Assessment of post-thaw semen quality in two conserved Norwegian rooster lines

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Norway’s laying-hen gene bank

— Live animal gene bank established in 1973.

— Housed at Hvam Upper Secondary School, Nes, Akershus.

— Responsible for conservation of 12 laying hen lines/breeds
- Last five production lines bred in Norway.
- Only national breed Jærhøns.

— Since 2022, 336 enriched cages; each line/breed has 23-29 cages/families.

— Rotational breeding structure to minimize increase in inbreeding.

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Norway’s laying-hen gene bank

- Strategic plan for Norwegian gene bank (2018-2027):
    1. Secure genetic material from the only Norwegian chicken breed and 5 production lines.
    2. Contribute to the good health status of Norwegian poultry production, by selling live animals to hobby breeders.
    3. Deliver material to research.

- Strategic use of effective conservation methods, including ***cryopreservation***, documentation of the material, and facilitating value creation based on the preserved line:
    - Long-term conservation of genetic material.
    - Active use in breeding.

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Background and motivation

— Anticipatory preparedness: climate change, geopolitical instability, and emerging animal diseases → local-regional-national food security also in Nordic countries.

— Action to complement live-animal safeguarding
  - two semen collection and cryopreservation trials in 2016 and 2018:
    - Apparent good motility but low fertilization success (3-5% fertility rate).
    - Subjective motility assessment.

→ Pilot study on objective quality assessment of semen on Jærhøns and NorBrid8 breeding line.

