CHARACTERIZATION OF THE SEMINAL MICROBIOM AND ITS ASSOCIATION WITH TESTOSTERONE LEVELS AND TESTICULAR MORPHOMETRY ACROSS DIFFERENT RAMS FERTILITY GROUPS

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INTRODUCTION

- The seminal microbiome plays a role in male reproductive function and semen quality.
- Advances in 16S rRNA sequencing enable detailed characterization of microbial communities.
- Fertility outcomes in livestock may be influenced by hormonal and morphometric factors, including testosterone and testicular size.
- The interaction between seminal microbiome composition and fertility classification in rams remains insufficiently defined.

AIM OF THE STUDY

To characterize the seminal microbiome of Naemi rams using 16S rRNA sequencing and evaluate its association with testosterone levels, testicular morphometry, and fertility classification.

MATERIALS AND METHODS

- 30 adult Naemi rams
- Managed under uniform conditions
- Fertility classified based on pregnancy rate
- Groups: Superior | Good | Acceptable

SAMPLE COLLECTION AND HORMONAL/MORPHOMETRIC ASSESSMENT

- Semen collected using artificial vagina
- Blood samples for testosterone (ELISA)
- Testicular morphometry recorded
- Pregnancy rate determined from breeding records

MICROBIOME PROFILING

- DNA extraction from semen samples
- 16S rRNA gene sequencing
- Taxonomic classification at family level
- Relative abundance analysis

STATISTICAL ANALYSIS

- Data analyzed using one-way ANOVA
- Post hoc comparisons between fertility groups
- Significance set at $P < 0.05$

RESULTS AND DISCUSSION

Table 1 :Effects of Season, Fertility, and Interaction of Season and Fertility on Testosterone Levels and Sexual and Reproductive Performance of Naemi Ram (Mean±SE)

Category	Parameters								
	Testosterone pg/ml	R time sec	Mounting sec	P Erection sec	Ejaculation	Mating Ability sec	Libido sec	Sexual Behavior	PR%
Effect of season									
Summer	1815.68	5.75	4.86	5.00	0.89	9.89	3.92	6.83	62.54
SE	230.1	0.08	0.07	0.76	0.05	0.05	0.04	0.05	0.62
P value	*0.0418*	*0.4874*	*0.7838*	*0.3099*	*0.6910*	*0.6910*	*0.6301*	*0.2844*	*0.4354*
Effect of fertility									
Superior	3343.75[a]	5.92	4.96[a]	5.58	1.00	9.92	4.00[a]	6.96	70.90[a]
Good	1868.30[b]	5.75	4.92[a]	3.92	0.92	10.00	4.00[a]	6.92	62.36[b]
Acceptable	1242.72[b]	5.71	4.67[b]	3.83	0.79	9.79	3.79[b]	6.75	57.16[c]
SE	281.81	0.10	0.09	0.94	0.06	0.06	0.05	0.07	0.76
P value	*<.0001*	*0.3267*	*0.0446*	*0.3381*	*0.0551*	*0.0551*	*0.0046*	*0.0734*	*<.0001*
Effect of interaction by season									
SS	2315.18	5.92	5.00	3.92	1.00	9.92	4.00	6.92	70.15
SG	1842.86	5.75	4.92	3.92	0.92	10.00	4.00	6.92	65.90
SA	1289.02	5.58	4.67	3.83	0.75	9.75	3.75	6.67	58.41
SE	398.54	0.15	0.12	0.12	0.09	0.09	0.07	0.09	1.08
P value	*<.0001*	*0.5906*	*0.2545*	*0.3854*	*0.2717*	*0.2717*	*0.0404*	*0.2098*	*<.0001*

[a, b, c] Means under the same category carrying different superscript letters within the same column differed statistically at $P<0.05$. PR: Pregnancy Rate, R: Reaction, P: Penile, SS: Summer Superior, SG: Summer Good, SA: Summer Acceptable, SE: Standard Error

Superior rams exhibited significantly higher testosterone levels

- Testosterone differed significantly across fertility groups ($P < 0.05$)
- Superior group showed the highest mean concentration
- Elevated testosterone may contribute to improved reproductive performance

