06 ± 0.28^{a}	63.39 ± 0.35^{b}	64.7 ± 0.37^{a}	66.4 ± 0.45^{c}	61.6 ± 0.41^{d}	64.88 ± 0.52^{a}	< 0.022
STR (%)	87.03 ± 0.25^{a}	83.03 ± 0.32^{b}	83.45 ± 0.33^{b}	84.57 ± 0.40^{c}	81.35 ± 0.40^{d}	83.99 ± 0.47^{bc}	< 0.024
WOB (%)	73.13 ± 0.21^{a}	74.12 ± 0.22^{bc}	74.51 ± 0.24^{c}	75.56 ± 0.28^{d}	73.55 ± 0.30^{ab}	75.13 ± 0.36^{cd}	< 0.018
ALH (μm)	1.64 ± 0.01^{a}	1.61 ± 0.01^{b}	1.60 ± 0.01^{b}	1.59 ± 0.01^{b}	1.55 ± 0.01^{c}	1.47 ± 0.01^{c}	< 0.038
BCF (Hz)	7.52 ± 0.05^{a}	6.54 ± 0.05^{b}	6.79 ± 0.06^{c}	7.25 ± 0.07^{d}	5.35 ± 0.07^{e}	6.46 ± 0.08^{b}	< 0.002

Different letters indicate significant difference within rows ($p < 0.05$).

RESULTS

Table 1 summarizes the motility characteristics of sperm in six birds. Overall, studied subjects indicated high mean values for live/dead (85.41 ± 1.22) and total motility (83.70 ± 1.43). A higher proportion of live and total motile sperms were observed in Falcon E as compared to Falcon A and F ($p < 0.05$; Table 1). All velocity parameters significantly differed among subjects with the highest VSL and VAP observed in falcons A and C ($p < 0.05$; Table 1). Mean VCL was highest in Falcon A and D while Falcon E and F had consistently lower velocity parameters compared to the rest of the birds ($p < 0.05$). Sperms of falcon E indicated the lowest values for straightness and linearity ($p < 0.05$; Table 1).

Cluster analysis of kinematic data resulted in the separation of sperms into 3 subpopulations with distinct differences in motility features as indicated by significant differences in all kinematic parameters among clusters (Table 2; $p < 0.000$). Higher mean values for velocity, ALH and BCF were observed in subpopulation 1 followed by subpopulation 2, as compared to subpopulation 3 ($p < 0.05$). Sperms of cluster 3 moved along the least forward trajectory as compared to sperms in cluster 1 and 2 which did not differ significantly with each other in STR mean values ($p > 0.05$).

The proportion of sperms in each defined cluster showed a significant degree of association across different samplings (Fig. 1). The 2 by 2 regression analysis of sperm proportions within each cluster indicated a variable degree of association (R^2 = 30.9 to 0.827; p = 0.019 to $p < 0.0001$) which proved to be significant among replicates in all clusters, indicative of moderate to high repeatability of measurements in the subjects.

Table 2. Mean values (±SEM) and ranges of the kinematic parameters in identified motility subpopulations of Gyr falcon semen

Kinematic parameters	Sperm subpopulation		
	1	2	3
No. sperm (%)	3303 (15.91%)	9410 (45.34%)	8040 (38.74%)
VCL (μm/s)	61.13 ± 0.19^{a} (46.30–157.10)	41.48 ± 0.06^{b} (26.20–65.70)	23.63 ± 0.09^{c} (10.00–51.70)
VSL (μm/s)	40.17 ± 0.17^{a} (0.00–99.20)	29.35 ± 0.07^{b} (0.00–48.50)	11.81 ± 0.06^{c} (0.00–28.40)
VAP (μm/s)	42.22 ± 0.12^{a} (10.30–73.70)	31.71 ± 0.05^{b} (7.40–50.80)	16.42 ± 0.06^{c} (3.10–59.40)
LIN (%)	67.78 ± 0.34^{a} (0.00–100)	72.26 ± 0.19^{b} (0.00–100)	53.66 ± 0.25^{c} (0.00–100)
STR (%)	92.43 ± 0.26^{a} (0.00–100)	91.00 ± 0.15^{b} (0.00–100)	72.61 ± 0.25^{c} (0.00–100)
WOB (%)	70.71 ± 0.25^{a} (15.00–100)	77.43 ± 0.13^{b} (12.00–86)	71.80 ± 0.19^{c} (17.00–81)
ALH (μm)	2.18 ± 0.01^{a} (0.60–6.50)	1.59 ± 0.05^{b} (0.40–4.90)	1.34 ± 0.04^{c} (0.30–3.90)
BCF (Hz)	9.96 ± 0.05^{a} (0.00–18.80)	8.05 ± 0.02^{b} (0.00–18.00)	3.88 ± 0.03^{c} (0.00–15.00)

