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Preliminary results on the antioxidant capacity of the *Coffea arabica* grounds extract on semen parameters of Fleckvieh cattle in the Amazonas region

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Electronic Supplementary Material (ESM)

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Table S1. Evaluation of the antioxidant activity of the extracts

Essay	Procedure
Radical scavenging assay using DPPH	The DPPH free radical scavenging assay was used to determine the antioxidant activity. A DPPH solution was prepared, and its absorbance was measured using a UV-VIS spectrophotometer (PEAK T9200, USA) at a wavelength of 516 nm before and after adding the sample extract. The results were expressed as milligrams of Trolox equivalent per-100 mg of lyophilised coffee grounds.
FRAP assay	The antioxidant activity of coffee grounds was evaluated using the DPPH free radical scavenging assay. The absorbance was measured at a wavelength of 593 nm before and after adding the sample extract. The results were expressed as milligrams of Trolox equivalent per 100 mg of lyophilized coffee grounds. Additionally, a calibration curve with ferrous sulfate was used to express the results in milligrams of gallic acid equivalents per 100 mg of lyophilized coffee grounds.
Total phenols	The phenolic compounds in the coffee grounds were determined using the Folin-Ciocalteu colorimetric assay. The sample extract was added to test tubes along with the Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3), and the mixture was incubated at 50 °C for 5 minutes. Then, a UV-Visible spectrophotometer was used to quantify the phenolic compounds at a wavelength of 765 nm. The results were expressed as milligrams of gallic acid equivalents per 100 mg of lyophilized coffee grounds.

DPPH = 2.2-diphenyl-1-picrylhydrazyl; FRAP = ferric reducing antioxidant power

Table S2. Kinematic and morphological definitions of sperm and definitions

Variables	Unit	Definitions
Description of Spermatozoa		
Total motility (TM)	%	percentage of total motile spermatozoa
Progressive motility (PM)	%	percentage of total progressively motile spermatozoa
Curvilinear velocity (VCL)	μm/s	velocity of spermatozoa along their actual path per unit of time
Average path velocity (VAP)	μm/s	average velocity of spermatozoa along their trajectory
Straight-line velocity (VSL)	μm/s	average velocity of spermatozoa along a straight line from their first to last position
Straightness (STR)	%	ratio of VSL to VAP (× 100)
Linearity (LIN)	%	relación entre VSL y VCL (× 100)
Wobble (WOB)	%	ratio of VAP to VCL (× 100)
Amplitude of lateral head displacement (ALH)	μm/s	standard deviation of extreme lateral head movement of spermatozoa during each beat cycle beat cross
Beat cross frequency (BCF)	Hz	frequency of tail beat crossings based on VCL crossing VAP per second
Sperm integrity		
Membrane functionality (MF)	%	percentage of spermatozoa with intact membrane
Acrosome integrity (AI)	%	percentage of spermatozoa with intact acrosome

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Figure S1. Zootechnical genealogical record of genealogical register (No. 1070)
JDE WALDBRAND REPTEIT HANS TE

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LABORATORIO DE ENFERMEDADES INFECCIOSAS Y PARASITARIAS DE ANIMALES DOMÉSTICOS
INSTITUTO DE INVESTIGACIÓN EN GANADERÍA Y BIOTECNOLOGÍA

CERTIFICADO DE PRUEBA DE DIAGNOSTICO 00034

DATOS GENERALES

PROPIETARIO	Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas
PROVINCIA	Chachapoyas
FECHA DE RECEPCIÓN DE LA MUESTRA	18 de julio del 2023
FECHA DE ANÁLISIS DE LA MUESTRA	20 de julio del 2023

DATOS DE LA MUESTRA

DATOS DEL ANIMAL					ANALISIS: ENFERMEDAD / PRUEBA						
N°	IDENTIFICACIÓN DEL ANIMAL	ESPECIE	SEXO	RAZA	BRUCELOSIS BOVINA	LEUCOSIS BOVINA ENZOOTICA	LENGUA AZUL	IBR	DVB	PARATUBER-CULOSIS	NEOSPOROSIS
					ELISA COMPETITIVA	ELISA	ELISA	ELISA	ELISA	ELISA INDIRECTA	ELISA COMPETITIVA
1	HANS - RG N° 1070	Bovino	M	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
2	ZAPFHAHN - RG N° 1591	Bovino	M	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
3	RADAMEL - RG N° 1585	Bovino	M	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
4	NEVADO - RG N° 1071	Bovino	M	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
5	EROS - RG N° 90109	Bovino	M	Angus N	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
6	BOLT - RG N° 14653	Bovino	M	Brown swiss	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
7	GIANLUCA	Bovino	M	Brahman	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
8	LUCI - I	Bovino	H	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
9	LUCI - II	Bovino	H	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
10	JUANITA	Bovino	H	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
11	MATIAZA	Bovino	H	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO
12	BRENDA	Bovino	H	Simmental	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO	NEGATIVO

