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Sperm motility patterns and metabolism in Catalonian donkey semen

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Abstract

The Sperm-Class Analyzer® detected four subpopulations of spermatozoa with different motility characteristics in the ejaculate of the Catalonian donkey. Significant differences ($P < 0.001$) in the distribution of these subpopulations, as well as in total sperm number and percentage total motility, were seen in the diluted semen of four sampled donkeys. All the ejaculates evaluated showed excellent semen quality characteristics; the sperm they contained was more rapid than horse sperm. Principal components analysis showed sperm L-lactate production to be a good predictor of semen condition. This, plus the characteristics of the motility patterns of the different sperm subpopulations, provides an excellent overall indicator of semen quality.

Keywords: Sperm subpopulations; Motility pattern; Donkey spermatozoa; L-lactate production

1. Introduction

For centuries the Catalonian donkey has been highly regarded for breeding mules. Documents from as far back as the 9th century mention the impressive height and

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exceptional characteristics of this animal [23] now in danger of extinction. In the last census only about 100 were recorded (one third of which were males), all living in the Pyrenean and pre-Pyrenean areas of Catalonia (north-east Spain) [9].

Numerous research projects have described and characterised domestic horse semen, but studies on donkey semen are almost non-existent. One recent report involving five animals of different breeds studies the preservation of donkey semen in tropical conditions [12], and in another, [25–27] examine the cryopreservation of Poitou jackass sperm. Greater knowledge of the reproductive features and semen characteristics of the Catalonian donkey will be required (e.g., on sperm motility descriptors and other variables of semen quality), however, if breeding programs to increase its numbers are to be designed.

The ejaculates of many mammalian species, e.g., the common marmoset, cattle, goats, gazelles, dogs, horses and pigs [1,2,7,14,15,18,19,24] are known to contain subpopulations of motile sperm. These subpopulations have been analysed using a computerised motility analysis system (CASA) which defines sperm motion characteristics with an unprecedented degree of sophistication. The existence of sperm subpopulations in animals of quite different phylogenetic origin suggests this is a highly conserved characteristic [14,15]. The characterization of these subpopulations could lead to new ways of improving mammal semen analysis techniques.

This aim of this study was to determine whether sperm subpopulations with specific motion characteristics exist in diluted Catalonian donkey ejaculates. Priority was given to the optimisation of CASA by clustering the motion variables that might better define sperm motion characteristics. In addition, relationships were sought between the sperm subpopulations found, the total motility of the sperm ejaculates, sperm concentration, and L-lactate production. The results obtained may contribute towards increasing the population of this breed of donkey and help avoid its extinction.

2. Materials and methods

2.1. Animals, mating behaviour and semen collection

This work was performed between April 2002 and January 2003 at the experimental farm belonging to the Veterinary Faculty of the Autonomous University of Barcelona. Semen was collected from four healthy, mature Catalonian donkeys aged 4, 5, 8 and 12 years, all of which were known to be fertile. Collections were performed at 2–3 days intervals using an artificial vagina and an ovariectomised female donkey brought into oestrus with estrogens.

2.2. Sperm morphologies and functional evaluation

An aliquot of each collected ejaculate was used to determine sperm concentration and pH. Gel-free semen was immediately diluted 1:1 with dry skimmed milk extender [11] kept at 37 °C in a water bath. These samples were maintained at room temperature, rediluted to a final concentration of 25×10^6 sperm/mL and stored in air-free, sterilise, polyethylene bags. Aliquots were then taken as needed for analysis. Sperm viability, the number of

altered acrosomes and any morphological abnormalities were determined in a sample of 200 spermatozoa by optical microscopy (magnification 1000X). Samples were stained with Eosin-nigrosin as described by [3]. The sperm concentration of the ejaculates was determined using a haemocytometer [13].

The hypoosmotic swelling test (HOST) was performed as described by [8,6]. Aliquots (100 μl) of fresh semen samples were added to 900 μl of hypo- and isoosmotic medium previously warmed to 37 °C. The isoosmotic medium ($\prod$ = 300 mOsm) contained 2.7% (w/v) fructose and 1.47% (w/v) sodium citrate in distilled water; the hypoosmotic medium ($\prod$ = 152 mOsm) contained 1.35 (w/v) fructose and 0.735 (w/v) sodium citrate in distilled water. The suspensions were maintained at 37 °C in a water bath for 20 min and then centrifuged at 600 × *g* for 2.5 min. Spermatozoa survival and swelling reactions were evaluated using 10 μl of the pellet and 10 μl of vital Eosin-nigrosin stain. To relate the swelling reaction to sperm viability, the percentage of true swollen tails (TST) was defined by Kumi-Diaka and Badtram [28]. The percentage of TST was defined as:

