

# Semen freezability in Italian chicken breeds

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**AIM** - Assessment of the sensitivity to semen cryopreservation in the Italian chicken breeds *Mericanel della Brianza* (MB) and *Milanino* (MI).

**MATERIALS AND METHODS** - MB (n=27) and MI (n=19) cockerels were housed in outdoor pens and the semen was regularly collected twice a week from April to June. Soon after semen collection, few ejaculates were pooled into one semen sample, diluted to 1.5x10<sup>9</sup>/ml in pre-freezing Lake diluent, refrigerated at 5° C and transferred to the DiMeVet laboratory (University of Milan) for further cryopreservation processing.

In brief, semen samples were further diluted to 1x10<sup>9</sup>/ml in pre-freezing Lake diluent added with DMA (6% final dilution) and trehalose (0.1 M), incubated 1 min at 5° C, loaded into 0.25 mL French straws and frozen in nitrogen vapours. Thawing was performed in water bath at 38° C for 30 sec. Ejaculate volume and concentration were recorded. Sperm viability (SYBR14-PI staining), motility and motion parameters (SCA System) were assessed in pooled semen samples before and after cryopreservation. The recovery rate (%) of viable, motile, progressive motile sperm after cryopreservation were calculated (mean on thawed semen\*100/mean on fresh semen). Analysis of variance was performed to study the effect of the breed and cryoprocessing on semen quality.



**Mericanel della Brianza**



**Milanino**



**Sperm viability**

Table 1 – Sperm quality parameters (LSMeans) measured in fresh (FR) and criopreserved (CR) semen of Mericanel della Brianza (MB) and Milanino (MI) cockerels.

Sperm Parameters	MB breed		MI breed	
	FR	CR	FR	CR
Viability (%)	77.43 ± 4.33a	29.25 ± 3.06b	91.10 ± 2.17c	41.32 ± 1.50d
Motility (%)	51.20 ± 6.57a	15.33 ± 4.64b	85.07 ± 3.28c	30.38 ± 2.27d
PM* (%)	7.93 ± 3.13a	2.75 ± 2.71a	21.84 ± 1.64b	3.34 ± 1.08a
VCL (µm/s)	37.73 ± 5.62a	33.00 ± 3.97a	56.92 ± 2.81b	35.91 ± 1.94a

\* PM = Progressive Motility

Table 2 – Sperm kinetic parameters (LSMeans) measured in fresh (FR) and criopreserved (CR) semen in local breeds.

Kinetic Parameters	Semen sample	
	FR	CR
VSL (µm/s)	18.55 ± 1.56a	11.13 ± 1.10b
VAP (µm/s)	29.62 ± 2.21a	18.79 ± 1.55b
LIN (%)	38.87 ± 1.67a	31.27 ± 1.18b
STR (%)	62.29 ± 1.78a	57.59 ± 1.25b
WOB (%)	62.07 ± 1.34a	53.70 ± 0.95b
ALH (µm)	3.02 ± 0.23a	2.25 ± 0.16b
BCF (Hz)	7.48 ± 0.62	6.19 ± 0.44

**RESULTS and DISCUSSION** - Good semen production was obtained only from MI cockerels and the total pooled semen samples processed for cryopreservation were 12 and 3 in MI and MB breed respectively. The quality of fresh semen was higher in MI compared to MB males, and the same condition occurred after cryopreservation. Analysis of variance showed a significant effect of the breed, cryopreservation and the relative interaction on sperm viability, motility, progressive motility and VCL (Table 1). Sperm viability and motility were significantly different between breeds in both fresh and cryopreserved semen samples; in contrast, progressive motility and VCL did not change after freezing/thawing in MB semen and significantly decreased in MI semen (Table 1). All other sperm kinetic parameters were significantly affected only by cryopreservation, and the mean values recorded before and after freezing/thawing are reported in Table 2. The proportion of viable and motile sperm recovered after cryopreservation was similar in both breeds and, in contrast, the recovery of progressive motile sperm was significantly higher in ME semen (Table 3). It's interesting underline the large variation found in the recovery values calculated within the MI semen samples (n=12); in particular, the proportion of motile sperm recovered after cryopreservation ranged from 14 to 74%, and of progressive motile sperm from 1 to 74%. These results suggest great differences in semen sensitivity to cryopreservation between males. The quality of cryopreserved semen assessed in the Italian chicken breeds is comparable, or even higher, to the quality of semen collected from other local breeds and processed in similar conditions (Siudzinska and Lukaszewicz, 2008; Santiago-Moreno et al., 2012).

**CONCLUSION** - The present data are considered preliminary results and further studies are required to confirm the different sperm sensitivity to cryopreservation found between and within breed. The functional and biochemical characterization of semen samples with extreme (highest vs lowest) sensitivity to cryopreservation is of undeniable interest to identify biochemical markers and will be investigated.

Table 3 – Recovery of viable, motile and progressive motile sperm after cryopreservation in Mericanel della Brianza (MB) and Milanino (MI) semen.

Recovery Values (%)	Breed	
	MB	MI
Viability	38.42 ± 4.22a	45.60 ± 2.07a
Motility	32.87 ± 6.92a	37.23 ± 3.39a
PM*	51.27 ± 10.25a	18.16 ± 5.23b

\* PM = Progressive Motility

## REFERENCES

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