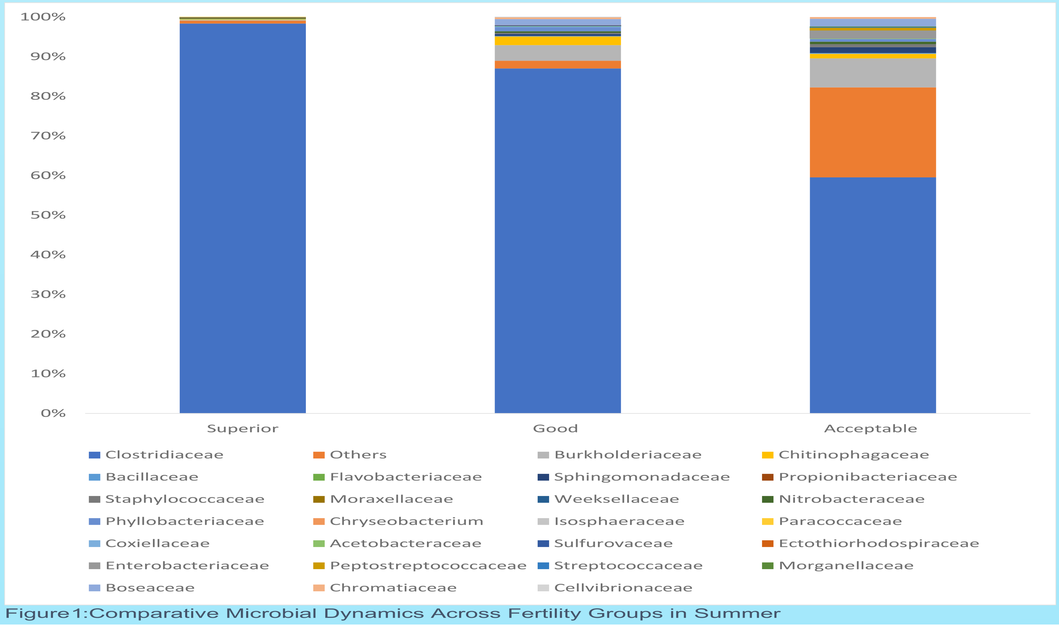

Figure 1:Comparative Microbial Dynamics Across Fertility Groups in Summer

Distinct microbial signatures characterize fertility groups

- Relative abundance varied across fertility classifications
- Clostridiaceae predominated in Superior rams
- Microbial composition may reflect reproductive physiological status

Table1:Effects of Season, Fertility, and Interaction of Season and Fertility on Testicular Morphometry of Naemi Ram (Mean±SE)

Category	Parameters						
	Testicular						Scrotal
	Length Left (cm)	Length Right (cm)	Length TL (average)	Volume (cm^3)	Width (cm)	Circumference (cm)	Circumference (cm)
Effect of season							
Summer	22.96	20.39	21.56	1186.04	6.60	22.01	36.90
SE	0.17	0.22	0.17	32.16	0.07	0.23	0.50
P value	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*0.0001*
Effect of fertility							
Superior	25.79	22.84	24.33	1459.140	7.35	23.52	38.58
Good	25.72	23.17	24.38	1454.13	7.28	23.37	38.20
Acceptable	25.24	22.37	24.01	1409.05	7.39	23.31	38.25
SE	0.21	0.27	0.21	38.76	0.08	0.27	0.60
P value	*0.1421*	*0.1200*	*0.3879*	*0.6101*	*0.5119*	*0.8652*	*0.8952*
Effect of interaction							
SS	23.27	20.83	21.89	1202.27	6.50	22.05	37.05
SG	22.99	20.57	21.62	1212.11	6.65	22.19	37.00
SA	22.62	19.76	21.14	1143.73	6.63	21.80	36.67
SE	0.30	0.38	0.29	55.69	0.12	0.40	0.87
P value	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*<.0001*	*0.0079*

[a, b, c] Means under the same category carrying different superscript letters within the same column differed statistically at $P<0.05$. SS: Summer Superior, SG: Summer Good, SA: Summer Acceptable, SE: Standard Error

Testicular morphometry differed significantly across fertility groups

- Superior rams exhibited larger testicular measurements
- Morphometric variation aligns with fertility classification
- Structural differences may support hormonal and microbiome patterns

Seasonal variation modulated testicular morphometry across fertility classes

- Significant Season × Fertility interaction observed
- Superior rams maintained higher measurements across season
- Environmental factors may influence reproductive morphology

Testicular morphometry varied significantly with fertility classification

- Fertility group significantly influenced testicular measurements ($P < 0.05$)
- Superior rams showed larger morphometric values
- Structural variation may contribute to endocrine and fertility differences

Table 3: Pregnancy Rate

Fertility Group	Season	No of Ewes	No of Pregnancy	Pregnancy Rate (%)
Superior	Summer	122	86	70.49
Good	Summer	138	83	61.59
Acceptable	Summer	126	70	55.54

Higher fertility classification corresponded with increased pregnancy rate

- Superior rams achieved the highest pregnancy outcome
- Fertility grouping aligned with reproductive success
- Microbiome and hormonal patterns may support this association

CONCLUSION

Fertility classification in Naemi rams is associated with distinct seminal microbiome profiles.

- Superior rams exhibited higher testosterone concentrations and favorable testicular morphometry.
- A significant Season × Fertility interaction influenced morphometric parameters.
- Clostridiaceae predominance characterized superior fertility groups.
- Integrated microbial, hormonal, and structural markers may support improved breeding selection strategies.

FUTURE WORK

- Perform longitudinal studies to evaluate temporal stability of seminal microbiome profiles.
- Conduct functional metagenomic analysis to identify microbial metabolic pathways influencing reproductive physiology.
- Investigate causal relationships between dominant bacterial families and testosterone regulation.
- Expand sampling across different breeds and geographic regions to validate biomarker potential.
- Develop predictive models integrating microbiome, hormonal, and morphometric indicators for fertility selection.

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