Different capital letters indicate significant difference in rows ($p < 0.000$).

Fig. 2 compares the frequency distribution of sperms with different motility features among the subjects. A significantly higher proportion of subpopulation 1 sperms were observed in Falcon A (19.82 ± 5.74), C (19.33 ± 5.26), and D (20.80 ± 1.55) as compared to Falcons B (7.89 ± 5.26), E (5.43 ± 1.32) and F (9.35 ± 3.82) ($p < 0.05$; Fig. 2). Highest proportion of subpopulation 3 sperms were observed in falcons F and E which also had the lowest pro-

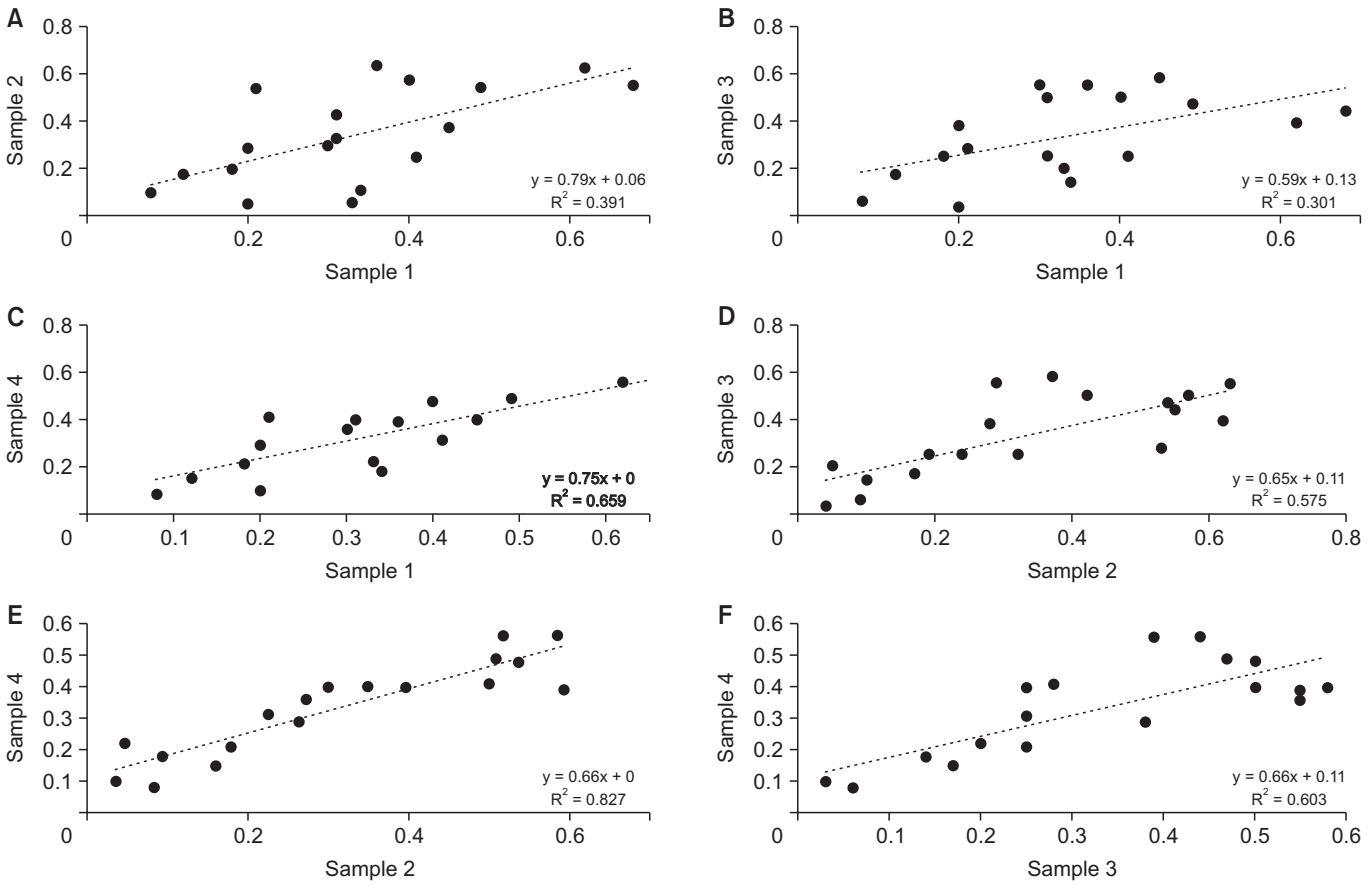

Fig. 1. Corelation analysis among proportion of sperms in clusters 1 to 3 across repeated samplings. (A) (R^2 = 0.391, p = 0.006); (B) (R^2 = 0.301, p = 0.019); (C) (R^2 = 0.659, p = 0.000), (D) (R^2 = 0.575, p = 0.000); (E) (R^2 = 0.827, p = 0.000); (F) (R^2 = 0.603, p = 0.000).

