Comparative Effect of Hormonal and Vitamin Treatments on Reproductive Performance in Rembi Ewes during the Low Sexual Activity Period in Western Algeria

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Abstract. *Reproductive efficiency is a key determinant of the profitability and sustainability of sheep farming systems, particularly in arid and semi-arid regions. In Algeria, declining flock productivity, exacerbated by recurrent droughts, forage scarcity, and thermal stress, has raised concerns about the resilience of extensive and semi-intensive sheep production. This study aimed to compare the efficacy of hormonal synchronization, vitamin AD_3E supplementation, and their combination on the reproductive performance of Rembi ewes during seasonal anoestrus. Conducted in spring 2024 on a semi-intensive farm in Aïn Bouchakif (Tiaret, Algeria), the trial involved 60 healthy pluriparous ewes randomly assigned to three groups (n = 20). Group 1 received hormonal treatment (intravaginal progestogen-impregnated sponges followed by PMSG), Group 2 received the same hormonal protocol combined with monthly AD_3E injections, and Group 3 received vitamins only. Fertility, fecundity, prolificacy, birth weight, and neonatal mortality were evaluated. Principal Component Analysis (PCA) was applied to explore multivariate patterns.*

The combined treatment (Group 2) yielded the best reproductive outcomes: 100% fertility, 160% fecundity, and 0% neonatal mortality. Group 1 recorded the lowest fertility (80%) and highest neonatal mortality (16.66%), while Group 3 achieved the highest average birth weight (4.25 kg) but lower prolificacy. PCA revealed three distinct reproductive profiles, clearly differentiating the effectiveness of the protocol.

These results underscore the synergistic benefit and practical advantage of combining hormonal synchronization with vitamin supplementation to improve fertility and prolificacy while reducing neonatal losses. This integrative strategy offers a viable and sustainable approach to enhancing reproductive performance in Rembi ewes under the challenging conditions of Western Algeria's steppe regions.

Introduction

The profitability of sheep farming fundamentally relies on the reproductive performance of females, particularly fertility rate, prolificacy, and the number of lambings per year. In Algeria, sheep production is largely dominated by a traditional extensive system accounting for over 90% of all farms (Gani, 2023). This low-input management model often results in modest reproductive outcomes, with fewer than one lamb produced per ewe per year. The underlying causes include the seasonal nature of reproduction, minimal zootechnical monitoring, and high dependence on climatic variability (Zidane et al., 2023).

According to the National Statistics Office (ONS, 2020), the national sheep population was estimated at 30.9 million head, representing about 81% of Algeria's total ruminant livestock. However, this population has shown a worrying decline, with unofficial sources reporting a drop to around 17 million by 2023 (Le Jeune Indépendant, 2023). This decrease is largely attributed to the cumulative effects of climate change, prolonged drought, rising feed costs, and suboptimal reproductive performance. These stressors are further aggravated by ecological degradation, including the loss of steppe vegetation, reduced forage availability, and worsening female body condition, all of which impair fertility and herd renewal capacity (FAO, 2021).

The Tiaret region, located in the semi-arid steppe zone of northwestern Algeria, illustrates this dynamic. It hosts a significant sheep population, predominantly composed of the indigenous Rembi breed, which is valued for its hardiness, meat conformation, and reproductive potential. Nonetheless, the region has not been spared from the impacts of climatic stress and structural constraints, leading to a marked deterioration in reproductive indicators (Bouacha, 2019).

To overcome the constraints of seasonal reproduction, oestrus synchronization using intravaginal

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sponges impregnated with progestagens, followed by equine chorionic gonadotropin (eCG or PMSG) injection at sponge removal, is widely applied in sheep reproductive management. This approach allows induction and synchronization of oestrus during anoestrus periods, facilitating flock planning and enhancing reproductive outcomes (Hameed et al., 2021).

However, the repeated application of this hormonal protocol poses several challenges. Economically, in Algeria, the cost of a full synchronization cycle, including sponge insertion and hormonal injections, can exceed $17 per ewe, representing a major limitation for smallholder and extensive systems (Meziane et al., 2024). Biologically, studies report that repeated exposure to PMSG may induce antibody formation against the molecule, thereby reducing ovarian responsiveness and compromising fertility (Abecia et al., 2012; Sun et al., 2019). Additionally, intravaginal sponges may provoke local adverse effects such as irritation, metritis, or alterations in vaginal microbiota, especially under suboptimal hygiene or prolonged use (Martemucci and D'Alessandro, 2011; Fashemi et al., 2013). Ethically, PMSG extraction involves repeated blood collection from pregnant mares, raising serious welfare concerns and leading several European countries to ban its use (EFSA, 2011; Veterinary Medical Ethics, 2022).

