

COMPARATIVE STUDY ON SPERM MOTILITY IN TWO ENDANGERED CATALAN RAM BREEDS

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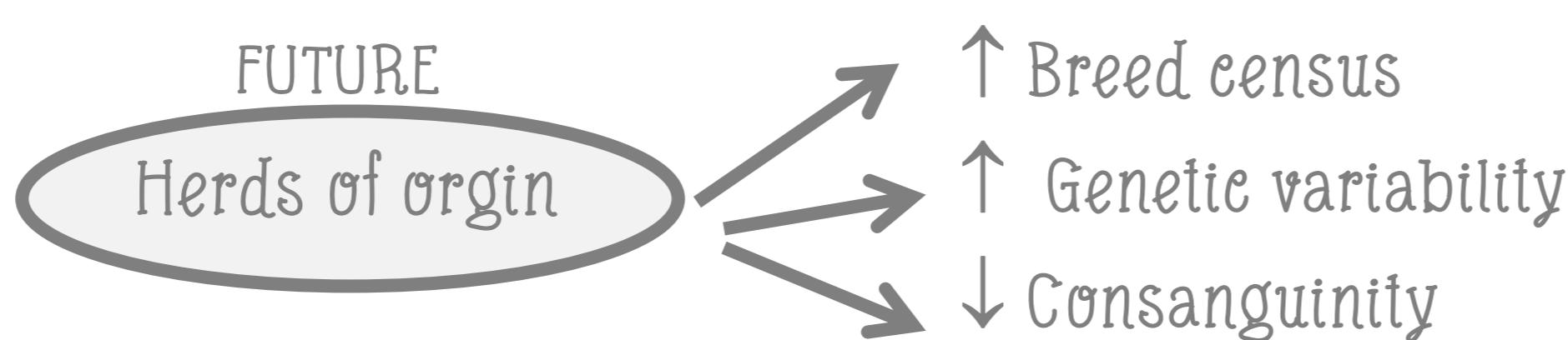
CATALUNYA	
Aranesa	Xisqueta
2.188♀ 103♂	16.649♀ 332♂

INTRODUCTION

INDIGENOUS BREEDS

- Environmental importance
- Socio-economic importance
- Genetic importance
- Cultural heritage

PROJECT RZ2009-00008-00-00 Institut Nacional d'Investigacions Agràries i Alimentàries (INIA): "Creation of a sperm bank for Catalan endangered breeds"



XISQUETA: 4 ♂, 3 years old
Num: 70, 71, 74 and 76



ARANESA: 5 ♂, 3 years old
Num: 78, 79, 80, 81 and 82



SAMPLING:

- Artificial vagina
- 2 months autumn
- 2 extractions/once or twice week



MATERIALS AND METHODS

REFRIGERATION:

- Washing
- Cryopreservaaition dilution: TGC + 5% glicerol + 15% powdered egg yolk

5°C 4h



SPERM EVALUATION:

- Sperm motility analisis (CASA)
- Sperm tracks cleaning

STATISTICAL ANALYSIS:

1. ANOVA + t-Student
2. Principal components + clustering
3. ANOVA + t-Student

JMP® Pro 12.0.1

TABLE 1. Seminal parameters measured in refrigerated ejaculates from Aranesa and Xisqueta males.

♂	XISQUETA	ARANESA
MT (%)	38,1 ± 23,4	43,9 ± 28,4
MP (%)	24,2 ± 17,7	24,7 ± 16,5
VCL (μm/s)	100,0 ± 22,1	95,6 ± 22,6
VSL (μm/s)	63,7 ± 20,4	56,5 ± 18,3
VAP (μm/s)	77,6 ± 22,3	70,6 ± 19,5
LIN (%)	63,1 ± 11,9	59,3 ± 14,4
STR (%)	81,5 ± 6,4	79,2 ± 7,8
WOB (%)	77,0 ± 10,2	74,0 ± 12,5
ALH (μm)	3,0 ± 0,7	3,0 ± 1,0
BCF (Hz)	9,5 ± 1,9	9,4 ± 2,5
N	3.044	4.454

Mean ± Standard Deviation (SD)

Significant differences ($p<0,05$) not found

TABLE 2. Result of Principal components analysis

	PRIN1	PRIN2	PRIN3
Eigenvalues	3,933	2,26	1,015
Variance explained	49,159	28,258	12,686
Eigenvectors			
VCL	0,339	0,482	-0,084
VSL	0,486	0,082	-0,087
VAP	0,446	0,231	-0,231
LIN	0,412	-0,367	0,036
STR	0,337	-0,293	0,345
WOB	0,375	-0,318	-0,234
ALH	0,073	0,608	0,053
BCF	0,146	0,125	0,868

MT = total motility, MP = progressive motility, VCL = curvilinear velocity, VSL = straight velocity, VAP = average velocity, STR = straightness, LIN = linearity, WOB = wobble, ALH = amplitude of lateral head, BCF = beat cross frequency, N = number of spermatozoa.

