National Rural Development Programme 2014-2022
Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)
REPORT ON THE ACTIVITIES PERFORMED DURING THE SECOND YEAR

PA UniMI

The report describes the activities performed from 01.05.2022 to 30.04.2023. The activities are described by Action, according to the original programme.

Action 1 – Phenotypical characterization of autochtonous breeds and species

Task 1.1 – Phenotipic characterization of Mericanel della Brianza (MB) chickens
Rearing activities related to the Mericanel della Brianza nucleus population continued during the second year of the project. Chickens hatched in March-April 2022 (n=138) were reared in order to select young breeders. Chickens were marked and weighted on hatch and reared in floor pens with controlled environment up to 225 days of age. The growth curve of both sexes is reported in Figure 1.1. All males (n=61) were sampled (feathers) and genotyped (external service UniTO) in order to set the mating plan according to molecular kinship and individual heterozygosis index. Selected breeders were organized in 4 familiar lines (A, BC, DEF, G), with the loss of 3 lines compared to the previous year. Each familiar line included 1 male and 10-12 females, and was reared in a floor pen with controlled environment. Sexual maturity occurred at 24-26 weeks of age (September-October 2022).
A small population of MB birds (20 males + 20 females) was introduced on November 2022 at the Poultry Center to identify new familiar lines. A quarantine period was observed to run sanitary tests and assess morphological traits; 26 birds were selected for genotyping. The results of genetic analysis provided 2 new familiar lines (5, 6) and a mating plan for all lines, including both males hatched at the Center and new ones.
Composition and characteristics of familiar lines in reproduction from February to May 2023 are reported in Table 1.1. Egg production and weight was recorded from September 2022 to May 2023. Weekly oviposition rate (%) is shown in Figure 1.2. Overall mean egg weight was 38,5 g (SD=3,64 g), and the range recorded in the period October 2022-May 2023 was 31,4 - 34,5 g.
So far, eggs have been set twice in order to select young breeders. Fertility and hatchability are reported in Table 1.2. Large variability was observed in fertility and hatchability according to the familiar line (Table 1.3).

Task 1.2 Phenotipic characterization of Modenese (MO) chickens
Thirty days old MO chickens (n=10) were housed at the Poultry Center (UniMI, Lodi) on July 2022. A quarantine period was observed to run sanitary tests. Body weight was recorded in 8 birds, 2 females and 6 males, and the growth curve is reported in Figure 1.3. Consistent reproductive data were not collected, since only one couple was available for reproduction. Further female Modenese hens have to be introduced to organize several familiar goups, as it is required for the phenotypical characterization of the breed.

Task 1.3 Phenotipic characterization of Nero d'Italia (NI) and Brianzolo (Br) turkeys
BR breed - Eleven week old BR turkeys (n=10) were housed at the Poultry Center on July 2022. A quarantine period was observed to run sanitary tests and 6 turkeys (3 males + 3 females) were kept for the reproductive period. Turkeys were organized in couples and reared in floor pens with controlled environment. Body weight was recorded at 14, 30 and 47 weeks of age; mean body weight of males and females is shown in Table 1.4. Sexual maturity in female turkeys occurred on 27 weeks af age; the weekly oviposition rate (%) recorded from October 2022 to May 2023 is shown in Figure 1.4. Overall mean egg weight was 69 g (SD=5,9 g), egg weight progressively increased from 64 g to 76 g in the period November 2022 - May 2023. Egg settings have been planned in May and June to hacth the progeny for young breeder selection.

NI breed – The search for NI turkeys has continued all the second year long; young turkeys are planned to be housed at the Poultry Center on next July 2023.

Task 1.4 Phenotipic charcaterization of semen in chicken and turkey breeds

Laboratory analyses were performed to assess the proteomic and lipid profiles of sperm and seminal plasma (previously stored samples) in the following breeds: Bionda piemontese (BP), Bianca di Saluzzo (BS), Pepoi (Pe) and Robusta maculata (RM). Furthermore, semen production and quality were recorded in Ermellinata di Rovigo (ER) and Robusta lionata (RL) chicken breed.

Proteome - Sperm and seminal plasma samples were analysed by mass-spectrometry using a shot-gun proteomic approach. Protein mixture was extracted from the samples, prepared, digested with trypsin and then analysed using nano-LC-MS/MS spectrometry (Orbitrap Fusion Tribrid, Thermo Fisher Scientific). Raw data were analysed using MaxQuant software (versione 1.6.1.0) for identification and quantification of proteins in each dataset.