Experiment and analyses

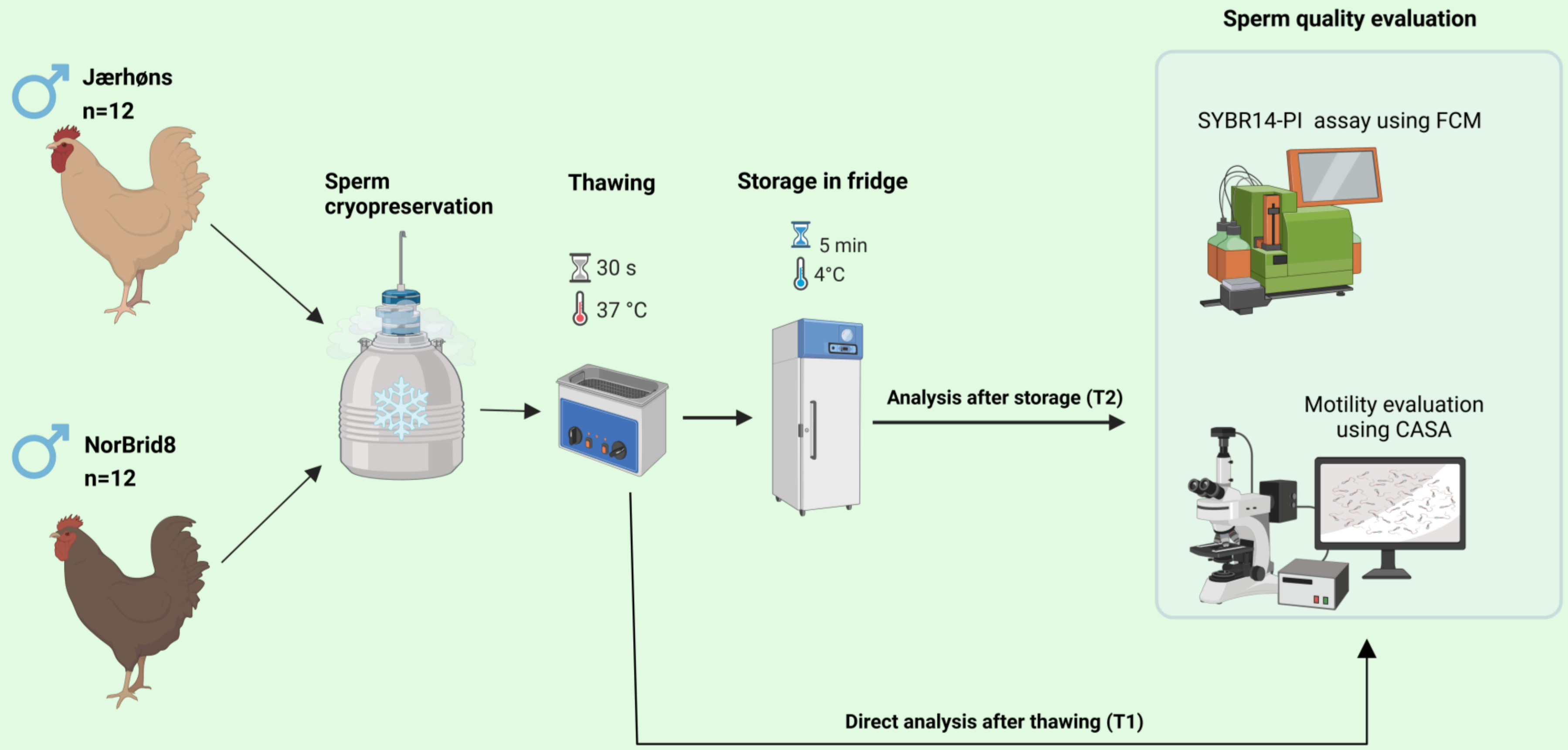

Illustration: Amin Sayyari

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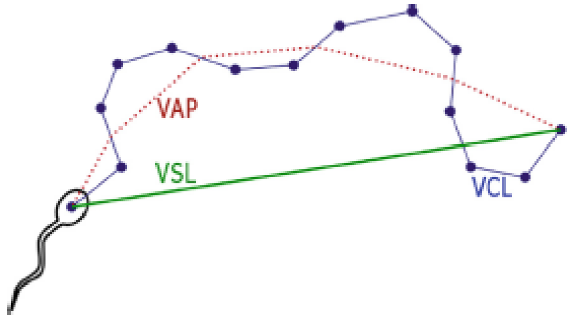
Traits and statistics

- CASA: total and progressive motility (MOT, PROG), velocity traits (VCL, VAP, VSL) and straightness (STR, LIN).



$STR = (VSL/VAP) * 100$

$LIN = (VSL/VCL) * 100$

App Development for Measuring Seminal Quality of Bovine Sperm *in situ*

Matías R. Castorina[1], Demian Noseda[1], Francisco J. Buchelly Imbachi[1], Virginia L. Ballarin[1], and Juan I. Pastore[1,2](✉)