Given these constraints, vitamin supplementation, especially with the AD_3E complex (vitamins A, D_3, and E), is increasingly considered as an alternative to support reproductive function. These vitamins are essential for ovarian activity, hormonal regulation, embryonic development, and immune function (Bozkurt et al., 2023; Efe et al., 2023). Their administration is cost-effective, easy to implement, and devoid of the immunogenic or ethical concerns associated with hormonal interventions.

While the individual effects of hormones or vitamins have been studied, a direct comparison of these strategies, and especially their synergistic combination, is lacking for indigenous breeds under North African steppe conditions. This study was, therefore, designed to rigorously compare the effects of a conventional hormonal protocol, a vitamin AD_3E supplementation, and their combination on the reproductive performance of Rembi ewes during the seasonal anoestrus. The objective was to identify a viable and affordable strategy to enhance reproductive efficiency and resilience in the face of climatic and nutritional challenges typical of traditional sheep farming systems in Western Algeria.

Materials and methods

Study area and environmental conditions

The trial was conducted on a private farm located in the Sersou plain, between the municipalities of Tiaret and Aïn Bouchakif (Tiaret province), in the northwestern high plateaus of Algeria. The site lies at 964 meters above sea level, with coordinates 35°21′21″N and 1°30′38″E. The region experiences a cold semi-arid Mediterranean climate (BWk according to the Köppen-Geiger classification), characterized by an average annual temperature of 14.9°C, with hot, dry summers (often exceeding 30°C) and cold winters with minimum temperatures frequently below 0°C. The average annual rainfall is approximately 362 mm, mainly concentrated in the autumn and winter months.

The farm follows a semi-intensive production system and is equipped with appropriate infrastructure, including ventilated sheepfolds, covered enclosures, and automatic watering systems. This experimental site was selected for its agro-ecological representativeness of the Algerian high plateaus and the genetic homogeneity of its Rembi ewe flock.

During April, the reproductive activity of Algerian sheep flocks is markedly reduced. This anovulatory state is principally governed by increasing photoperiod, which suppresses gonadotropic activity. The situation is compounded by nutritional stress from declining forage quality and the prevalence of lactational anoestrus. Consequently, inducing oestrus during this period necessitates the application of exogenous hormonal protocols rather than occurring through natural means.

Experimental animals and herd management

A total of 60 clinically healthy pluriparous Rembi ewes, aged between 3 and 5 years and with a postpartum interval of at least 80 days, were selected for the trial. Body condition was evaluated using the Body Condition Scoring (BCS) system, with all animals scoring between 3.0 and 3.5 on a 5-point scale. The ewes were managed under a semi-intensive system involving daily grazing and evening supplementation with 600 g per head of crushed barley and ad libitum access to barley straw and water. A nutritional flushing programme was implemented during March and April 2024, consisting of an additional 230 g per head per day of whole barley.

Experimental design and treatments

The ewes were randomly allocated into three experimental groups of 20 animals each.

In Group 1 (hormonal treatment only), ewes were treated with intravaginal sponges impregnated with 30 mg flugestone acetate (Syncro-part®, CEVA Santé Animale, Libourne, France) for 14 days. At sponge removal, each ewe received an intramuscular injection of 500 IU of pregnant mare serum gonadotropin (PMSG) (Syncro-part®, CEVA Santé Animale, Libourne, France). No vitamin supplementation was administered.

In Group 2 (combined hormonal and vitamin treatment), ewes received the same hormonal protocol as in Group 1, along with monthly intramuscular injections of a vitamin AD_3E complex (Adecon®

1.9%, FATRO S.p.a, Ozzano Emilia "Bologna", Italia: 10,000,000 IU vitamin A, 2,500,000 IU vitamin D_3, 10,000 mg vitamin E per 100 mL), administered at a dose of 5 mL per ewe from March to October 2024.

In Group 3 (vitamin treatment only), ewes were exposed to natural mating without hormonal induction but received the same AD_3E supplementation as in Group 2, using identical dosage and timing.

Eight fertile Rembi rams, aged 5 to 7 years and originating from the same flock, were selected as sires. These rams were kept separate from the females until the designated mating period to prevent premature contact.

All animals were individually identified using numbered ear tags. Prior to inclusion in the study, each ewe and ram underwent a comprehensive health check, including general clinical examination, inspection of external genitalia, and assessment of limb integrity. Only animals meeting the inclusion criteria were retained to ensure the internal validity of the experiment.