The preliminary analysis allowed to identify datasets (4 for each breed) that underwent differential expression analysis (Table 1.5, Table 1.6) aimed to identify shared and exclusive proteins of the different chicken breeds (Figure 1.5, Figure 1.6). The same proteins will be used in the next functional informatic analysis.

Lipids – Total lipids were extracted according to the Folch method and separated with TLC procedure in the following classes: total phospholipids, triacylglycerols, free fatty acids, cholesteryl ester. Esterified lipid classes were transmethylated by refluxing and the fatty acid composition determined by GC with FID detector on capillary column. Free cholesterol was quantified using a commercial kit. Analysis of raw data and statistical analyses are in progress.

Semen production and quality – Roosters of ER (n=20) and RL (n=20) breed were housed in single cages with controlled environment at the Poultry Center. Birds were trained to semen collection, then routinely milked twice weekly from February to May 2023. Mean values of semen quality parameters are reported for each breed in Table 1.7. After centrifugation, sperm and seminal plasma aliquots were stored at -20°C for further proteomic and lipid analyses.

Action 2 - Genetic characterisation of Italian autochthonous breeds and species using also genomic tools

Task 2.1 - Genetic characterisation using microsatellite markers

See UniTO report.

Task 2.3 Sequencing of genome in Meleagris gallopavo species

The sample for genomic analyses included the following breeds (n. birds): Castano precoce (8), Bronzato comune (7), Ermellinato di Rovigo (7), Romagnolo (9), Tacchino di PR&PC (6). DNA samples were extracted and sent to Novogene lab (Novogene, Company Limited, UK) after quality control for sequencing and bioinformatic analyses. Data were provided on May 2023 and statistical analyses are in progress.

Action 4 - Estimation of genetic and genomic indices and reproductive management

Task 4.1 - Selection of males for reproduction and production of semen

See UniTO report.

Task 4.2 - Reproductive management

See UniTO report.

Action 8 – Collection of biological samples and germplasm

Task 8.2 Collection of germplasm for the Semen Cryobank

The Italian semen cryobank of autochthonous chicken and turkey breeds was implemented and semen collected from the breeds described in Action 1 task 1.4 was stored in liquid nitrogen. Semen doses were prepared

according to the cryopreservation procedure for chicken semen described in the Cryobank SOP. Seven male semen donors were selected according to semen production (both quantity and quality variables) in both ER and RL. The preparation and storage of semen doses is still running and will end in June. At present, 158 and 35 semen doses have been stored for ER and RL breed respectively.

Action 10 – Communication and dissemination activities

Several activities were developed and briefly described:

- Breed technical forms, *Gallus gallus* species, were planned, produced and published online. The form structure was planned in accordance with all PA, who provided the data to fill in. Afterwards, genetic data were also included in the form, in agreement with the results on nucleus populations provided by UniTO. The forms were published online in www.pollitaliani.it and a print version is available as *booklet* pdf.
- The web site www.pollitaliani.it was regularly updated. Major changes were in: publications, events, results and annual meetings.
- Two flyers on project activities and breeds were prepared and printed. Flyers have been provided to all PA and to the consultant UniTO and ANCI. Flyers will be distributed to the audience in public events occurring at local and national level.
- Project results were discussed in scientific manuscripts published in open access journals and presented in national and international congresses. All scientific publications were reported in the project web site pollitaliani.it.

FIGURES AND TABLES

Figure 1.1 – Growth curve recorded from 1 to 32 weeks of age in Mericanel della Brianza chickens (F=females; M=males)