$$\mathrm{TST}(\%) = \frac{(\mathrm{HTS} - \mathrm{ITS}) \times \mathrm{HV}}{\mathrm{IV}}$$

where HTS is the percentage of swollen tails in the hypoosmotic medium, ITS the percentage of coiled or curled tails in the isoosmotic medium, HV the percentage viability in the hypoosmotic medium, and IV the percentage of viable spermatozoa in the isoosmotic medium.

L-lactate production in the isoosmotic medium was determined enzymatically [20,21]. Fresh semen of 100 μl were added to 900 μl of the isoosmotic medium and incubated at 37 °C in a water bath for 1 h. The suspension was then homogenised and centrifuged at 1000 × *g* for 2 min. Aliquots of supernatant were frozen. At analysis, all samples were defrosted at 37 °C and the L-lactate concentration determined using a computerised biochemical analysis system (COBAS). To normalise the results, the values obtained were divided by the sperm count.

2.3. Computed-assisted motility analysis

The motion characteristics of the samples were determined using a Sperm-Class Analyzer® (Microptic, Barcelona, Spain). A 5 ml aliquot of the diluted semen was incubated for 5 min in a water bath at 37 °C. Three consecutive 5 μl drops of each of the studied ejaculates were then observed using a phase contrast microscope with a heatable stage (37 °C). Two fields per drop were analysed. The total number of spermatozoa analysed in each sample (including those not motile) was 25–50. The CASA system is based on the analysis of 16 consecutive, digital images of a single field at a magnification of ×200 (dark ground). These 16 images were obtained over 0.64 s—an image capture rate of 1 photograph every 40 ms.

2.4. Statistical analysis

Data were processed using the SAS statistical package (2000) [22]. Normality was assessed by the Shapiro–Wilks test (W) included in the UNIVARIATE procedure. When

results were not distributed normally they were subjected to square root or base 10 logarithm transformation [5,10]. The sperm motility descriptors (21 in total) obtained from CASA were clustered in separate groups by the VARCLUS procedure [14] to reduce the number of variables (data not shown). This allowed the variables that best explained overall sperm movement to be selected. These analyses grouped the tested motion variables into six clusters that accounted for 78.6% of the total variation (data not shown). Further analysis of the relationships among the variables in each cluster led to the choice of a single variable from each that provided the most information. Those selected were:

- Mean velocity (VAP): the mean trajectory of sperm per unit time. Units are μm/s.
- Mean lateral head displacement (mean LHD): the mean head displacement along the curvilinear trajectory around the mean trajectory (units are μm).
- Linear coefficient (LIN): the coefficient between linear velocity and curvilinear velocity (units are %).
- Frequency of head displacement (BCF): the number of lateral oscillatory movements of the sperm head around the mean trajectory (units = Hz).
- Minor harmonic oscillation of the head (HLO): the minimum distance between the curvilinear trajectory and the mean trajectory (units = μm).
- Algebraic angular mean displacement (MADalg): the algebraic value of the advancing angle of the sperm trajectory, provided that negative values indicate a clockwise displacement. Units are angular degrees.

The FASTCLUS clustering procedure was used for separating the spermatozoa into subpopulations [14,15]. Spermatozoa with very similar motility characteristics were assigned to the same cluster. The total number of spermatozoa in the diluted semen samples–2556 motile sperm from 78 samples-were subjected to this procedure. A general linear model (PROC GLM) was used to determine the differences ($P < 0.05$) between the sperm motility descriptors of these subpopulations. The LSMEANS procedure was used to compare the sperm subpopulations. Finally, the Chi-square test was used to relate the distribution of the sperm subpopulations in fresh semen with percentage total motility (i.e., the percentage of spermatozoa with a VAP > 10 μm/s; Thurston et al. [15]) and overall sperm concentration. For this, whole ejaculates were first categorised by the FASTCLUS procedure as follows:

According to total motility:

Group 1 whole ejaculates: total motility $\leq 50\%$.
Group 2 whole ejaculates: total motility $> 50\% \leq 80\%$.
Group 3 whole ejaculates: total motility $> 80\%$.

