Milking order of healthy and subclinically mammary infected dairy ewes in mid lactation

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TECH CARE
Integrating innovative TECHnologies along the value Chain
to improve small ruminant welfARE management

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Milking order: Feasible Early Warning System (EWS) for mastitis detection in dairy ewes?.

Hypothesis:

- **Healthy and high welfare ranked ewes come earlier** to milking parlor (rewarded by oxytocin and concentrate).
- **Sick** (mastitic) **animals will come on delay** (malaise).

But, mastitis in small ruminants has a…

- Low prevalence for **clinical** (<5% on <u>whole lactation</u>).
- High prevalence for **subclinical** (>30%, mainly CNS).
- **Controversial link with SCC (>500·10³ cells**/mL?).
- Udder morphology and imaging (IR) show **past mastitis**.
- **IMI (intramammary infection)** <u>needs to be assessed</u> by **bacterial culture** (**>3-5 CFU**?).

Materials & Methods:

- UAB experimental farm: Lambing Sept 2022.
- **112 dairy ewes** (34 Manchega and 78 Lacaune) grazing and supplemented indoors with TMR.
- Milked twice daily (0700 and 1730 h) from parturition.
- Experimental period: **52 d in mid lactation (88 to 140 DIM)**.
- 2 x 12 milking parlor (DeLaval) with 12 electronic milk meters (MM25SG).
- Ewe e-ID with ceramic boluses (22 g, Datamars, ES) and 2 reading tunnels with ISO readers (DeLaval, SE).
- **Milking order and yield at each milking** (Alpro, DeLaval).
- **Milk composition and SCC** (ALLIC laboratory, ES) **by month**.
- **Bacterial culture on next week** when **SCC > 500·10³ cells**

Materials & Methods: Flock and milk recording

5 L jar

Milk sampling for **composition and SCC** analyses

Materials & Methods:
Low frequency e-ID reading system and milk meters

e-ID by bolus (134.2 kHz) and tunnel reader

Fixed order by stall number

Flow

Milk flow meter (NIR)

Key point:
Reading efficiency 96-99%
(Nieddu & Caja, 2017) line errors.
Manual correction?
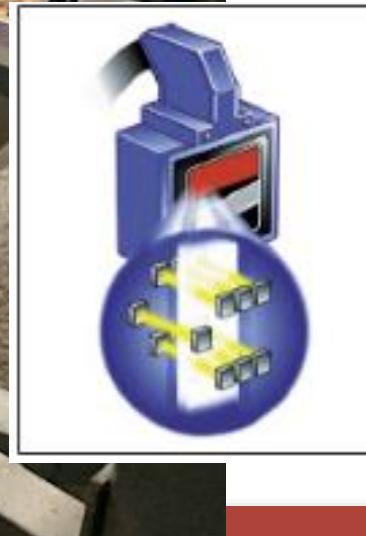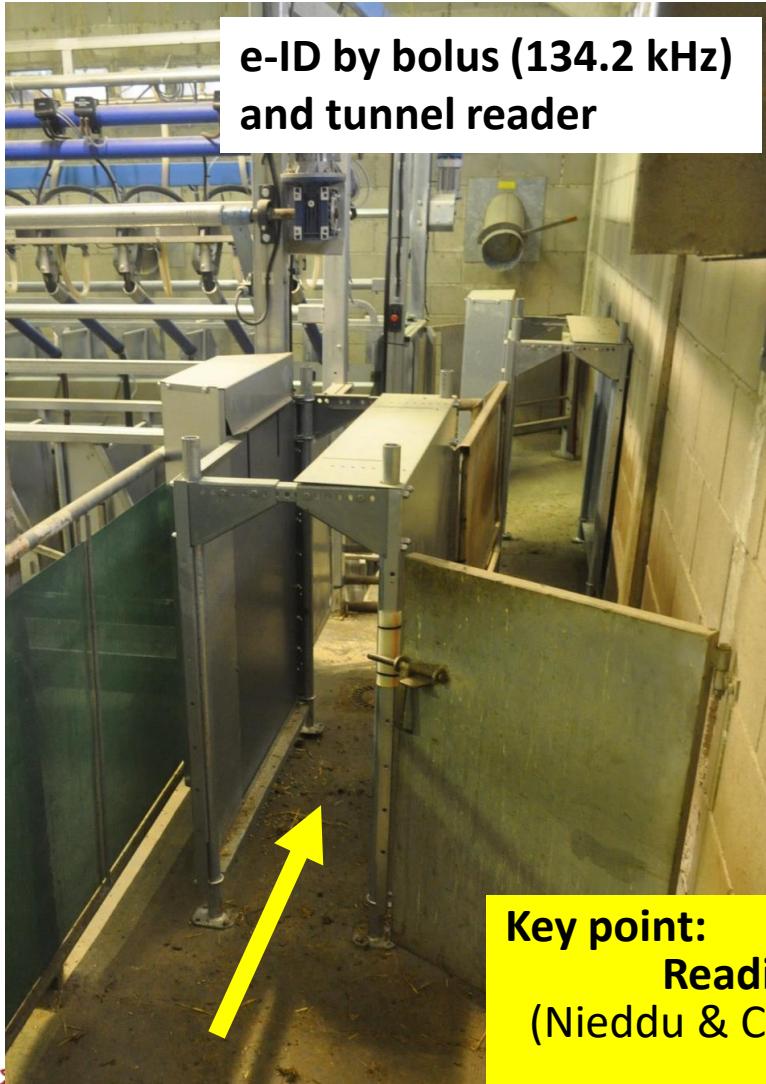
Materials & Methods: Sampling for bacterial culture