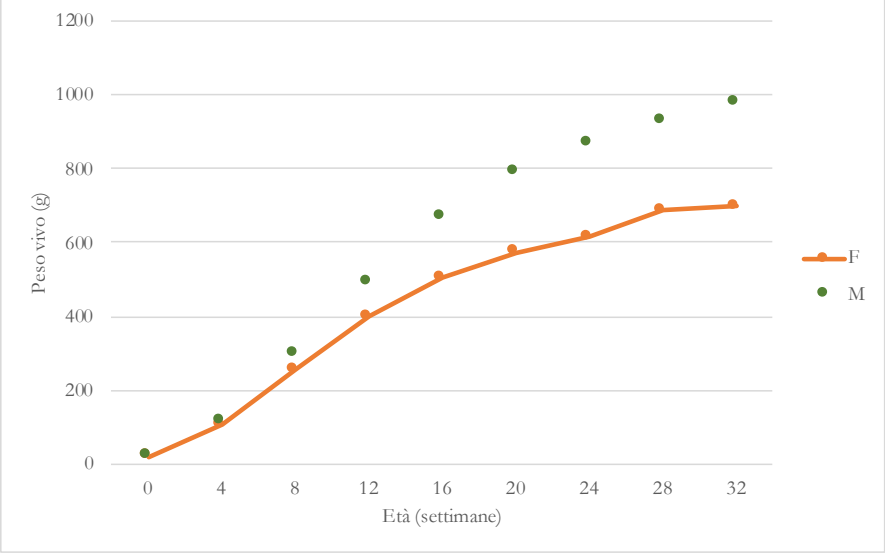

Table 1.1 – Number and body weight of breeders, and molecular kinship (IPM) in familiar lines of Mericanel della Brianza nucleus population

Familiar line	N. birds		Body weight (g)		IPM
	male	females	male	females	
A*	1	10	1040	655	0,48
BC*	1	11	910	645	0,45
DEF*	1	12	950	641	0,60
G*	1	12	905	784	0,58
5**	1	8	850	829	0,57
6**	1	7	800	727	0,58

* internal familiar line; ** new familiar lines

Table 1.2 – Incubation parameters recorded on April-May 2023 in the Mericanel della Brianza breeders

Hatch	Fertile eggs (%)	Dead embryos (%)	Hatched chicks (% on egg set)	Hatched chicks (% on fertile eggs)
1*	74.1	12,1	62,0	83,8
2**	85,0	10,0	75,0	88,2

* eggs stored for 14 days; ** eggs stored for 7 days

Figure 1.2 – Oviposition rate (%) recorded weekly from October 2022 to May 2023 in Mericanel della Brianza hens of nucleus and new lines

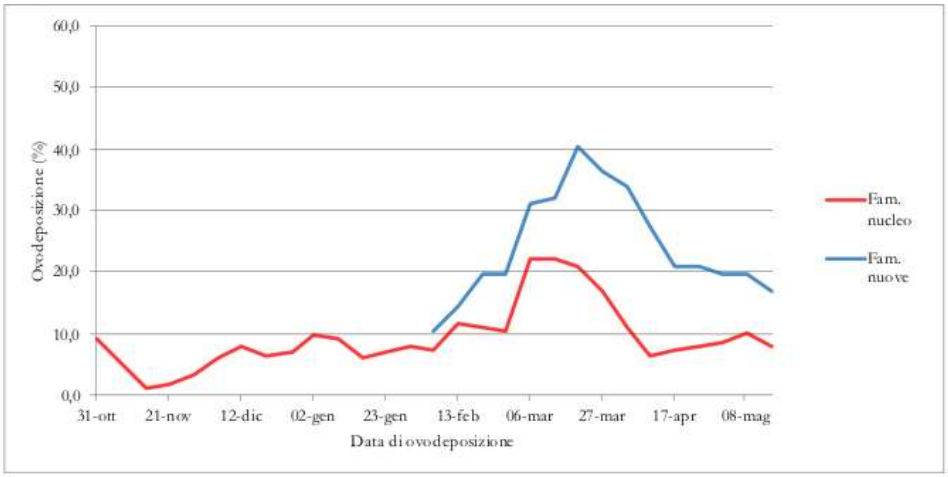

Table 1.3 – Incubation parameters of familiar lines recorded in Mericanel della Brianza breeders on April-May 2023

Familiar line	Fertility (%)	EM[1] (%)	Hatchability (% on egg set)	Hatchability (% on fertile eggs)
A	97	10	87	89
BC	86	14	72	84
DEF	61	6	56	91
G	43	7	36	83
5	64	13	51	80
6	79	13	66	83

[1] EM dead embryos

Figure 1.3 – Body weight recorded in male and female Modenese chickens from 8 to 46 weeks of age