According to sperm count of diluted samples:

Group 1 whole ejaculates: sperm count $\leq 166 \times 10^9$ spermatozoa/mL.
Group 2 whole ejaculates: sperm count > 166 to $\leq 500 \times 10^6$ spermatozoa/mL.
Group 3 whole ejaculates: sperm count $> 500 \times 10^6$ spermatozoa/mL.

The main aim of these categories is to provide a reference that allows changes in the distribution of the motile subpopulations to be detected. Finally, the GLM procedure was used to determine the relationship between the different sperm subpopulations and these categories. The LSMEANS procedure was used to evaluate the significance of any differences.

Principle components analysis (PCA) was used to determine which sperm variables best explained the results obtained.

3. Results

3.1. Mean semen quality analysis of donkey ejaculates

Table 1 shows the mean values for the semen quality variables. Sperm movement and morphology values were good, although viability diminished after incubation in isoosmotic medium. The composition of semen and sperm motility descriptors varied between donkeys and even between ejaculates of the same animal ($P < 0.001$; data not shown).

3.2. Sperm subpopulation analysis

The FASTCLUS procedure detected four motile sperm subpopulations from the motility data (Table 2).

Table 1
General characteristics of Catalonian donkey semen

Variable	Mean ± S.E.	Confidence interval (95%)
Filtered volume (mL)	56.61 ± 23.18	55.68–57.55
Sperm count ($\times 10^6$ /mL)	280.88 ± 228.94	271.12–290.63
Total motility, VAP > 10 μm/s (%)	68.40 ± 16.59	67.73–69.06
Ph	7.77 ± 0.35	7.75–7.78
Sperm viability (%)	70.06 ± 16.46	69.40–70.72
Sperm immature tail (%)	10.22 ± 4.80	10.03–10.41
Sperm coiled-tail (%)	1.22 ± 1.16	1.17–1.26
Sperm head abnormality (%)	2.5 ± 2.37	2.41–2.60
Tailless spermatozoon (%)	2.95 ± 2.17	2.86–3.04
Immature sperm with proximal cytoplasmic droplet (%)	1.36 ± 1.42	1.31–1.41
Immature sperm with distal cytoplasmic droplet (%)	0.39 ± 0.55	0.37–0.42
Total abnormalities	18.99 ± 8.62	18.30–18.99
L-lactate production (μ mol/mg de protein × 60 min)	0.85 ± 1.32	0.74–0.95
HOST test (%)	24.19 ± 19.26	22.64 ± 25.74
Sperm curvilinear velocity (VCL, μm/s)	80.20 ± 51.72	78.21 ± 82.20
Sperm linear velocity (VSL, μm/s)	49.80 ± 43.18	48.13 ± 51.46
Mean velocity (VAP, μm/s)	59.39 ± 43.04	57.74 ± 61.05
Linear coefficient (LIN, %)	60.50 ± 28.79	61.60 ± 59.39
Straightness coefficient (STR, %)	79.21 ± 24.50	78.28 ± 80.14
Wobble coefficient (WOB, %)	71.97 ± 20.27	71.20 ± 72.74
Mean lateral head displacement (mean ALH, μm)	2.36 ± 2.05	2.28 ± 2.44
Frequency of head displacement (BCF, Hz)	12.71 ± 5.20	12.51 ± 12.91
Minor harmonic oscillation of the head (HLO, μm)	0.49 ± 1.30	0.44 ± 0.54

Table 2
Sperm subpopulations and motility descriptors in Catalonian donkey semen

Sperm motility descriptors	Sperm subpopulations			
	1	2	3	4
N°	845	547	447	818
(%)	31.8	20.6	16.8	30.8
VAP (μm/s)	114.96 ± 0.74^{a}	25.37 ± 0.98^{c}	65.03 ± 1.05^{b}	25.73 ± 0.84^{c}
LIN (%)	86.85 ± 0.61^{a}	29.84 ± 0.80^{c}	28.38 ± 0.87^{c}	69.70 ± 0.70^{b}
ALHmed (μm/s)	1.94 ± 0.06^{c}	2.64 ± 0.08b	4.94 ± 0.08^{a}	1.36 ± 0.07^{d}
MADalg (μm)	-1.68 ± 0.64^{c}	-28.40 ± 0.82^{d}	12.85 ± 0.89^{a}	0.87 ± 0.70^{b}
BCF (Hz)	13.54 ± 0.20^{a}	13.27 ± 0.25^{a}	11.93 ± 0.27^{b}	11.01 ± 0.21^{c}
HLO (μm)	0.66 ± 0.05^{a}	0.40 ± 0.06^{b}	0.81 ± 0.07^{a}	0.23 ± 0.05^{b}

Motility descriptors are described in Section 2. Different superscripts (a, b, c and d) in the same row indicate significant differences ($P < 0.05$). Results are expressed as means ± S.E. from 78 semen samples from four Catalonian donkeys. The total number of motile spermatozoa analysed was 2556.