Milk sampling for **bacterial culture (blood-agar)** at 37ºC (24 and 48 h)

Materials & Methods: Ewe's management

- Semi-intensive: 6 h/d grazing + TMR (F:C = 60:40) + 0.1 kg concentrate at each milking.
- **Joined as an only flock** for grazing (6 h/d) and milking (2x).
- Free entrance to milking and penned at random during the night.
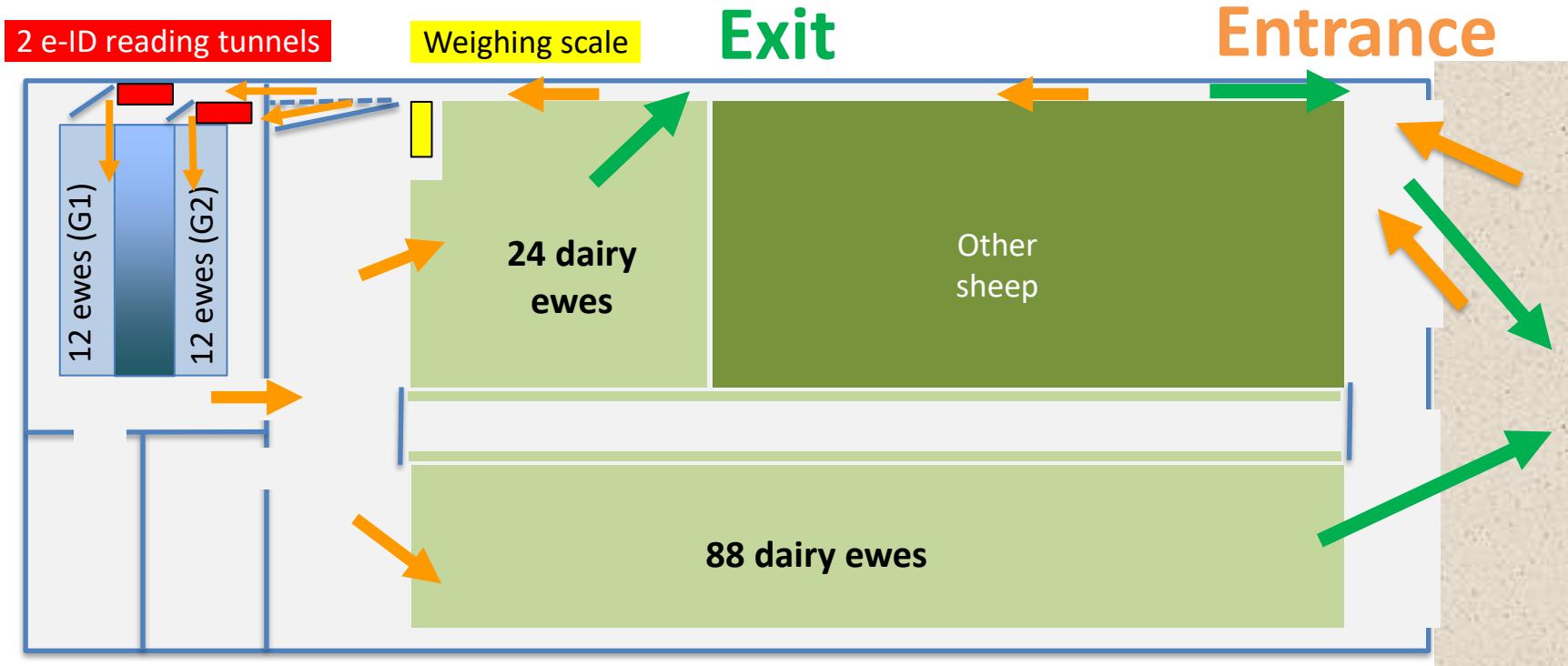
Materials & Methods: Statistical analysis

- Use of **R Core Team (2021)** packages.

- Association of quantitative variables (milk yield, milk composition, SCC, BW) by Spearman correlations.

- Ordinal variables (milking order, IMI, parity, breed) medians compared by Wilcoxon Mann-Whitney test.

- Milking order consistency by intraclase correlations.