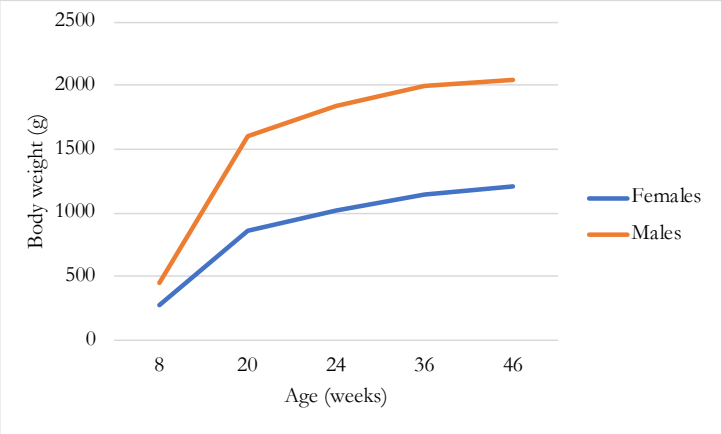

Table 1.4 – Body weight (mean±SD) recorded in males and females Brianzolo turkeys during the growing period

Age (weeks)	Males	Females
14	1779 ± 88	1379 ± 96
30	4357 ± 145	2713 ± 204
47	4527 ± 186	2658 ± 30

Figure 1.4 – Oviposition rate (%) of Brianzolo hens recorded weekly from October 2022 to May 2023

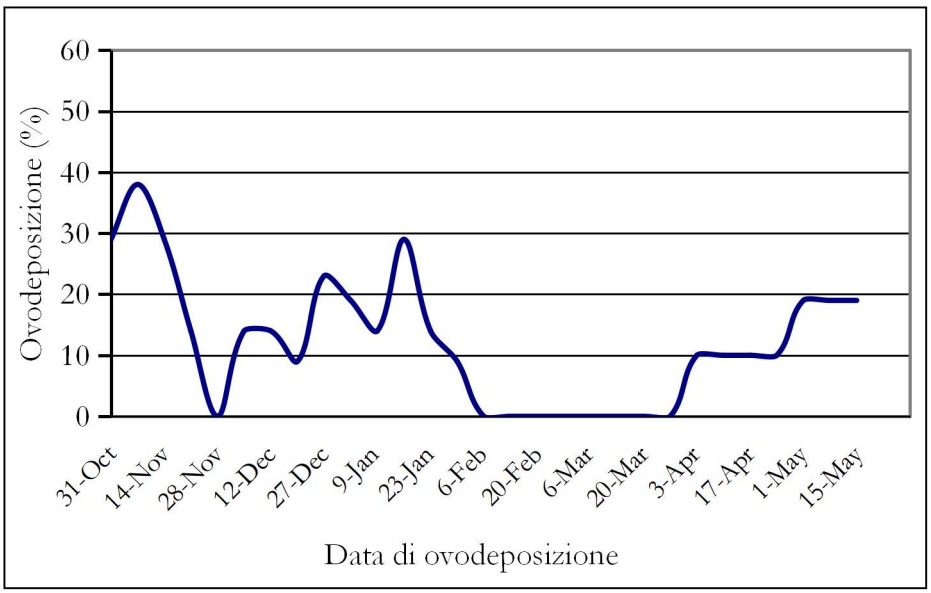

Table 1.5 – Number of proteins, proportion of MS/MS, value of LFQ signal related to dataset identified for differential analysis

	Cellule spermatiche			Plasma seminale		
	Numero di proteine identificate	% di MS/MS effettuate	Somma dei valori di intensità del segnale LFQ	Numero di proteine identificate	% di MS/MS effettuate	Somma dei valori di intensità del segnale LFQ
Mericanel della Brianza						
Dataset 1	997	14,88	22665	686	9,29	15642
Dataset 2	1020	13,04	23186	677	10,27	15307
Dataset 3	993	12,51	22602	689	10,37	15616
Dataset 4	1044	14,61	23643	633	8,79	14454
Bionda Piemontese						
Dataset 1	1000	11,72	22737	719	7,11	16103
Dataset 2	1009	11,21	23073	675	8,04	15274
Dataset 3	1006	10,88	22965	703	8,75	15818
Dataset 4	998	9,95	22854	688	6,61	15468
Bianca di Saluzzo						
Dataset 1	1035	10,98	23504	670	10,13	15170
Dataset 2	1038	11,00	23567	584	8,18	13430
Dataset 3	1086	13,74	24449	590	8,05	13454
Dataset 4	1039	16,07	23619	607	8,13	13875
Pepoi						
Dataset 1	955	11,55	21829	509	5.52	11719
Dataset 2	967	13,22	22064	543	4,37	12418
Dataset 3	977	15,31	22162	545	3,83	12509
Dataset 4	858	12,28	19815	571	4,26	12998
Robusta Maculata						
Dataset 1	858	12,25	19804	525	5,31	11937
Dataset 2	822	10,53	19023	613	6,77	13870
Dataset 3	927	15,47	21260	514	4,91	11736
Dataset 4	982	17,03	22332	543	5,23	12339