3.2.1. Subpopulation 1

The spermatozoa of this subpopulation showed the greatest progressiveness; they were highly active (as inferred from the very high LIN and VAP values) with a low mean ALH and high BCF. The MADalg implied a negative, clockwise displacement. About 31.8% of the spermatozoa were assigned to this group.

3.2.2. Subpopulation 2

The spermatozoa of this group showed non-linear trajectories, low progressiveness and a low VAP value. The MADalg was very high; displacement was negative and clockwise. Some 20.6% of motile spermatozoa were included in this subpopulation.

3.2.3. Subpopulation 3

This contained rapid spermatozoa with high VAP values, although with trajectories with low linear coefficients. The mean ALH was higher than that for the other subpopulations; the BCF was the lowest of all. The MADalg implied a positive clockwise displacement. About 16.8% of the total motile spermatozoa were assigned to this subpopulation.

3.2.4. Subpopulation 4

This group was characterised by spermatozoa with high progressiveness and high LIN values; VAP and ALH values were, however, low. These results show these spermatozoa have complex but overall straight trajectories. About 30.8% of the total motile spermatozoa were included in this subpopulation.

Significant ($P < 0.01$) differences were seen between the four donkeys sampled with respect to the distribution of these subpopulations in whole fresh ejaculates. The percentage of Subpopulation 1 spermatozoa ranged from 28.78 to 42.69%, Subpopulation 2 from 14.82 to 24.69%, and Subpopulation 4 from 24.07 to 33.02%. Subpopulation 3 was similar in all four donkeys (14.42–16.86%).

Significant ($P < 0.001$) differences were also seen when the distribution of the four subpopulations was compared to the classification of the ejaculates according to adjusted

Table 3
Relationship between the proportions of motile sperm subpopulations in diluted ejaculates of donkeys and the total motility of the whole ejaculates

Sperm subpopulations	Total motility groups		
	≤50%	>50% to ≤80%	>80%
1	37.74[a]	32.19[a,b]	26.14[b]
2	22.44[a]	19.23[a]	21.07[a]
3	17.83[a]	16.89[a]	15.86[a]
4	21.99[c]	31.69[b]	36.93[a]

Sperm subpopulations are described in Section 3. The table shows the percentages of motile spermatozoa in each subpopulation depending upon the total motility values of the whole ejaculates. Different superscripts (a, b and c) in the same row indicate significant differences ($P < 0.05$; Chi-squared test). The results were obtained from 78 semen samples; 33 ejaculates were from donkey 1, 19 from donkey 2, 13 from donkey 3 and 13 from donkey 4. The total number of motile spermatozoa analysed was 2556.

percentage total motility. Subpopulation 1 decreased from about 37.7% in ejaculates with a total motility of ≤50% (Group A ejaculates, see Section 2) to about 26.1% in ejaculates with total motility >80% (Group C ejaculates, see Section 2 and Table 3). Subpopulation 4 progressively increased with total motility. Subpopulations 2 and 3 were not affected by semen total motility and showed a similar distribution in ejaculates with high and low sperm movement. This classification shows a clear relationship between the percentage distribution of motile subpopulations and total motility.

The distribution of the sperm subpopulations also varied depending upon the total number of spermatozoa in the whole ejaculates, although in this case the variations were less marked. Differences ($P < 0.05$) in sperm number were observed in Subpopulations 1 and 2 (from about 6–8% in sperm Subpopulation 1 and 6% in sperm Subpopulation 2) (Table 4). The linear coefficient decreased significantly (6.5%) in semen samples with a sperm count of over 375×10^6 sperm/ml ($P < 0.05$).