- Milking order SD assessed by non-linear segmented regression ("splines")

- Significancy declared at $P < 0.05$

Results: Reading efficiency and lactational data

- Dynamic reading efficiency (52 d): DRE = 11,403/11,648 = **97.9%**

- Milk yield and composition in mid lactation (n = 112 ewes):

Time (DIM)	Breed	Yield (L/d)	SCC (x10³)	Log$_{10}$ SCC	Composition (%)			Urea (mg/L)	Mean order
					Fat	Protein	Lactose		
1 (d 110)	MN	0.50	389	4.90	9.44	6.84	4.53	327	70
	LC	0.89	1,622	5.73	7.16	5.92	4.57	353	58
	Mean	**0.77**	**1,248**	**5.48**	**7.85**	**6.20**	**4.55**	**345**	**61**
	±SE	**±0.04**	**±210**	**±0.08**	**±0.14**	**±0.07**	**±0.04**	**±8**	**±7**

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C2 (d 140)	MN	0.49	799	5.69	9.24	6.82	4.64	670	71
	LC	0.89	1,674	5.94	6.91	5.94	4.55	585	42
	Mean	**0.74**	**1,409**	**5.87**	**7.62**	**6.21**	**4.58**	**611**	**58**
	±SE	**±0.13**	**±173**	**±0.05**	**±0.13**	**±0.07**	**±0.04**	**±9**	**±3**

Results: Mammary infection and SCC value

IMI (Bacterial culture) and SCC (n = 112 ewes):

- Milk recording C1 (110 DIM):
- Milk recording C2 (140 DIM):

CFU vs SCC

$R^2 = 0,0162$

$1,248 \pm 210 \times 10^3$

CFU vs SCC

$R^2 = 0,0283$

$1,409 \pm 173 \times 10^3$
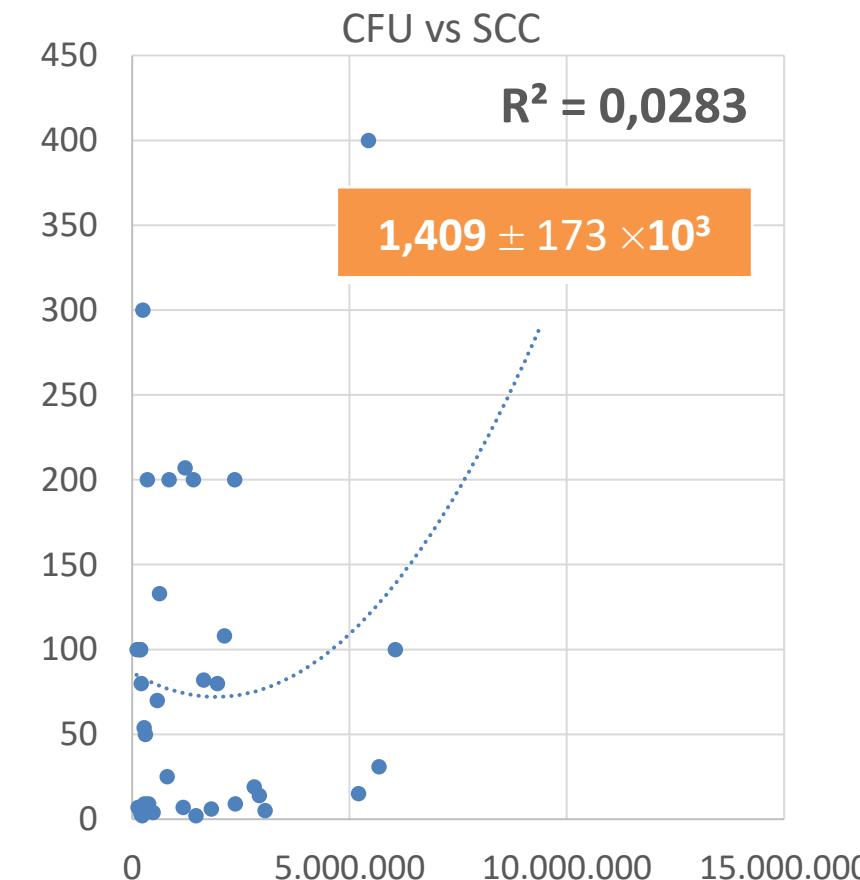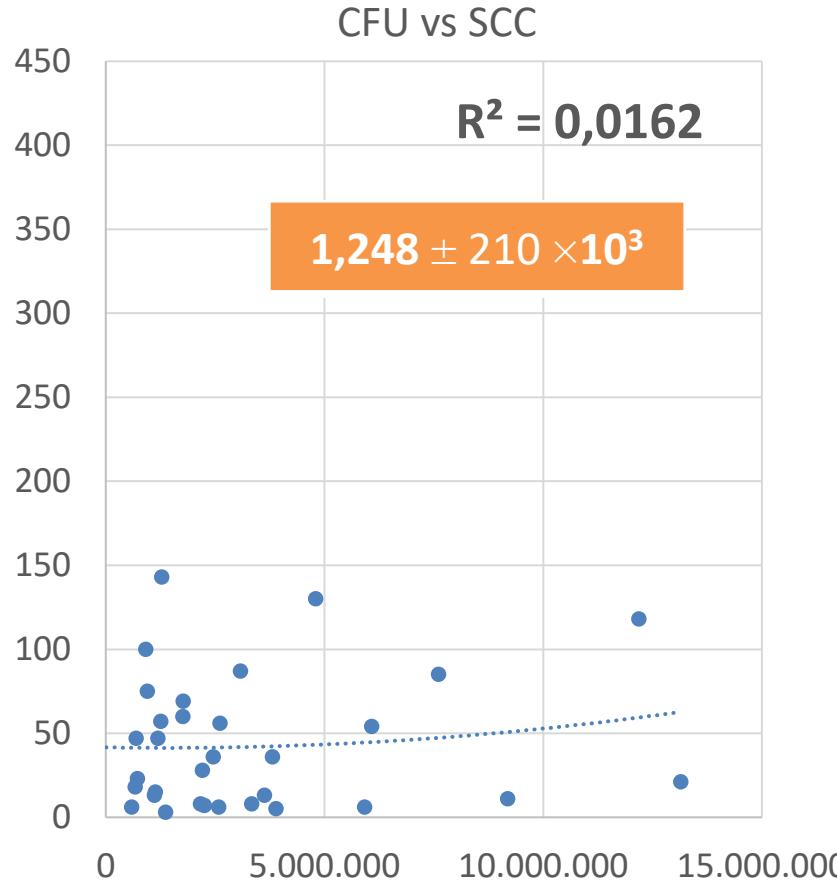
Results: Mammary infection and Log_{10} SCC