- Flow cytometry: Viability%.
- Mixed-effects model, breed and treatment effects, random rooster intercept.
- Sensitivity analyses: retrospect statistical power simulations and minimum detectable effect (MDE).
- Correlations: covariation of parameters → kinematic profiles.
- Principal component analysis (PCA).

Results: mixed models

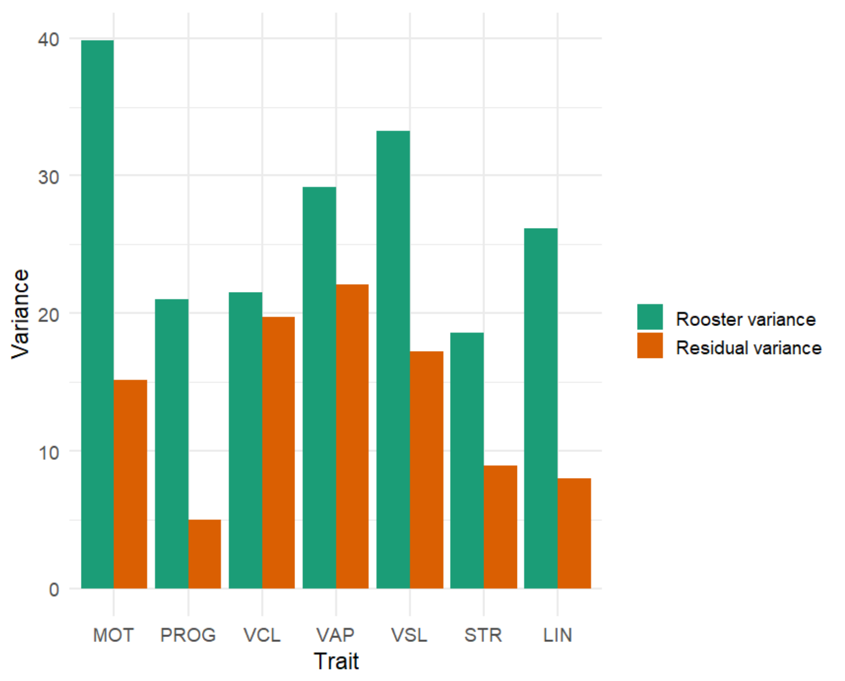
— Mean total and progressive motility: 36.46% and 19.94%
  - JH had higher average motility and progressive motility (p=0.011 and p=0.017).