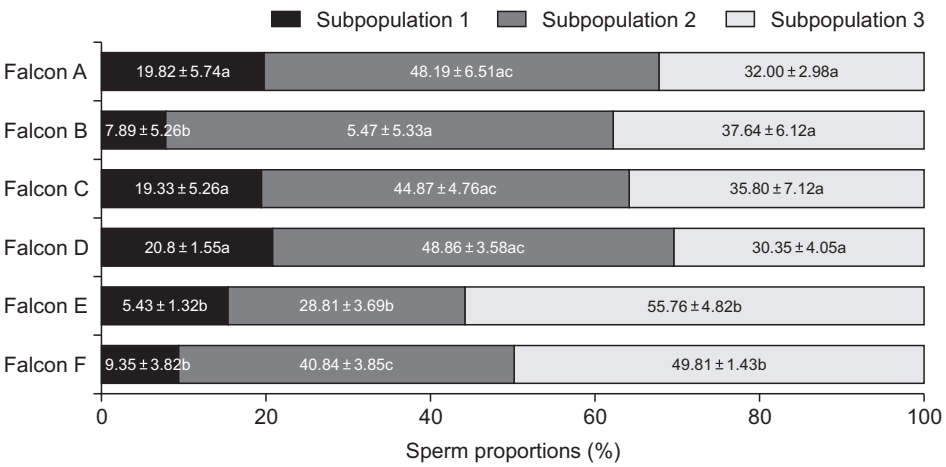

Fig. 2. Frequency distribution of sub populations within Falcons A to F (Figures represent percentage ± SEM).

portion of subpopulation 2 sperms (p < 0.05; Fig. 2).

DISCUSSION

The present study investigated the presence of motility subpopulations of sperms in Gyr falcon and individual variations in the motility pattern of sperm among the males. The results showed for the first time that different subpopulations of motile sperms in this species can be identified using kinematic parameters generated by CASA.

The proportion of sperm with distinctive motility features seemed to be moderately to highly repeatable among ejaculates in a given male while showing a considerable variation among birds.