MEDICO VETERINARIO RESPONSABLE DE TOMA DE MUESTRA:

Jhordy Rojas Tafur
MÉDICO VETERINARIO
CMVP 13359

MEDICO VETERINARIO RESPONSABLE DE LABORATORIO

Figure S2. Health record of genealogical register (No. 1070)
JDE WALDBRAND REPTEIT HANS TE

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SCA EVOLUTION

Referencia: NATIVO45 **Código:** RG 1070

Fecha (día/mes/año): 5/03/2024
Centro: Laboratory

Animal: HANS

Fresco (05/03/2024 10:47)

Concentración

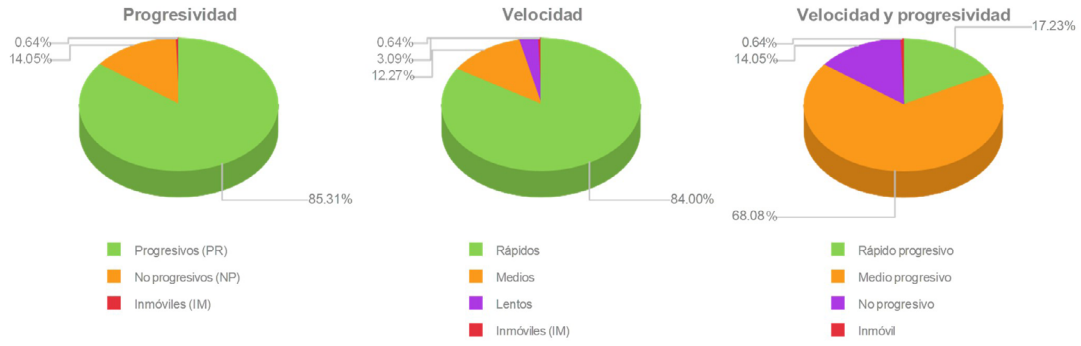
188.69 M/mL	471.73 M/muestra	Volumen (mL): 2.50
(≥15M/mL)	*(≥39M/muestra)*	Dilución 1:0

Progresividad		Total	%	M/mL	M/muestra
Progresivos (PR)	*(≥32%)*	2788	85.31	160.98	402.44
No progresivos (NP)		459	14.05	26.50	66.26
Inmóviles (IM)		21	0.64	1.21	3.03

		Total	%	M/mL	M/muestra
Móviles	*(≥40%)*	3247	99.36	187.48	468.69

Velocidad	Total	%	M/mL	M/muestra
Rápidos	2,745	84.00	158.49	396.23
Medios	401	12.27	23.15	57.88
Lentos	101	3.09	5.83	14.58
Inmóviles (IM)	21	0.64	1.21	3.03

Velocidad y progresividad	Total	%	M/mL	M/muestra
Rápido progresivo	563	17.23	32.51	81.27
Medio progresivo	2,225	68.08	128.47	321.17
No progresivo	459	14.05	26.50	66.26
Inmóvil	21	0.64	1.21	3.03



	Media	Inmóviles (IM)	Lentos	Medios	Rápidos	Unidades
Área de la cabeza	39.69	23.29	30.87	41.89	38.77	μm^2

	Concentración	
Células redondas	0.41	M/mL

	Total	%
Recorridos circulares	2,961	90.61 %

Figure S3. Sperm analysis evaluation using sca evolution – sperm class analyzer of genealogical register (No. 1070) JDE WALDBRAND REPTEIT HANS TE

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Referencia: NATIVO45 Código: RG 1070 Animal: HANS Fecha (día/mes/año): 5/03/2024
Centro: Laboratory