— No breed differences in kinematic traits or viability.

— Short-term refrigeration had an adverse effect on all traits, except straightness.

— Clear rooster-specific differences across all traits.

Trait	p-value	
	Breed	Treatment
MOT (%)	**0.011***	**0.000*****
PROG (%)	**0.017***	**0.000*****
VCL (µm/s)	0.677	**0.007****
VAP (µm/s)	0.929	**0.005****
VSL (µm/s)	0.734	**0.002****
STR (%)	0.762	0.240
LIN (%)	0.780	**0.040***
Viability (%)	0.149	NA



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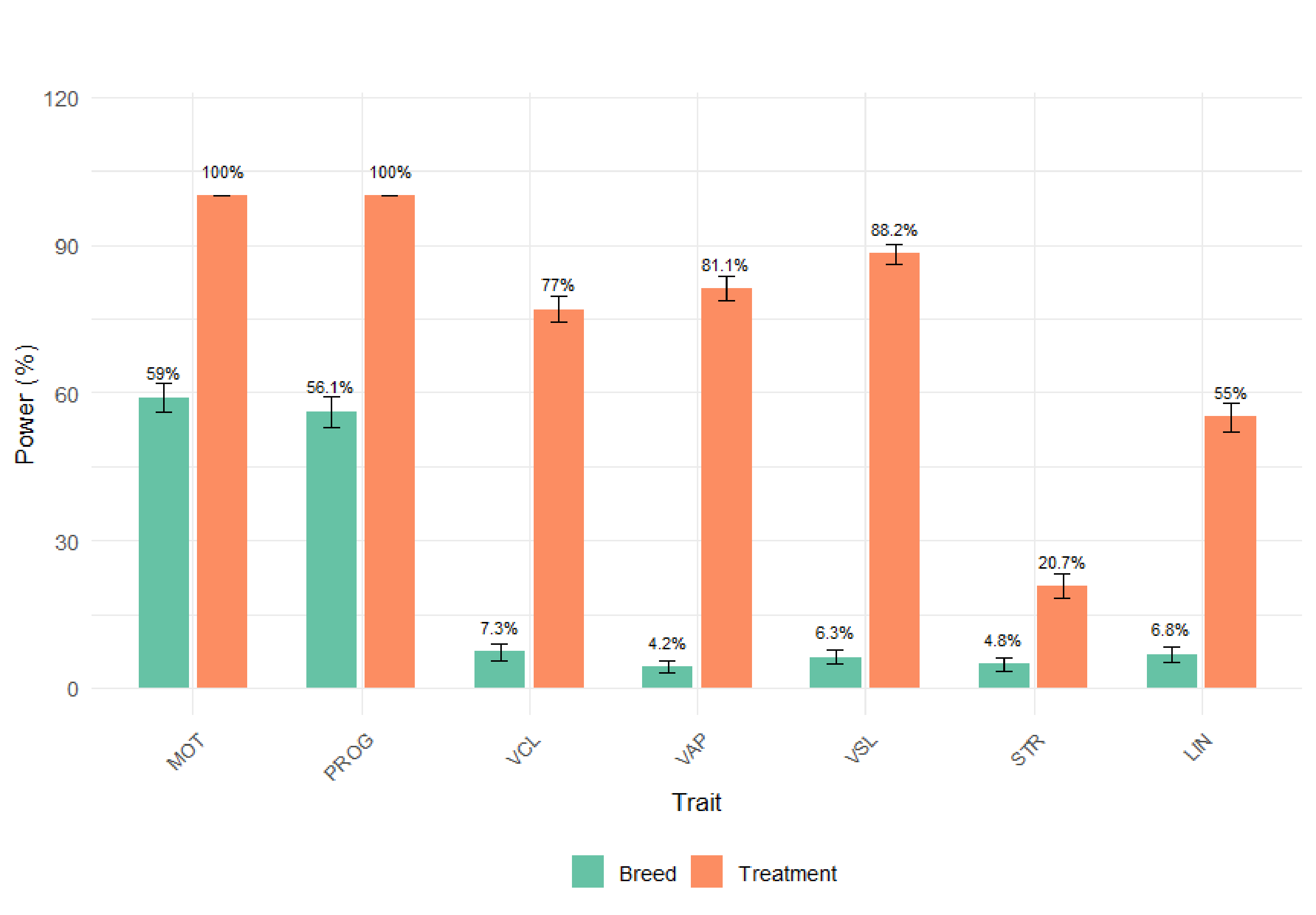
Results: power and MDE