Oestrus synchronization and mating management

Oestrus synchronization was induced in Groups 1 and 2 through the intravaginal insertion of progestogen-impregnated sponges on April 14 and 18, 2024, respectively (Table 1). Following sponge removal, oestrus detection was conducted at 6-hour intervals for 48 hours by experienced technicians. Oestrus identification was based on standardized behavioural and physiological indicators including restlessness, vulvar hyperaemia and oedema, presence of cervical mucus discharge, and standing reflex when mounted by teaser rams.

The same cohort of eight proven fertile Rembi rams was utilized across all experimental groups. For Groups 1 and 2, controlled mating was implemented immediately after oestrus confirmation using a 1:5 ram-to-ewe ratio during a concentrated 48-hour breeding period, with rams systematically rotated between these groups. For Group 3 (non-synchronized), natural mating was conducted throughout April 2024 to ensure coverage of at least one complete oestrous cycle (approximately 17 days), thereby accounting for individual variation in cycle onset and maximizing conception opportunities in the absence of hormonal synchronization.

Gestation monitoring and data collection

Gestation status in ewes was monitored using a combined approach of non-return to oestrus records and systematic behavioural assessment. This weekly post-mating surveillance served to confirm initial pregnancy and to diagnose reproductive failures, including early and late embryonic mortality, as well as abortions. Ewes that did not return to oestrus were provisionally classified as pregnant, a status that was subsequently confirmed through documented behavioural indicators such as increased docility, voluntary separation from the flock, and a quantifiable increase in feed intake. All lambing events were systematically recorded. For each birth, the lambing date, litter size, viability status (live or stillborn), and individual birth weights were documented. Neonatal weights were obtained within hours of delivery using calibrated hanging scales to ensure precise measurement.

Reproductive performance indicators

The following indicators were calculated:

- Fertility rate (%) =
$$= \frac{\text{Number of ewes that lambed}}{\text{Number of ewes exposed to mating}} \times 100$$
- Fecundity rate (%) =
$$= \frac{\text{Total number of lambs born (live and stillborn)}}{\text{Number of ewes exposed to mating}} \times 100$$
- Prolificacy rate (%) =
$$= \frac{\text{Total number of lambs born (live and stillborn)}}{\text{Number of ewes that lambed}} \times 100$$

Table 1. Timeline of the experimental protocol applied to the three groups of Rembi ewes

Parameter	Group 1 (Hormonal only)	Group 2 (Hormonal + AD_3E)	Group 3 (AD_3E only)
Number of ewes	20	20	20
Synchronization status	Synchronized	Synchronized	Non-synchronized
AD_3E supplementation	None	5 mL monthly (March–October 2024)	5 mL monthly (March–October 2024)
Sponge insertion date	14 April 2024	18 April 2024	-
Sponge removal date	28 April 2024	02 May 2024	-
PMSG administration	500 IU (28 April 2024)	500 IU (02 May 2024)	-
Sponge retention issues	1 ewe lost sponge	No issues	-
Effective synchronized ewes	19	20	-
Mating period	29 April–01 May 2024	03–05 May 2024	Throughout April 2024
Expected lambing period	30 Sept–06 October 2024	04–07 October 2024	Starting 12 September 2024

- Neonatal mortality rate (%) = $= \frac{\text{Number of stillborn lambs}}{\text{Total number of lambs born}} \times 100$
- Average birth weight (kg): The mean birth weight of lambs was calculated and compared across groups, considering the type of birth (single or multiple).

Statistical analyses

Statistical analyses were conducted using the software PAST version 4.08 (Hammer et al., 2001). Descriptive statistics were calculated for all quantitative variables. Intergroup comparisons were performed using the Mann-Whitney U test, with a significance threshold set at $P < 0.05$. A Principal Component Analysis (PCA) was applied to explore multidimensional associations.

Ethical considerations

The entire experimental protocol was conducted in accordance with international guidelines for the ethical use of animals in research. All procedures adhered to good veterinary practices to minimize stress and ensure animal welfare.

Results

The reproductive performance of Rembi ewes under different treatment protocols is summarized in Table 2, with comprehensive statistical analysis presented in Table 3. The experimental cohort for analysis consisted of 19 ewes in Group 1 (following exclusion of one ewe that expelled the intravaginal sponge), 20 ewes in Group 2, and 20 ewes in Group 3, as detailed in Table 1.

Reproductive efficiency parameters

The combined hormonal-vitamin treatment (Group 2) yielded optimal reproductive outcomes, achieving complete fertility (100%) with absence of neonatal mortality. Vitamin supplementation alone (Group 3) resulted in moderate fertility (85%), while exclusive hormonal treatment (Group 1) showed reduced fertility (80%) accompanied by significant neonatal mortality (16.7%).