IMI (Bacterial culture) and Log SCC (n = 112 ewes):

- Milk recording C1 (110 DIM):
- Milk recording C2 (140 DIM):

CFU vs SCC

$R^2 = 0,0118$

5.48 ± 0.08

CFU vs Log SCC

$R^2 = 0,0093$

5.87 ± 0.05
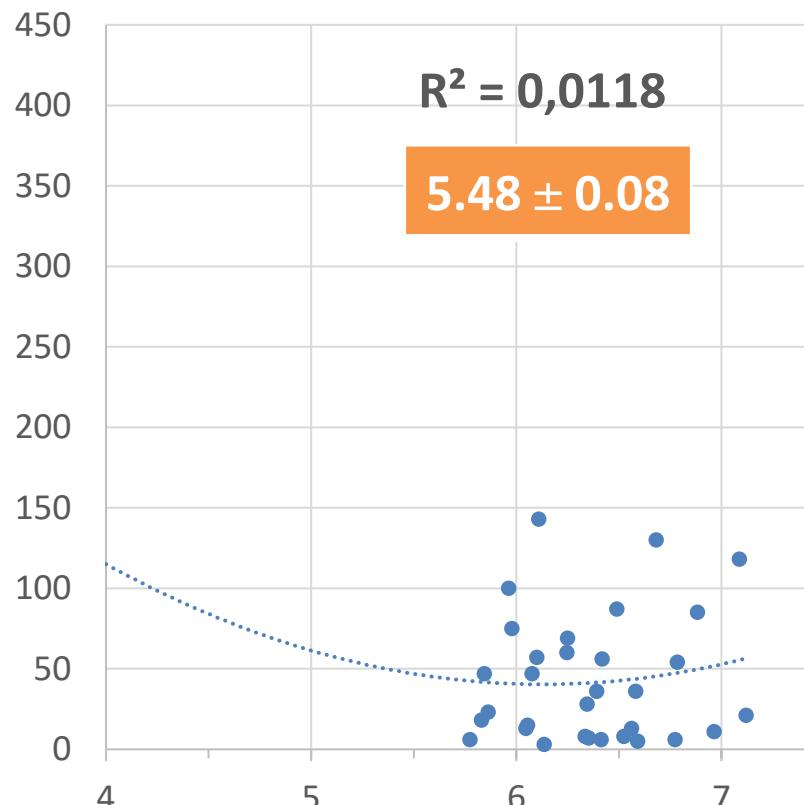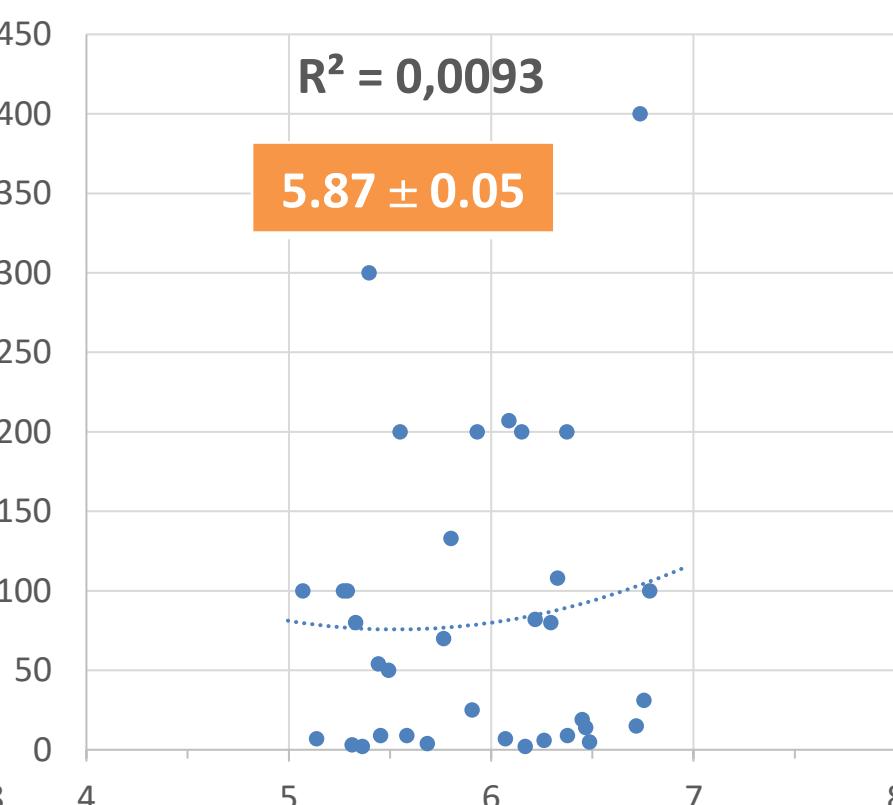
Results: Mammary infected ewes (subclinically sick)