- Retrospective power analysis allowed us to determine whether the experiment was adequately powered to detect the observed breed and treatment effects
  - Simulated trait-wise power estimates.

- Minimum detectable effect: how large a breed or treatment difference must be for our study to detect it with confidence, given the sample size and trait variability.



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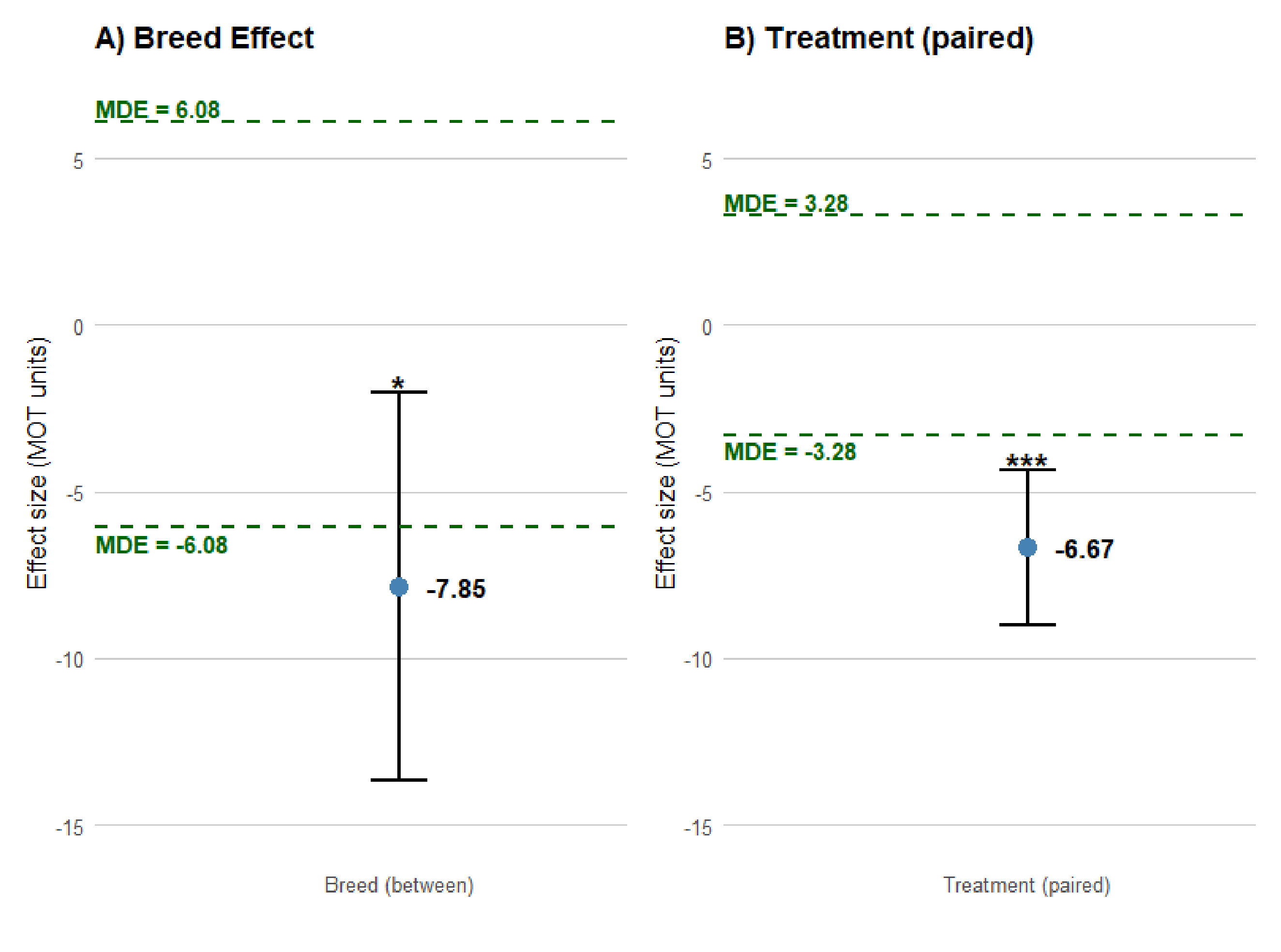
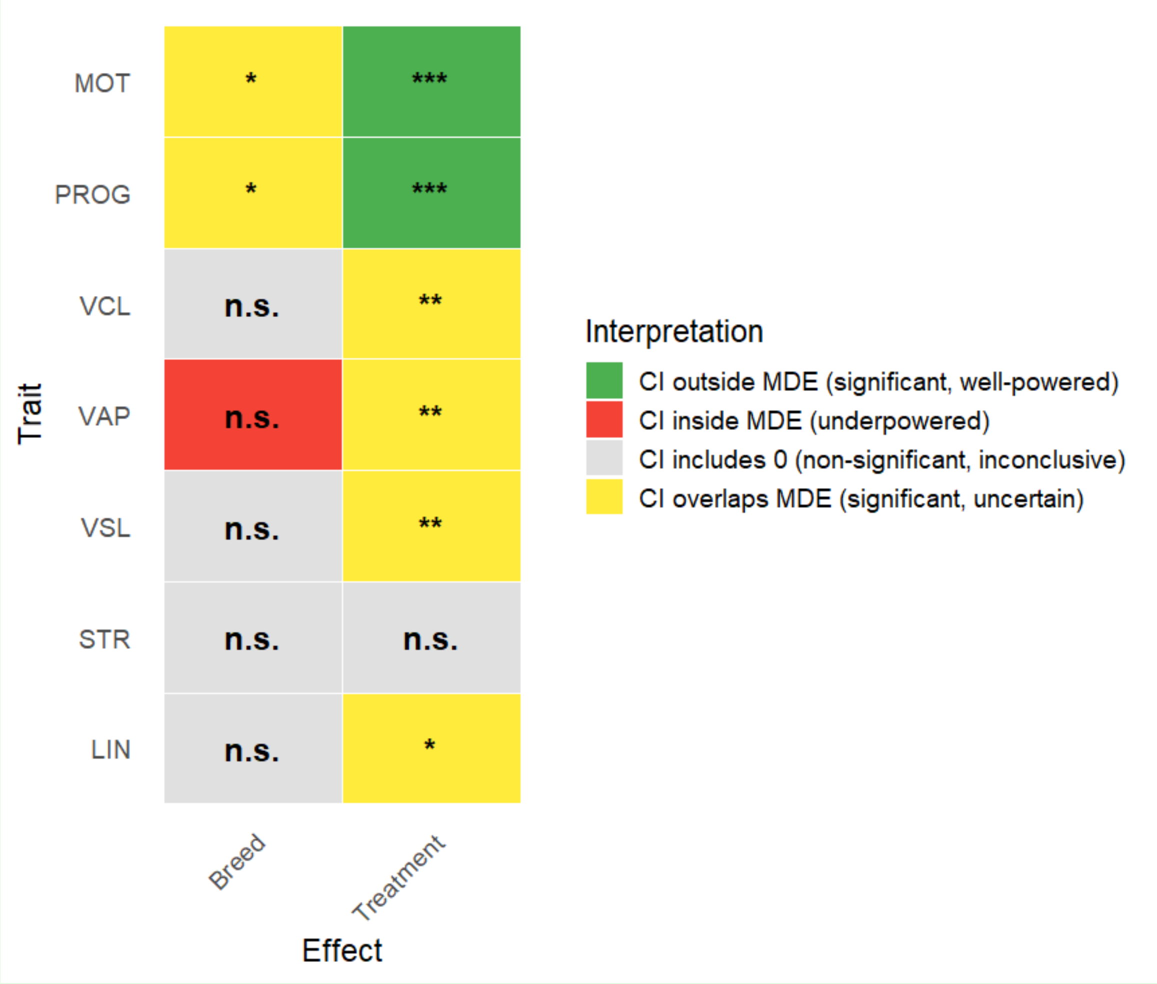