Finally, the sperm subpopulations showed differences with respect to L-lactate production. Although all the ejaculates sampled showed low L-lactate production values,

Table 4
Relationship between the proportions of motile sperm subpopulations in fresh and diluted ejaculates and total spermatozoa in whole ejaculates

Sperm subpopulation	Total spermatozoa-number groups		
	$\leq 166 \times 10^6$ sperm/mL	$>166 \times 10^6 \leq 375 \times 10^6$ sperm/mL	$\geq 375 \times 10^6$ sperm/mL
1	30.01[b]	38.79[a]	32.79[b]
2	21.34[a]	14.94[b]	21.70[a]
3	16.51[a]	15.23[a]	18.60[a]
4	32.13[a]	31.03[a]	26.92[a]

Sperm subpopulations are described in Section 3. The table shows the percentages of motile spermatozoa in each subpopulation depending upon the total motility values of the whole ejaculates. Different superscripts (a and b) in the same row indicate significant differences ($P < 0.05$; Chi-squared test). The results were obtained from 78 semen samples; 33 ejaculates were from donkey 1, 19 from donkey 2, 13 from donkey 3 and 13 from donkey 4. The total number of motile spermatozoa analysed was 2556.

Table 5
Partial correlation coefficients between normalised donkey semen quality variables

Parameters	Total motility	Sperm count	Host test	Viability	VAP	LIN	ALHmed
L-lactate rate (μ mol/mg de protein × 60 min) [a]	0.10*	−0.38***	0.56***	−0.09*	0.13**	0.13**	−0.04
Total motility (%)		−0.09***	−0.04	0.004	−0.11***	−0.02-	0.04*
Sperm count (×10^6 /ml) [b]			0.19***	0.41***	0.06**	−0.03	0.06**
Host test (%)				−0.12**	−0.06	−0.001	0.009
Viability (%)					−0.03	−0.05*	0.02
VAP (μm/s)						0.45***	0.15***
LIN (%)							−0.54***

[a] Normalised after logarithmic transformation.
[b] Normalised after square root transformation.
* $P < 0.05$.
** $P < 0.001$.
*** $P < 0.005$.

Subpopulations 1 and 3 produced more (1.11 μmol/mg protein × 60 min) than Subpopulations 2 and 4 (0.58–0.62 μmol/mg protein × 60 min). It is important to empathise that sperm Subpopulations 1 and 3 also showed the highest VAP, ALHmed and HLO values; there is therefore a close relationship between these variables.

Table 5 shows the correlations between a number of semen quality variables. In absolute terms, the logarithmic transformation of L-lactate production was strongly correlated with the sperm count, Host test, VAP and LIN. This indicates that the rate of formation of L-lactate is a consistently good indicator of semen quality compared to other descriptors, at least in the donkey. Moreover, PCA showed that the variables with the highest Eigenvalues were L-lactate production (2.1176), followed by total motility (1.6209), sperm count (1.3504) and Host test (1.3264) (Table 6). The descriptors of sperm viability and sperm motility (VAP, LIN and ALHmed) showed Eigenvalues of below 1—the idea of PCA analysis is to select variables with Eigenvalues equal to or above 1. The Eigenvalues showed that just four variables, L-lactate production, total motility, sperm count and Host test result, explained 80% of the total variance, with L-lactate production the most important for determining semen-quality.

Table 6
Total variance explained by each semen quality variable

Variable	Eigenvalues	Proportion	Cumulative proportion
L-lactate production (μ mol/mg de protein × 60 min)	2.1176	0.2647	0.2647
Total motility, VAP > 10 μm/s (%)	1.6209	0.2026	0.4673
Sperm count (×106 /mL)	1.3504	0.1688	0.6361
Host test (%)	1.3264	0.1658	0.8019
Sperm viability (%)	0.9195	0.1149	0.9169
VAP (μm/s)	0.3629	0.0454	0.9623
LIN (%)	0.1715	0.0214	0.9837
ALHmed (μm/s)	0.1304	0.0163	1.0000

4. Discussion

Sperm motion were examined using the FASTCLUS procedure, a clustering analysis that examines the heterogeneity of sperm swimming characteristics. This procedure has been previously used to define subpopulations of spermatozoa based on motion variables in the stallion and boar [14,15]. The PATN method [4] has been used to define sperm subpopulations based on motion parameters in the boar and gazelle [1,2].