Media de velocidad	Móviles	No progresivo	Medio progresivo	Rápido progresiv	Unidades
Velocidad curvilínea (VCL)	112.37	34.09	119.69	147.27	µm/s
Velocidad media (VAP)	60.05	17.60	61.95	87.17	µm/s
Velocidad lineal (VSL)	30.84	6.55	25.63	71.25	µm/s
Indice de rectitud (STR)	46.50	35.24	40.03	81.23	%
Índice de linealidad (LIN)	25.90	19.56	21.21	49.64	%
Indice de oscilación	53.30	51.99	51.66	60.84	%

Media de otros parámetros	Móviles	Medio progresivo	Rápido progresivo	Unidades
Amplitud lateral de la cabeza (ALH)	3.02	3.25	3.54	µm
Frecuencia de batida (BCF)	10.02	10.29	13.94	Hz

	Total	% (Móviles)	% (Total)	M/mL	M/muestra
Hiperactivados	721	22.21	22.06	41.63	104.07
Penetración de la mucosidad	291	8.96	8.90	16.80	42.00
Penetración de la mucosidad	291	8.96	8.90	16.80	42.00

Analista: Administrator
Comentarios:

Figure S4. Results obtained from the sperm evaluation of JDE WALDBRANT REPTEIT HANS TE – N°1070

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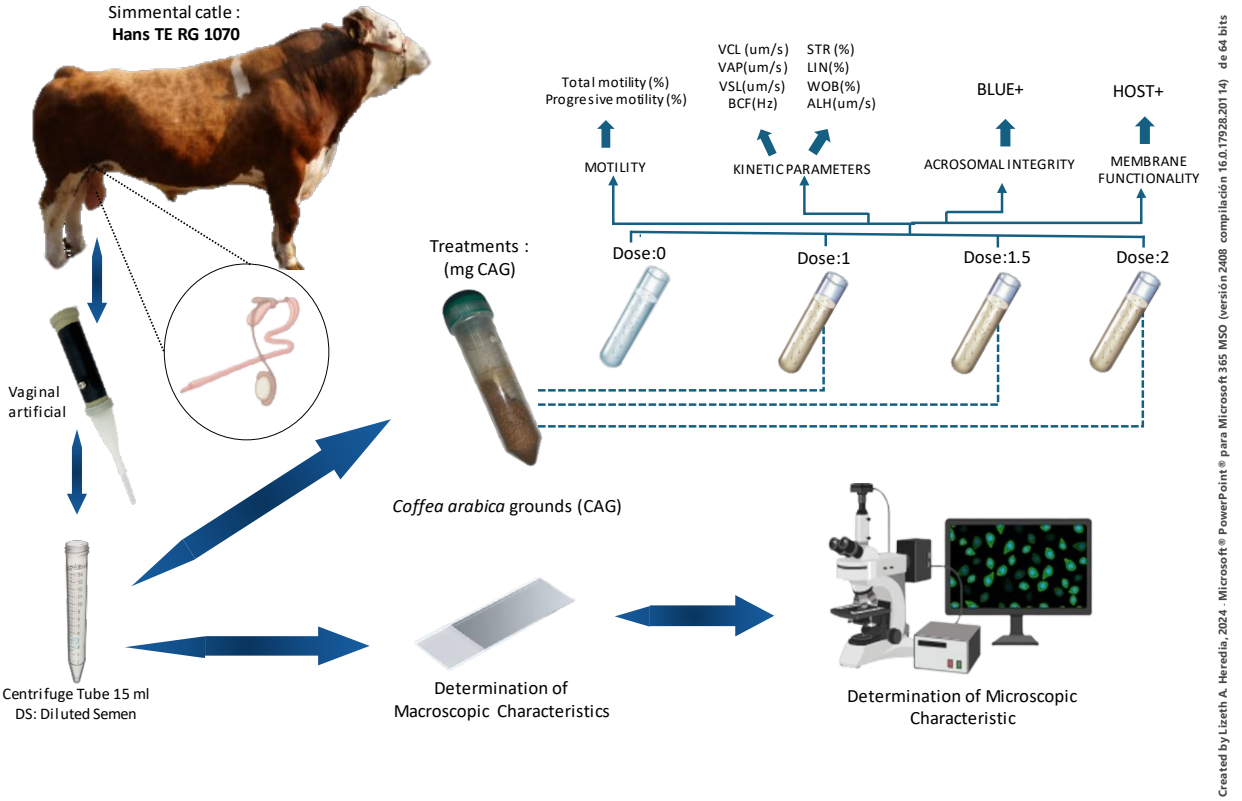

Figure S5. Graphical abstract