Fecundity was significantly enhanced in Group 2 (160%) compared with Group 3 (110%; $P = 0.035$), with Group 1 exhibiting intermediate performance (125%) that did not differ statistically from either group. Prolificacy rates were comparable between

Table 2. Effects of hormonal and vitamin treatments on reproductive parameters in Rembi ewes (Tiaret, Algeria, 2024).

Groups	Number of treated ewes	Ewes that lambed n (%)	Single births n	Twin births n	Triplet births n	Total lambs born n	Neonatal deaths n
Group 1 (Hormonal only)	20	16 (80.0)	8	7	1	25	5
Group 2 (Hormonal + AD_3E)	20	20 (100.0)	10	8	2	32	0
Group 3 (AD_3E only)	20	17 (85.0)	12	5	0	22	0
Total	60	53 (88.3)	30	20	3	79	5

Table 3. Reproductive performance parameters and statistical comparisons between experimental groups in Rembi ewes (Tiaret, Algeria, 2024).

Parameter	Group 1 (Hormonal only)	Group 2 (Hormonal + AD_3E)	Group 3 (AD_3E only)	Statistical comparisons (p-value)
Fertility rate (%)	80.0 ± 4.10^{a}	100.0 ± 0.00^{b}	85.0 ± 3.66^{ab}	1 vs 2 : 0.040* 1 vs 3 : 0.696 2 vs 3 : 0.080
Fecundity rate (%)	125.0 ± 8.50^{ab}	160.0 ± 6.80^{b}	110.0 ± 6.40^{a}	1 vs 2 : 0.214 1 vs 3 : 0.556 2 vs 3 : 0.035*
Prolificacy rate (%)	156.3 ± 6.29	160.0 ± 6.80	129.4 ± 4.69	1 vs 2 : 0.929 1 vs 3 : 0.206 2 vs 3 : 0.164
Neonatal mortality rate (%)	16.7 ± 3.16^{a}	0.0 ± 0.00^{b}	0.0 ± 0.00^{b}	1 vs 2 : 0.021* 1 vs 3 : 0.033* 2 vs 3: 1.000
Lamb birth weight (kg)	3.02 ± 0.62^{a}	3.48 ± 1.01^{b}	4.25 ± 0.70^{b}	1 vs 2 : 0.026* 1 vs 3 : <0.001*** 2 vs 3 : 0.976

Values are presented as mean ± standard deviation. Different superscript letters (^{a}, ^{b}) within a row indicate significant differences ($p < 0.05$); *= Significant ($p < 0.05$); *** = Very highly significant ($p < 0.001$)

hormonally treated groups (Group 1: 156.3%; Group 2: 160.0%), while Group 3 demonstrated lower prolificacy (129.4%), although these differences lacked statistical significance.

Lamb viability and development

Notably, vitamin supplementation eliminated neonatal mortality in both Groups 2 and 3, contrasting significantly with Group 1 ($P = 0.021$ and $P = 0.033$, respectively). Lamb birth weights were maximized in Group 3 (4.25 ± 0.70 kg), significantly exceeding Group 1 (3.02 ± 0.62 kg; $P < 0.001$) while comparable with Group 2 (3.48 ± 1.01 kg; $P = 0.976$).

Multivariate pattern analysis

Principal Component Analysis (PCA) revealed distinct reproductive profiles among treatment groups, with two principal components explaining 100% of total variance (Fig. 1). Group 2 (hormonal + AD_3E) clustered strongly with enhanced fertility (100%) and fecundity (160%) parameters. Group 1 (hormonal only) associated with neonatal mortality (16.7%), while Group 3 (AD_3E only) correlated with improved birth weights (4.25 kg). This clear separation demonstrates the differential efficacy of each treatment strategy and confirms the synergistic effect of combining hormonal synchronization with vitamin supplementation. The multivariate approach provides statistical validation of the hypothesis that combined intervention optimizes reproductive outcomes beyond single-treatment approaches in Rembi ewes during seasonal anoestrus.

Discussion

Synergistic enhancement of reproductive performance

The present study clearly demonstrates that the integration of hormonal synchronization with vitamin AD_3E supplementation generates a synergistic improvement in reproductive efficiency of Rembi ewes during seasonal anoestrus. Group 2, which received the combined treatment, achieved outstanding performance (100% fertility, 160% fecundity, and complete absence of neonatal mortality). These findings are consistent with previous reports emphasizing the complementary interactions between hormonal induction protocols and nutritional support in optimizing ovine reproductive outcomes (Hameed et al., 2021; Martinez-Ros et al., 2019; Al-Zubaidi et al., 2024; Benia et al., 2022). Importantly, the absence of neonatal losses in vitamin-supplemented groups reinforces the vital role of vitamins AD_3E in sustaining embryonic and foetal development, particularly under the challenging environmental conditions of the Algerian steppe (Gani, 2023; Bouacha, 2019).