Previous studies in mammals have focused on identification of sperm subpopulations in semen of animals by applying different methods of cluster analysis using different combination of kinematic parameters in the calculations (Martinez-Pastor et al., 2005). The majority of these studies have led to identification of three to four distinct subpopulations with specific motion characteristics (Abaigar et al., 2001; Dorado et al., 2010; Dorado et al., 2011; Flores et al., 2008; Muiño et al., 2008; Muiño et al., 2009; Ortega-Ferrusola et al., 2009; Quintero-Moreno et al., 2003; Ramió et al., 2008) suggesting that, the co-existence of three or four motile sperm subpopulations is a common feature of mammalian semen (Quintero-Moreno et al., 2003). Our results showed that 3 well separated groups of motile sperms can also be identified in Gyr falcon semen by k-means clustering procedure using CASA data (VCL, VAP, VSL, ALH and BCF) with minimal within-group and significant between-group variations in all kinematic parameters among clusters. Co-existence of sperm sub-groups in the semen of avian species was first documented in a study by García-Herreros (2016) in which sperm head morphometrics obtained by CASA were used to identify 3 and 5 subpopulations in rooster (*Gallus domesticus*) and Guinea fowl (*Numida meleagris*), respectively (García-Herreros, 2016). The study corroborated the objective analytical application of CASA to identify strong differences in morphometric parameter values and related subpopulation distribution among species. To the best of our knowledge, no study has reported the presence and characteristics of motility subpopulations in avian sperm and falcons in particular.

The qualitative interpretation of motion characteristics in each of these subpopulations reveals similarities to what has been previously described about motility pattern of mammalian sperm. Sub-population 1 contained the fastest forward-moving sperms as indicated by the highest mean velocity values and linearity in their movement trajectory; characteristics described to be necessary for being a part of fertilizing population of sperm in mammals (Table 2) (Sakkas et al., 2015). Given these features, 15.91% of the whole population of the sperms clustered in this subpopulation seem to represents the rapid progressive fraction of the sample (Table 2). This is particularly important because the conventional methods of subjective motility assessment in birds can result in a variation of 30-60% of the motility parameters for the same ejaculate (Fischer et al., 2014) and lead to less objective evaluation of the studied subjects. By contrast to subpopulation 1, subpopulation 3 sperms moved at a lower speed on a less linear trajectory as indicated by lower velocity and linearity values compared to the other groups (Table 2) and therefore, represent a metabolically compromised fraction of the population which are soon to lose their motility altogether (Dorado et al., 2010). Sperm in subpopulation 3 had higher mean velocity compared to subpopulation 3 and a comparable straightness in their motion trajectory to subpopulation 1 and therefore, can be nominated as the moderate progressive fraction of the ejaculate (Table 2).

It has been proposed that different subpopulations represent sperms in different physiological status (Abaigar et al., 2001). From physiological stand point, an orchestrated alteration in motility pattern of sperms is in progress in response to environmental changes or storage, so that all sperms undergo structural and biochemical changes which cause step by step impairment of their functionality. As for the subpopulations identified in the current study, it can be hypothesized that rapid progressive sperms compromise their velocity and over time, convert to medium progressive sperms, which later undergo further loss of metabolic integrity and membrane damage, just prior to losing their vigour and becoming poorly motile. This latter status represents the last stage of sperm motility deterioration. An interesting observation in our study is that poorly motile sperms of Gyr falcon, still resumed a relatively high degree of linearity and straightness (53.66 ± 0.25 and 72.61 ± 0.25, respectively; Table 2) when compared to their mammalian counterparts in which linearity of the medium and slow-moving subpopulations averages 18.91 ± 8.14 and 29.7 ± 13.48 in stallion (Gacem et al., 2021), 59.4 (range: 11.7-97.7) and 59.2 (range: 13.4-100.0) in cows (Muiño et al., 2008), 67.5 ± 5.3 and 38.2 ± 5.7 in boar , 22.8 ± 1.2 and 63.2 ± 1.1 in donkey (Flores et al., 2008).