In this study, four sperm subpopulations were identified in samples of Catalonia donkey semen. Group values for the individual Sperm-Class Analyzer® CASA-derived descriptors provide an indication of the motion behaviour of each sperm subpopulation, facilitating the physiological interpretation of data. A simple interpretation of this sperm subpopulation structure is that these four groups represent different levels of sperm quality, reflected in their very different swimming behaviours. Subpopulation 1 contained the most vigorous and progressive spermatozoa, these would probably attract the attention of a subjective observer. The other sperm subpopulations had high or low non-linear patterns of motility, and differed in their trajectory and vigour. Subpopulations 2 and 3, the smallest groups, were characterised by low-linear motion. Their low LIN values indicate that these cells might be either hyperactivated or show erratic and uncoordinated movements, but their mean ALH movements were not sufficiently large and did not seem consistent with hyperactivation frequencies. Population 2, with its low VAP and LIN values, might represent a subgroup of metabolically compromised sperm, soon to lose their motility altogether. Population 3 contained spermatozoa with non-linear and circular trajectories and with poor progressiveness, possibly representing hyperactivated or uncoordinated motility. This characteristic was evident in this subpopulation's higher ALHmed values. The definition of hyperactivated motility in stallion spermatozoa is a VCL of $\geq$180 μm/s and a mean ALH of $\geq$12 μm/s [17]. This probably means these cells were affected by sample management or by other intrinsic factors, and had begun a degenerative process. Subpopulation 4, whose members showed high progressiveness and low velocity, represented 31% of the total spermatozoa in the semen samples (the second largest subpopulation). Their characteristics were similar to the predominant subpopulations of other mammalian sperm, such as those of the gazelle, stallion and boar [1,2,14,15]. It should be emphasised that donkey spermatozoa are more rapid than stallion spermatozoa [14], although the progressiveness of their spermatazoa are relatively similar.

The specific subpopulation structure of an ejaculate depended on several variables such as the individual donkey, total motility and total sperm number/ml. This was expected since other species (e.g., horse and pig) are known to show such relationships [14,15]. The comparison between motile subpopulation structure, total motility and total sperm count indicates that sperm trajectories vary according to the sperm number and total motility. This is logical if it is assumed that the motility of a single spermatozoon depends on the different interactions established with other sperm cells [14]. These factors strongly affect both the percentages and the motility characteristics of the motile subpopulations of donkey ejaculates.

Similar motile sperm subpopulation structures have been recorded in the ejaculates of phylogenetically distinct mammals such as the common marmoset, gazelle, horse or pig [7,1,2,14,15]. The existence of three or four subpopulations may therefore be widespread

in mammals. In the boar, it is reasonable to assume that variation in sperm morphology originates during spermatogenesis, when genotype effects influence sperm structure [24]. Differences in sperm morphology are also found in the majority of mammalian species, and it is logical to assume that these could lead to spermatozoa with different motility patterns. Nevertheless, more studies are required involving more mammalian species, and in vitro fertility assays will be necessary to fully comprehend the physiological role of this subpopulation structure.

The production of L-lactate would seem to be a good indicator semen quality in the Catalonian donkey since this was the most important variable in PCA analysis. L-lactate production was also strongly correlated with other important variables such as sperm count, HOST, VAP and LIN. Rigau et al. [20] obtained similar results for boar ejaculates, and suggest L-lactate production should be examined in the analysis of fresh boar semen since it indicates the metabolic status of the spermatozoa more clearly than other variables such as viability, altered acrosomes, ORT or motility. With respect to boar semen, it has been described that percentage sperm viability, followed by ORT and HRT, can predict the conception rate [15]. In the present study, the donkey-sperm viability according to HOST was very low and decreased from 70 to 24%, although results were very variable. This shows that donkey spermatozoa are more sensitive to a hypoosmotic medium than are stallion spermatozoa. In fact, stallion spermatozoa are also sensitive when incubated in a hypoosmotic medium since viability decreases from 70 to 38%, but the results are less variable than those seen for donkey sperm [6].

A proper analysis of donkey semen quality requires two types of laboratory test, one that evaluates membrane integrity, such as HOST, HRT-test and sperm viability, and one that reflects the metabolic function of the spermatozoa, such as the production of L-lactate and the motility pattern. Including the sperm count and percentage of morphological abnormalities would add further strength to any diagnosis. The production of L-lactate by donkey spermatozoa is low in comparison to boar spermatozoa. In general, L-lactate production is better than the motility pattern for evaluating metabolic performance since this is less affected by external agents such as temperature, and since poor motility is not strictly associated with a poor metabolic performance. This indicates that L-lactate production is a good indicator of sperm metabolism status [20].

In conclusion, Catalonian donkey ejaculates show a characteristic motile sperm subpopulation structure similar to that of other mammalian species. The relationship between the distribution of the sperm subpopulations and total motility and sperm sperm count shows that the spermatozoa of each have different motility patterns. Subpopulation distribution may also vary according to the donkey sampled. L-lactate production should be included in the quality analysis of donkey semen since it indicates the metabolic status of the spermatozoa, and thus indirectly predicts its fertilizing potential.

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