RESULTS AND DISCUSSION

TABLE 3. Mean values ($\pm SD$) of kinematic parameters defining the four subpopulations identified in refrigerated Xisqueta and Aranesa ram semen.

	Subpopulation 1	Subpopulation 2	Subpopulation 3	Subpopulation 4
VCL (μm/s)	81,79 ± 41,52 ^a	133,72 ± 24,70 ^b	68,48 ± 20,03 ^c	111,69 ± 37,48 ^d
VSL (μm/s)	28,29 ± 19,65 ^a	81,2 ± 21,93 ^b	48,6 ± 18,47 ^c	93,12 ± 33,65 ^d
VAP (μm/s)	50,13 ± 30,78 ^a	96,69 ± 22,30 ^b	54,29 ± 19,42 ^c	105,12 ± 36,95 ^d
LIN (%)	35,36 ± 17,59 ^a	60,89 ± 12,37 ^b	70,33 ± 15,11 ^c	83,14 ± 8,36 ^d
STR (%)	57,42 ± 22,17 ^a	84,01 ± 10,89 ^b	88,97 ± 8,18 ^c	88,74 ± 7,43 ^c
WOB (%)	60,49 ± 18,32 ^a	72,41 ± 10,69 ^b	78,61 ± 13,81 ^c	93,45 ± 5,25 ^d
ALH (μm)	3,32 ± 1,66 ^a	4,53 ± 1,03 ^b	2,15 ± 0,66 ^c	2,43 ± 0,95 ^d
BCF (Hz)	7,27 ± 3,00 ^a	12,19 ± 2,70 ^b	11,57 ± 2,52 ^c	7,54 ± 2,32 ^d
N	901	768	576	692
Total average	30,68	26,15	19,61	23,56

Different superscript letters (a-d) in a row indicate significant differences among subpopulations ($p<0,05$)
Mean (%)

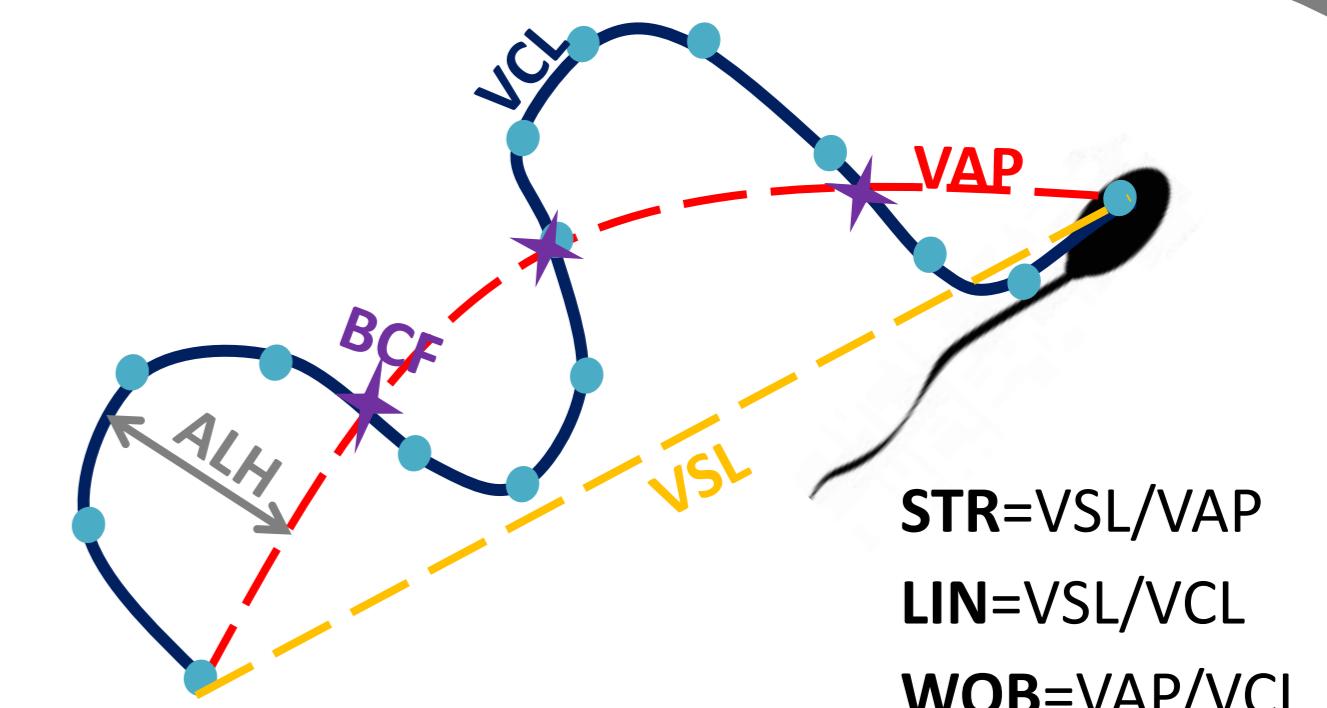


FIGURE 1. Schematic representation of different motility parameters evaluated by computer-assisted sperm analysis (CASA).