- Suspected mastitic ewes (subclinical): >500.000 cells/mL

Item	C1 (d 110)	C2 (d 140)	Change
Ewes	112	112	-
SCC, $x10^3$ cells/mL	1,248	1,409	161
Log10 SCC	5.48	5.87	0.39
Ewes >500, n (%)	**50 (44.6)**	**68 (60.7)**	**18 (16.1)**
Infected ewes >5 CFU	**32 (28.6)**	**36 (32.1)**	**4 (3.5)**
Sensitivity[1], %	64.0	52.9	–11.1
Healed, n (%)		7 (21.9)	
New infections, n (%)		10 (27.8)	

[1]Sensitivity, % = True positive/Sick individuals x100

Staphylococcus aureus

CNS (*Coagulase negative staphylococcus*)

SCC according to lactation

Intramammary infections (IMI)
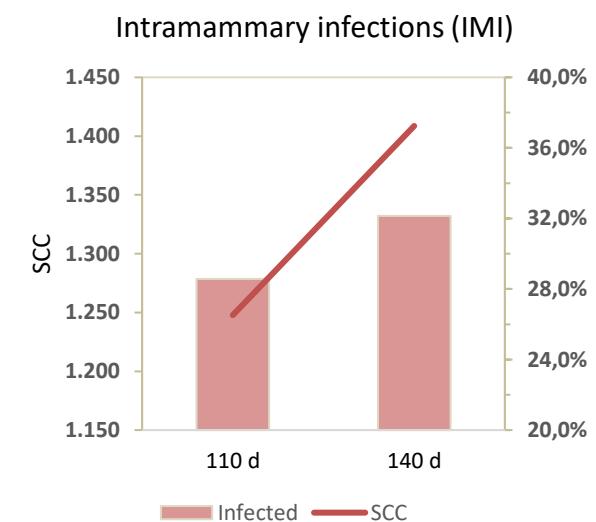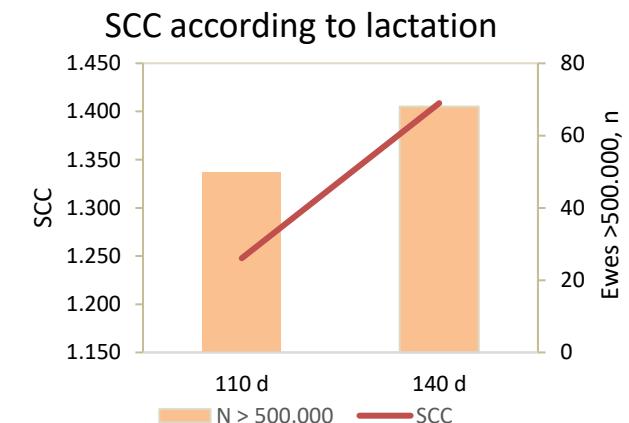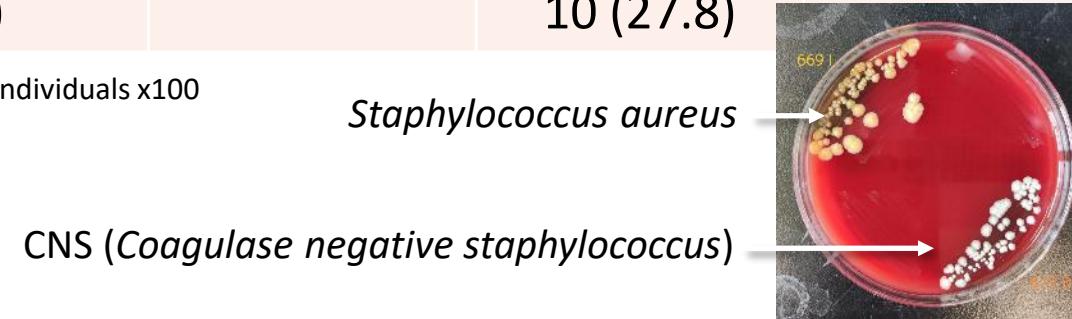
Results: Mammary infected udder halves

- Confirmed mastitic glands (subclinical): >499.000 cells/mL

Item	C1 (d 110)	C2 (d 140)	Change
Udder halves, n	66	66	-
Healthy, n	18 (27%)	22 (33%)	4 (6)
CNS, n	**22 (33%)**	**33 (50%)**	**11 (17)**
CPS, n	6 (9%)	0 (0%)	–6 (–9)
S. aureus, 20	**20 (30%)**	**11 (17%)**	**9 (–13)**
IMI, n	48 (73%)	44 (67%)	– 4 (–6)