A) Breed Effect
MDE = 6.08
MDE = -6.08
*
-7.85
Effect size (MOT units)
5
0
-5
-10
-15
Breed (between)
B) Treatment (paired)
MDE = 3.28
MDE = -3.28
***
-6.67
Effect size (MOT units)
5
0
-5
-10
-15
Treatment (paired)


Trait
MOT
PROG
VCL
VAP
VSL
STR
LIN
Breed
Treatment
Effect
MOT: Breed *, Treatment ***
PROG: Breed *, Treatment ***
VCL: Breed n.s., Treatment **
VAP: Breed n.s., Treatment **
VSL: Breed n.s., Treatment **
STR: Breed n.s., Treatment n.s.
LIN: Breed n.s., Treatment *
Interpretation
CI outside MDE (significant, well-powered)
CI inside MDE (underpowered)
CI includes 0 (non-significant, inconclusive)
CI overlaps MDE (significant, uncertain)

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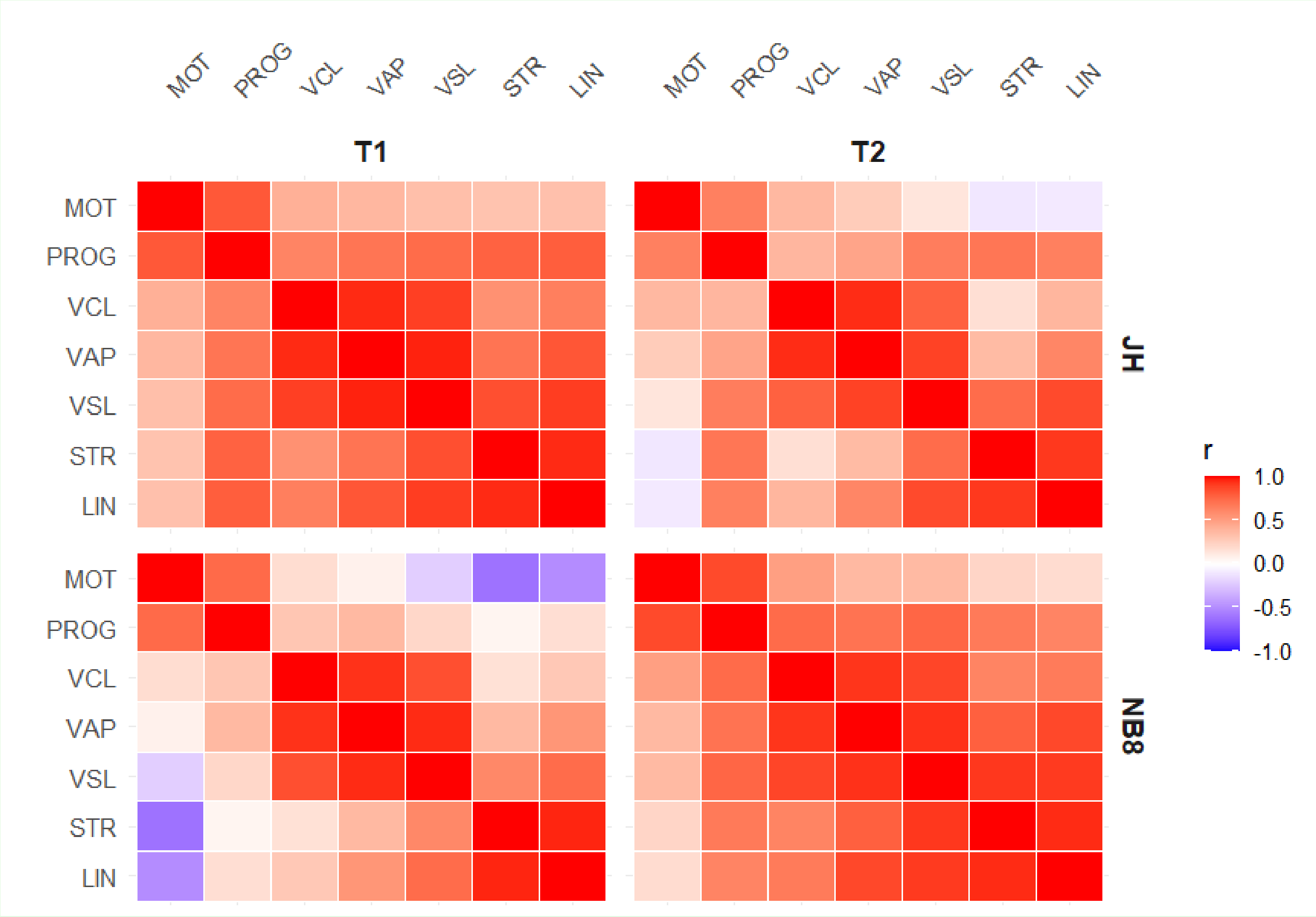
Results: correlations