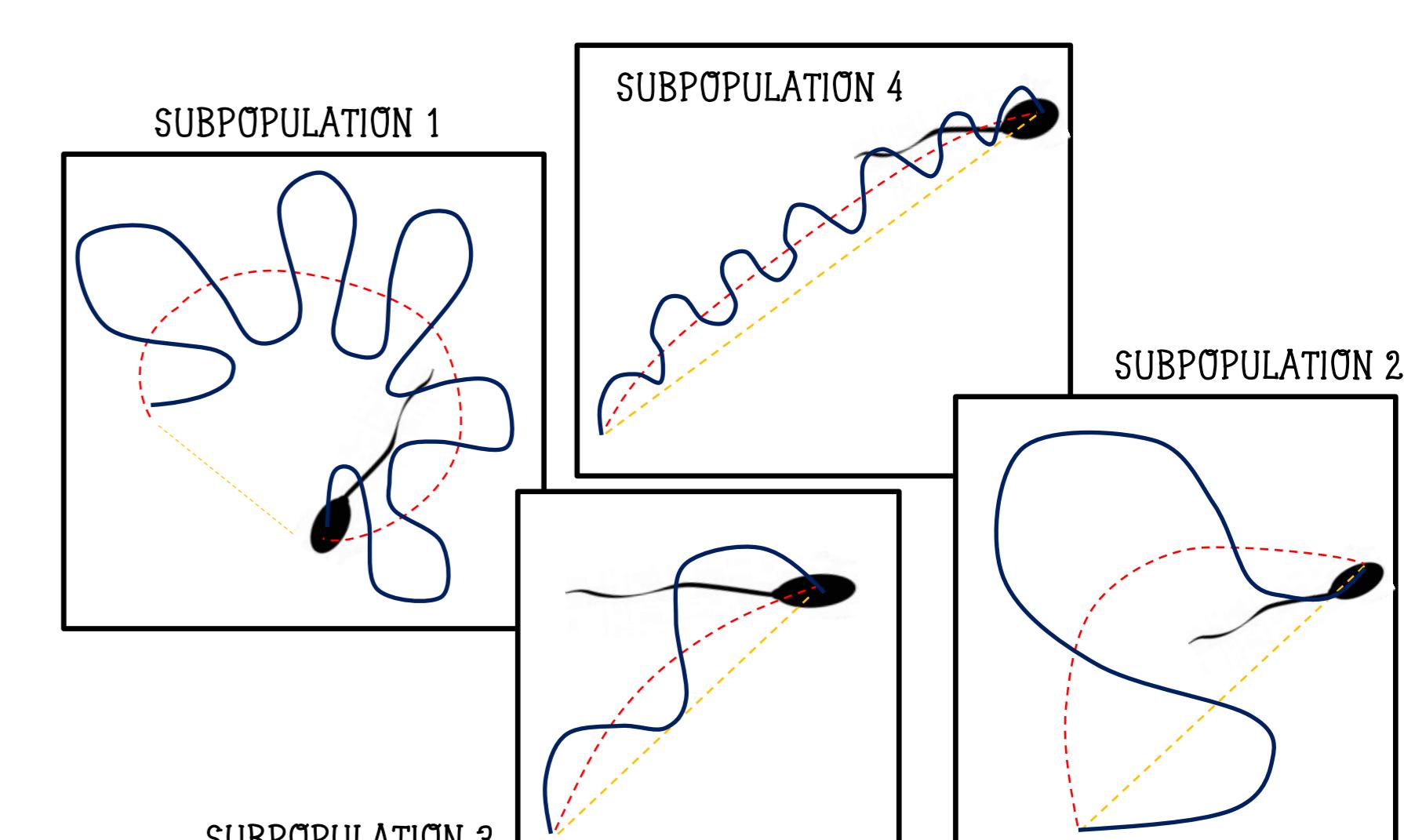


FIGURE 2. Schematic representation of different subpopulation tracks using mean values of kinematic parameters.

CONCLUSIONS

- No significant differences has been found between kinetic parameters and neither males nor breeds.
- 4 motile subpopulations has been defined.
- No significant differences has been found between subpopulations and neither males nor breeds.
- Subpopulation analysis shows deeper information than average comparison. It is a powerful tool that has to be developed.

REFERENCES

- Bravo JA, Montaner J, Galera R, Roy TJ. 2011. Identification of sperm subpopulations with defined motility characteristics in ejaculates from Ile de France rams. Anim. Reprod. Sci. 129:22-29.
 Dorado J, Molina I, Muñoz-Serrano A, Hidalgo M. 2010. Identification of sperm subpopulations with defined motility characteristics in ejaculates from Florida goats. Theriogenology 74:795-804.
 Garcia-Vera WC. 2014. Optimización de los protocolos de criopreservación de semen ovino de las razas autóctonas en peligro de extinción xisqueta y aranesa.
 Martínez-Pastor F, Tizaldo EJ, Garde JJ, Anel L, de Paz P. 2011. Statistical Series : Opportunities and challenges of sperm motility subpopulation analysis. Theriogenology 76:783-795.