Staphylococcus aureus →

CNS (*Coagulase negative staphylococcus*) →

C1 (d 110)
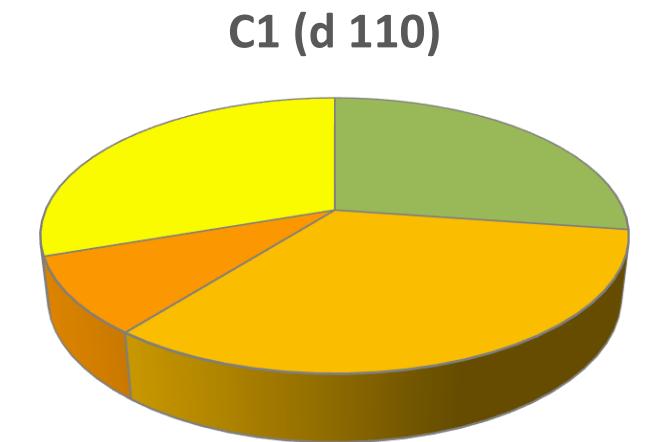
☐ Healthy ☐ CNS ☐ CPS ☐ S. aureus

C2 (d 140)
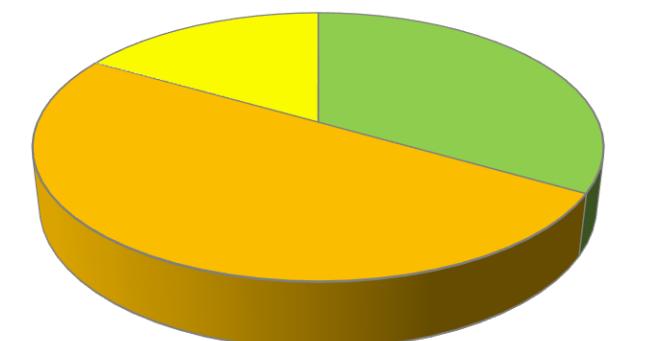
☐ Healthy ☐ CNS ☐ S. aureus 14
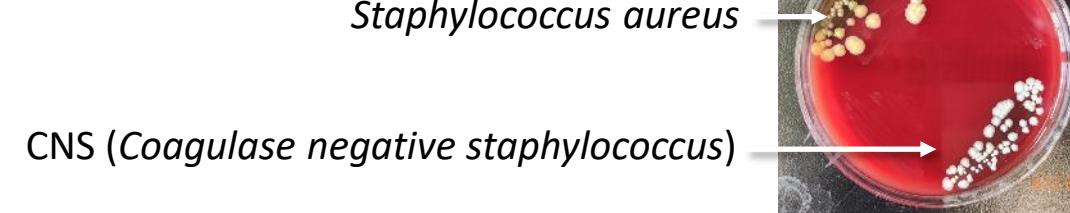
# Results: Milking order consistency

VC and SD coefficients (%) of dairy ewes' milking order (n = 112)