Table 1.6 –Pearson correlation coefficients between datasets identified for differential analysis

	Cellule spermatiche				Plasma seminale			
Mericanel della Brianza								
	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 1**	**Dataset 2**	**Dataset 3**	**Dataset 4**
Dataset 1	1	0.95	0.94	0.96	1	0.93	0.96	0.94
Dataset 2	0.95	1	1	0.96	0.93	1	0.98	0.96
Dataset 3	0.94	0.96	0.96	0.97	0.93	0.98	1	0.96
Dataset 4	0.96	0.96	0.97	1	0.94	0.96	0.96	1
Bionda Piemontese								
	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 1**	**Dataset 2**	**Dataset 3**	**Dataset 4**
Dataset 1	1	0.96	0.95	0.95	1	0.92	0.9	0.91
Dataset 2	0.96	1	0.98	0.97	0.92	1	0.94	0.95
Dataset 3	0.95	0.98	1	0.97	0.9	0.94	1	0.94
Dataset 4	0.95	0.97	0.97	1	0.91	0.95	0.94	1
Bianca di Saluzzo								
	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 1**	**Dataset 2**	**Dataset 3**	**Dataset 4**
Dataset 1	1	0.98	0.95	0.94	1	0.96	0.86	0.92
Dataset 2	0.98	1	0.93	0.94	0.96	1	0.84	0.91
Dataset 3	0.95	0.93	1	0.98	0.86	0.84	1	0.94
Dataset 4	0.94	0.94	0.98	1	0.92	0.91	0.94	1
Pepoi								
	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 1**	**Dataset 2**	**Dataset 3**	**Dataset 4**
Dataset 1	1	0.98	0.96	0.96	1	0.92	0.84	0.89
Dataset 2	0.98	1	0.97	0.96	0.92	1	0.85	0.89
Dataset 3	0.96	0.97	1	0.96	0.84	0.85	1	0.91
Dataset 4	0.96	0.96	0.96	1	0.89	0.89	0.91	1
Robusta Maculata								
	Dataset 1	**Dataset 2**	**Dataset 3**	**Dataset 4**	**Dataset 1**	**Dataset 2**	**Dataset 3**	**Dataset 4**
Dataset 1	1	0.97	0.97	0.96	1	0.9	0.92	0.93
Dataset 2	0.97	1	0.97	0.95	0.9	1	0.93	0.92
Dataset 3	0.97	0.97	1	0.97	0.92	0.93	1	0.95
Dataset 4	0.96	0.95	0.97	1	0.93	0.92	0.95	1

Figure 1.5 – Venn diagram on proteins identified in sperm and seminal plasma of Bionda piemontese (BP), Bianca di Saluzzo (BS), Mericanel della Brianza (MB), Pepoi (Pe), and Robusta maculata (RM) chicken breeds