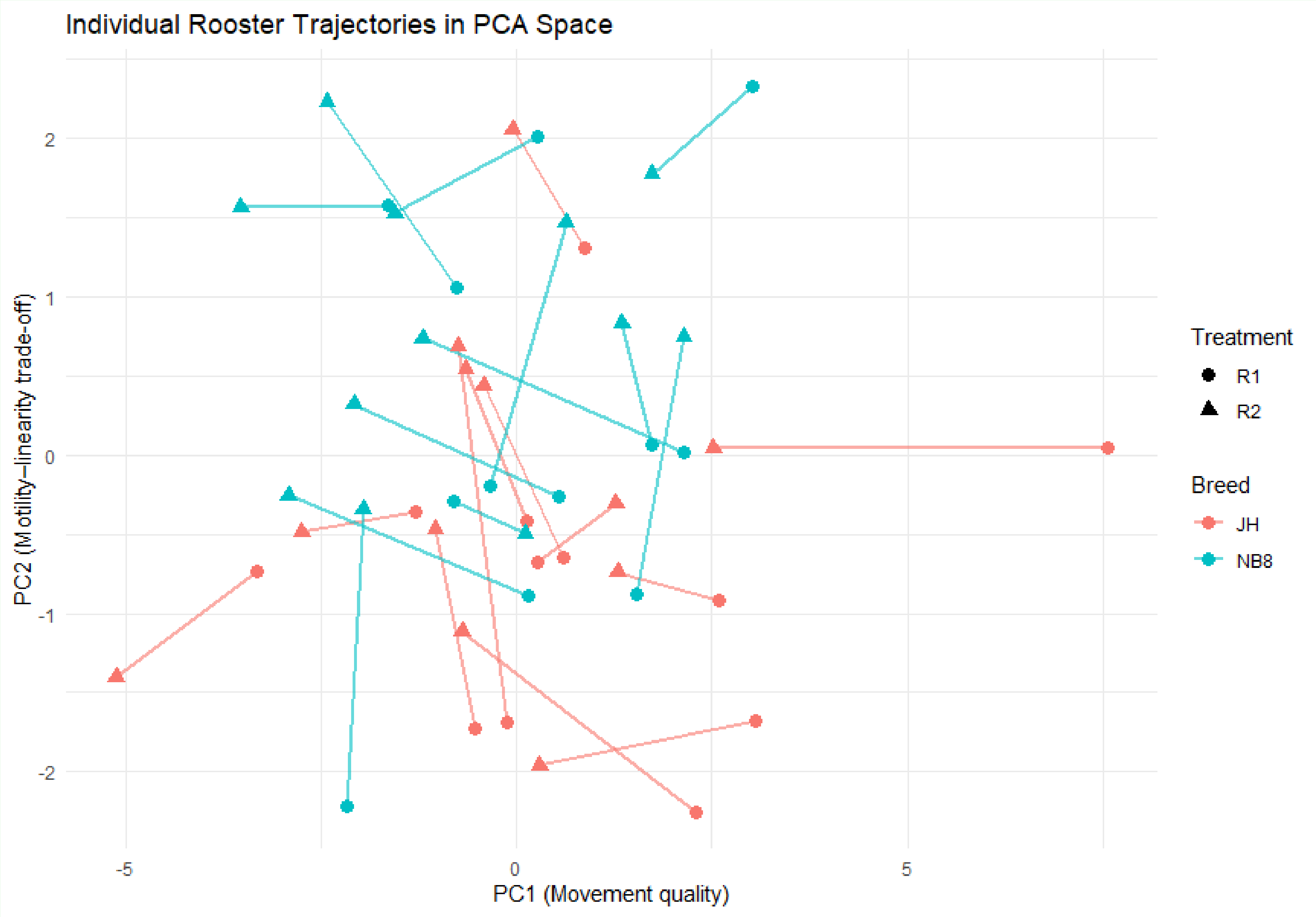


- Treatment affected trait covariation differently between breeds.
- Total motility: low insignificant correlation with other traits.
- Correlations between progressive motility and other traits in NB8 → short-term storage increased coherence.
- Viability% effectively independent from motility traits.

Results: PCA

- PC1-PC3 explained 98.3% of the variation
  - PC1: a general index of overall sperm movement quality.
  - PC2: motility-linearity trade-off.
  - PC3: variation in movement pattern.
- Treatment affected PC1 and PC2, breed only PC2.
- Conditional R^2 values (0.36–0.78) highlighted substantial rooster-to-rooster variation across all components.

- Spaghetti plots: clear differences in within-breed variability.


Individual Rooster Trajectories in PCA Space
PC2 (Motility–linearity trade-off)
PC1 (Movement quality)
2
1
0
-1
-2
-5
0
5
Treatment
R1
R2
Breed
JH
NB8

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Conclusions and implications: pilot

— Sperm quality is shaped by treatment effects, multivariate trait structure, and substantial individual-level variation.

— Rooster identity accounted for a large proportion of total variation, underscoring that sperm quality is an individual-specific phenotype rather than a uniform breed characteristic.

→ Need for careful donor selection, adequate sample sizes, and multivariate analytical approaches in future studies and cryobanking programs.

→ Fresh and frozen–thawed semen assessments with fertilization outcomes: essential for developing effective long-term cryoconservation strategies for endangered Norwegian poultry breeds.

Practical implications

- Value of cryopreservation is fully dependent on the fertilization success!
  - No reliable fertilization success = no justification for investing in cryopreservation.

- Our experience shows that cryopreservation is only viable when long-term storage is secured in advance, as relying on rented storage facilities is inherently risky and can result in the loss or disposal of valuable genetic material.

Thank you for your attention.

Norwegian University of Life Sciences

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