Mean milking order

b1 = 0,338 ± 0,119

b3 =–0,303 ± 0, 071

Morning

b2 =–0,214 ± 0,104

b1 = 0,190 ± 0,055

b3 =–0,605 ± 0,798
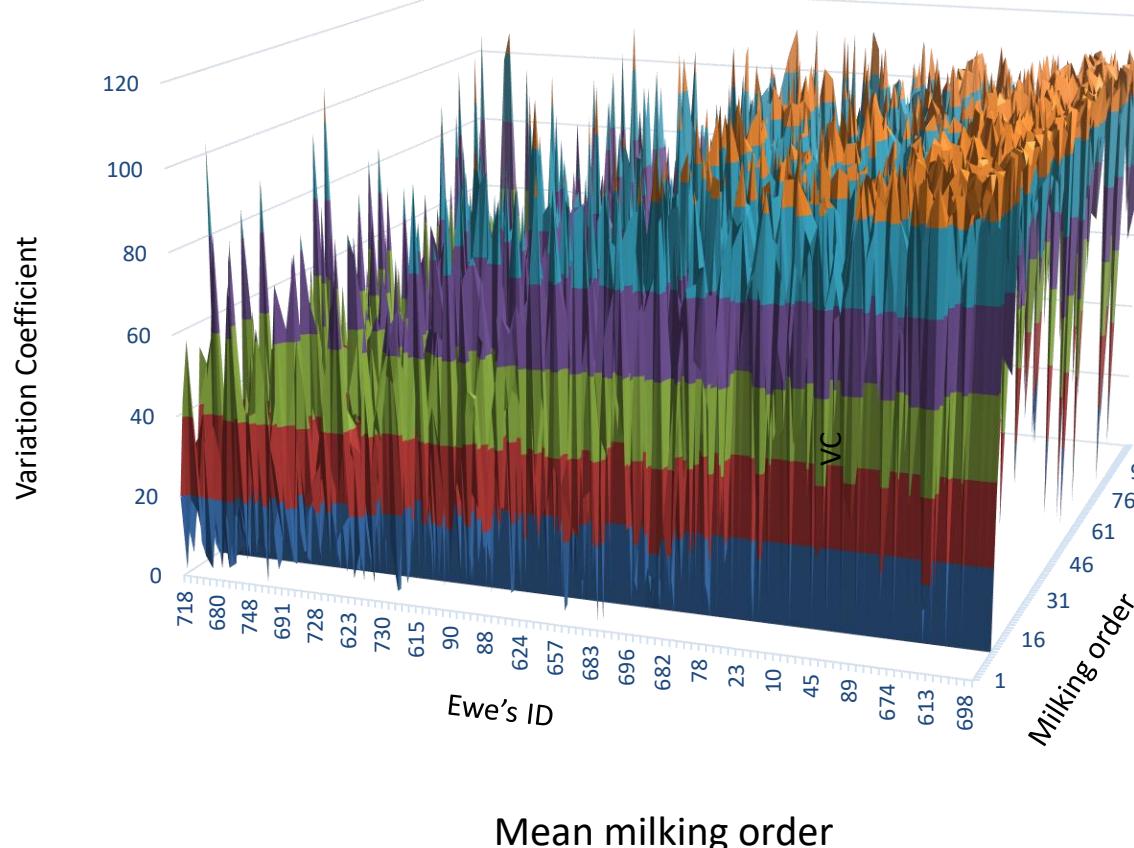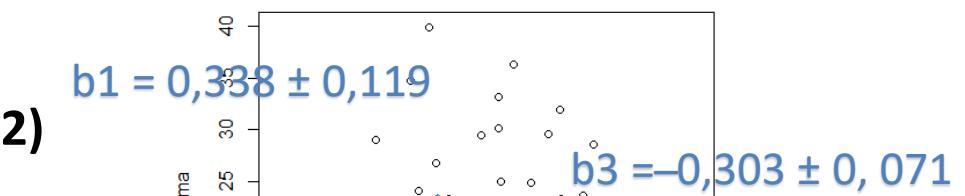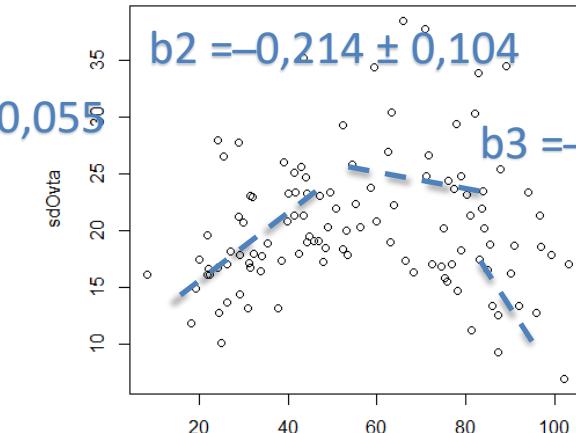
Afternoon

Results: Milking order consistency as EWS

- Consistency of milking order: Box-Plot outliers
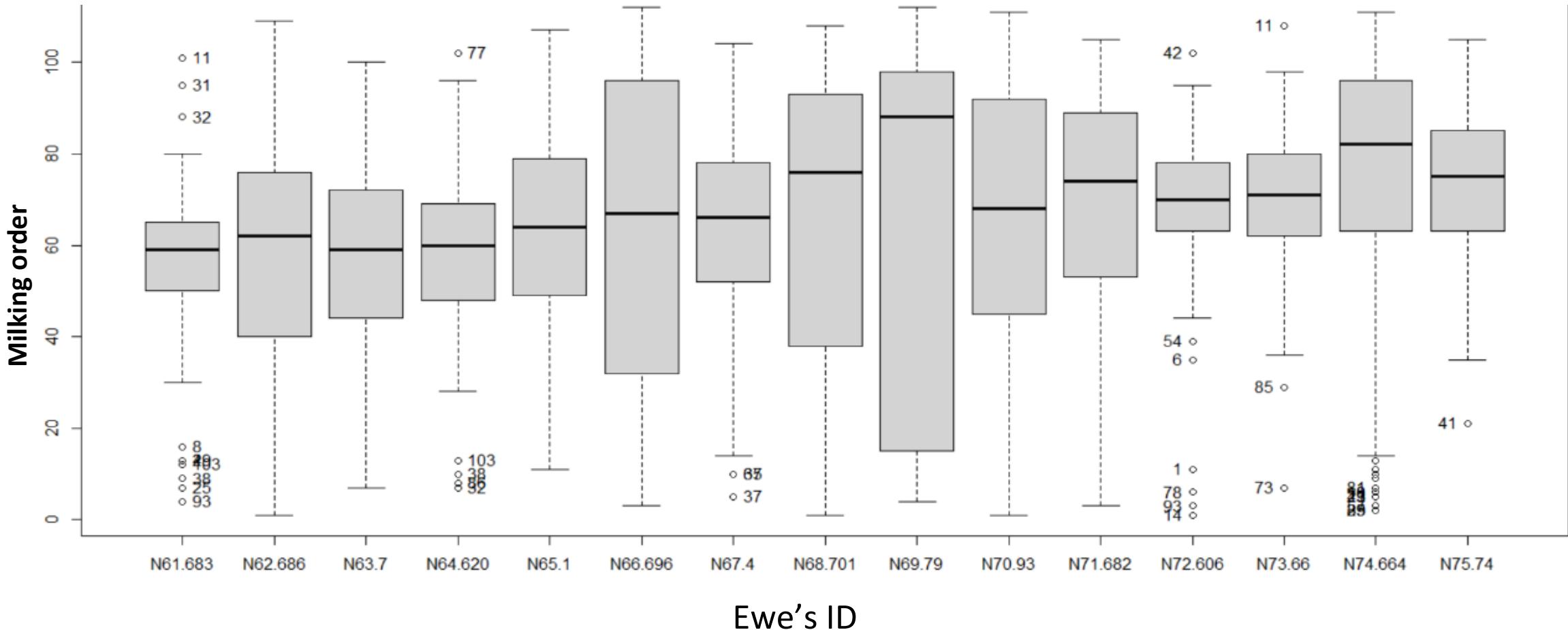
Ewe's ID