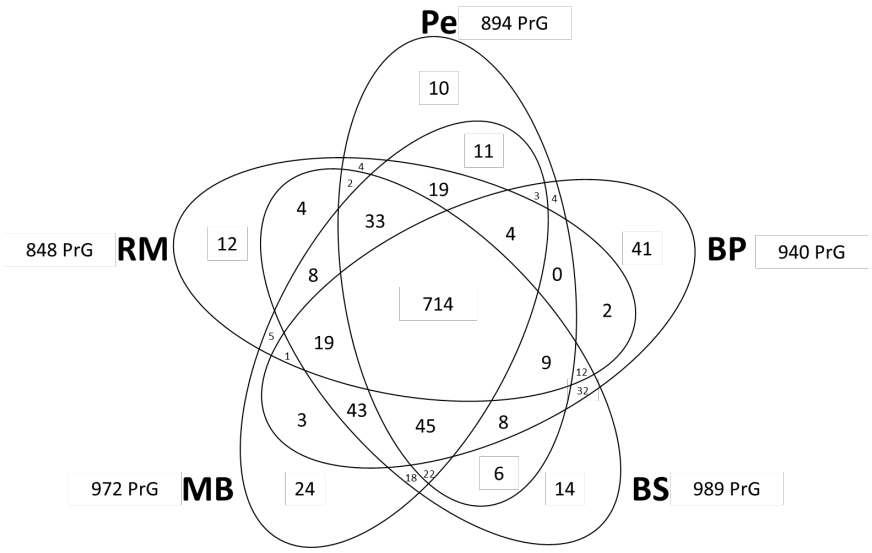

Figure 1.6 – Venn diagram on proteins identified in seminal plasma of Bionda piemontese (BP), Bianca di Saluzzo (BS), Mericanel della Brianza (MB), Pepoi (Pe), and Robusta maculata (RM) chicken breeds

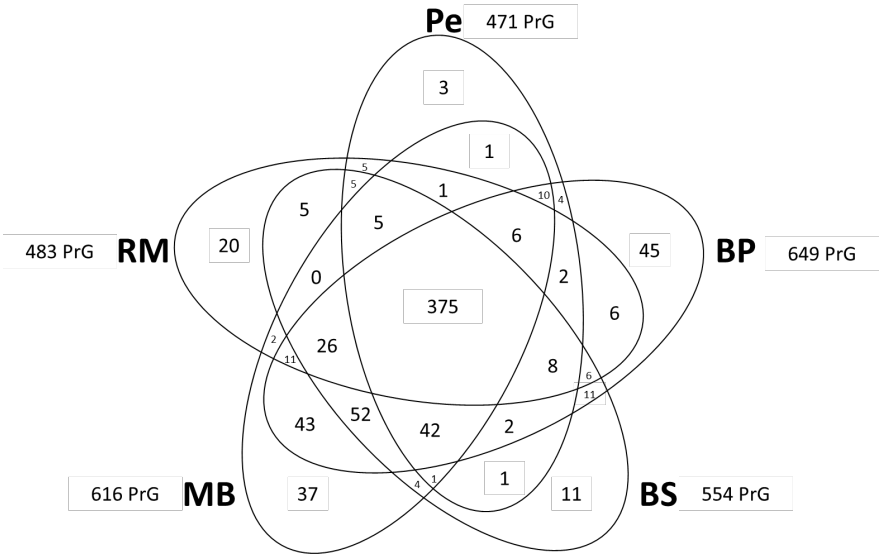

Table 1.7 – Quantitative and qualitative semen parameters in Ermellinata di Rovigo (ER) and Robusta lionata (RL) chicken breeds

Semen parameter*	Breed	
	ER	RL
Volume (mL)	0,248 ± 0,098	0,167 ± 0,073
Concentration (x10^9/mL)	1,35 ± 0,80	1,43 ± 0,39
Sperm/ejaculate (x10^9)	0,368 ± 0,309	0,255 ± 0,148
IM (%)	94,0 ± 3,20	93,0 ± 2,82
Motility (%)	97,2 ± 2,29	96,1 ± 4,35
MP (%)	38,3 ± 4,38	40,4 ± 8,27
VCL (μm/s)	103,1 ± 13,4	112,6 ± 14,5
VSL (μm/s)	43,9 ± 5,15	49,9 ± 8,49
VAP (μm/s)	68,4 ± 7,66	75,2 ± 9,46
LIN (%)	43,7 ± 4,47	45,3 ± 8,37
STR (%)	64,6 ± 3,67	66,5 ± 7,16
WOB (%)	67,0 ± 3,09	67,3 ± 5,69
ALH (μm)	4,18 ± 0,43	4,52 ± 0,52
BCF (Hz)	7,19 ± 0,52	7,54 ± 0,74

*IM: sperm membrane integrity, MP: progressive motility, VCL: curvilinear velocity, VSL: straight-line velocity, VAP: average path velocity, LIN: VSL/VCL, STR: VSL/VAP, WOB: VAP/VCL, ALH: amplitude lateral head displacement, BCF: beat cross frequency.