The findings indicated considerable variations among male falcons in terms of motility characteristics of their semen as indicated by significant differences in total motility and kinematic parameters of their respective

samples (Table 1). These variations were translated into significant shifts in frequency distribution of sperms in 3 subpopulations among the subjects (Fig. 2) while the proportion of sperms in all clusters were fairly consistent within each individual over repeated samplings (Fig. 1). To the best of our knowledge, there is no published data on sperm motility subpopulations in falcons and the only other study on sperm subpopulations in this specis have focused on categorization of sperms with regards to sperm head morphometrics (Villaverde-Morcillo et al., 2017). Authors documented the co-existence of 4 subtle subpopulations within Gyr falcons, European falcon subspecies (*F. p. peregrinus* and *F. p. brookei*) semen with subpopulations 1 to 4 containing long wide heads (8.8, 13.3 and 11.5%), long narrower heads (24.8, 29.3 and 18.5%) fusiform shaped heads (37.3, 33.3 and 37%) and oval shaped heads (28.4, 24 and 33%) in all three studied breeds, respectively. Measured morphometrics indicated significant between-species differences ($p < 0.001$) but a high heterogeneity in all ejaculates, fresh Gyrfalcon sperm in particular, as revealed by the large coefficients of variation (Villaverde-Morcillo et al., 2017). In contrast to high subject-related variation in sperm morphology, our findings suggest a consistently significant correlation between proportion of sperms in each cluster over measurements indicating a minimum within-subject variation in sperm motility characteristics in Gyr falcon (Fig. 1). The repeatability of sperm proportions in each cluster in a given male can give sperm subpopulation analyses a more reliable potential for selection of males with superior sperm quality and facilitate future comparative studies that aim to establish valid normative sperm evaluation values for Gyr falcon.

Comparison of sperm proportion in subpopulations indicates significant between-subjects variations with Falcons A, C and D having consistently higher number of fast progressive sperms as compared to Falcons B, E and F ($p < 0.05$; Table 2). Cumulative fertility data obtained from artificial inseminations during the same time frame of the study refers to a significantly higher proportion of fertilized eggs when females were inseminated with the semen from Falcon A and D as compared to falcons B and E (data not shown). This is of particular interest since Falcon E had the highest live percentage and total motility which did not seem to compensate for the lower kinematic values observed in the same bird. The crucial role of sperm motility in fertility of birds was investigated in a study by Froman et al. (2002) where they classified male roosters into two groups of high and low mobility based on metabolic activity of sperms and used the respective semen from 48 subjects of both groups for artificial insemination. By plotting fertility as a function of sperm mobility, they concluded that mobility is a primary determinant of fertility in the fowl and that birds differ due to mobility attributes inherent to their sperm (Froman et al., 1999; Froman et al., 2002). In another study on bovine *in-vitro* production of embryos the proportion of sperm in a subpopulation 4, which had the highest VCL, LIN and STR among clusters, and the number of spermatozoa was found to be associated with the proportion zona binding ($r^2 = 0.79$, $p < 0.01$). VCL of the samples were also corelated to the number of zona binding sperms ($r^2 = 0.62$, $p < 0.01$), but the proportion of cells in subpopulation 4 was the only parameter to predict pronucleus formation (Ferraz et al., 2014) which indicates the advantage of subpopulation evaluation over the use of multiple kinematic values when trying to interpret the results in terms of fertilizing capacity of ejaculates. Clarification on which subpopulation corelates the most with fertility outcome requires further experimentations on fertility data which also incorporate other sperm characteristics such as morphology and metabolic status into their design.

CONCLUSION

In conclusion, coexistence of different motility subpopulations of sperms (3 categories) was documented for the first time in Gyr falcon using kinematic parameters generated by CASA. Clustering sperm offers an advantage in semen analyses by incorporating all kinematic data into a single parameter and thereby simplifying further investigation of motility characteristics among individuals as well as between species comparisons. Significant between subject variations in kinematics and sub-populations will facilitate future studies to clarify the role of a particular subpopulation as the determinant of fertilizing capacity in male Gyr falcon. This is of practical value since the proportion of sperms within subpopulations showed an overall consistency in each individual bird which in turn allows for an objective evaluation of subjects and corelating their sperm motility features with fertility results in future studies.

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