Results: Milking order Spearman correlations

- Spearman correlations with milking order: 112 ewes

Item	C1 (d 110)		C2 (d 140)	
	Rho	*P* value	Rho	*P* value
Milk yield, L/d	− 0.07	0.44	− 0.17	0.07
Log$_{10}$ SCC	− 0.01	0.91	0.15	0.10
Fat, %	**0.25**	0.007	**0.26**	0.005
Protein, %	**0.21**	0.03	**0.24**	0.01
Lactose, %	**− 0.26**	0.006	**− 0.31**	0.001
Urea, %	0.09	0.32	0.24	0.01
Body weight, kg	**0.40**	0.001	**0.47**	0.001

Results: Milking order Spearman correlations

- Spearman correlations with milking order by breed: **Manchega (n = 34)**

Item	C1 (d 110)		C2 (d 140)	
	Rho	*P* value	Rho	*P* value
Milk yield, L/d	0.19	0.28	− 0.03	0.85
Log$_{10}$ SCC	− 0.06	0.71	0.05	0.79
Fat, %	0.20	0.26	0.09	0.43
Protein, %	0.10	0.59	0.31	0.08
Lactose, %	0.10	0.56	− 0.06	0.76
Urea, %	− 0.02	0.90	0.33	0.06
Body weight, kg	0.10	0.60	0.17	0.33

Results: Milking order Spearman correlations

- Spearman correlations with milking order by breed: **Lacaune (n = 78)**

Item	C1 (d 110)		C2 (d 140)	
	Rho	*P* value	Rho	*P* value
Milk yield, L/d	0.03	0.79	− 0.08	0.50
Log$_{10}$ SCC	**0.24**	**0.04**	**0.31**	**0.006**
Fat, %	0.11	0.36	0.18	0.31
Protein, %	0.15	0.20	0.14	0.22
Lactose, %	**− 0.36**	**0.001**	**− 0.40**	**0.001**
Urea, %	0.21	0.06	0.07	0.50
Body weight, kg	**0.50**	**0.001**	**0.54**	**0.001**

Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: 112 ewes

Item	W	*P* value	Median values	
C1 (d 110):				
Bacterial culture	254	0.49	IMI − = 56	IMI + = 55
Breed	**927**	**0.016**	**MN = 71**	**LC = 50**
Parturition nb.	1,666	0.001	Primp = 31	Multp = 64
C2 (d 140):				
Bacterial culture	**306**	**0.021**	**IMI − = 44**	**IMI + = 69**
Breed	**936**	**0.014**	**MN = 70**	**LC = 50**
Parturition nb.	1,745	0.001	Primp = 33	Multp = 65

Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: **Manchega (n= 34)**

Item	W	*P* value	Median values	
C1 (d 110):				
Bacterial culture	58	0.94	-	-
Parturition nb.	-	-	-	-
C2 (d 140):				
Bacterial culture	29	0.30	-	-
Parturition nb.	-	-	-	-

Results: Milking order correlations with udder health

- Wilcoxon Mann-Whitney correlations with milking order: **Lacaune (n = 78)**

Item	W	*P* value	Median values	
C1 (d 110):				
Bacterial culture	692	0.93	-	-
Parturition nb.	960	0.004	Primp = 31	Multp = 56
C2 (d 140):				
Bacterial culture	**366**	**0.001**	**IMI − = 35**	**IMI + = 68**
Parturition nb.	1,020	0.001	Primp = 33	Multp = 58

Conclusions: Is milking order feasible as EWS for mastitis in dairy ewes?

1) SCC has not enough sensitivity for subclinically infected sheep udders.

2) Milking order:

- Was efficiently recorded by **low RFID boluses and ISO readers** (134.2 kHz).
- Was **repeatable** with higher correlations for the earlier and later milking groups.
- **Does not discriminate subclinically IMI ewes.**
- Depends on **BW**, **breed** milkability and parity.
- **Last** entering ewes have **worst udder health.**

3)   Other indicators should be used as mastitis EWS.

Thank you for attention!

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