Molecular and physiological mechanisms

The enhanced efficacy of the combined protocol likely arises from a convergence of molecular mechanisms through which vitamins potentiate

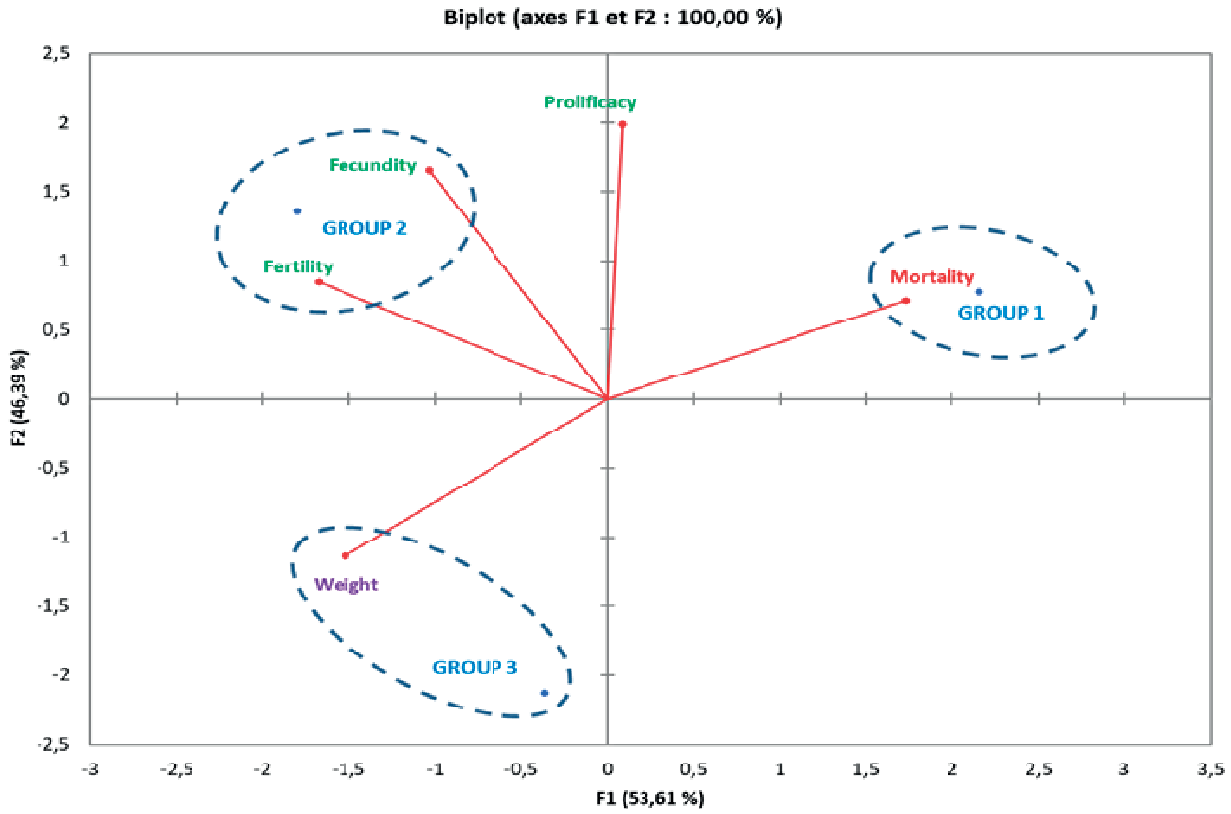

Fig. 1. Projection of three experimental ewe groups on the PCA factorial plane (F1 × F2) based on reproductive and productive performance ndicators

hormonal responsiveness. Vitamin D_3 has been shown to upregulate endometrial progesterone receptors, thereby strengthening luteal function and pregnancy maintenance (Meng et al., 2023). Vitamin E exerts protective effects on oocyte integrity by counteracting oxidative stress (Hatami et al., 2022), while vitamin A contributes to follicular growth and maturation (Abdulkareem et al., 2023; Li et al., 2024). Together, these micronutrients create a metabolic and endocrine milieu favourable to the successful action of exogenous hormones, explaining the superior reproductive outcomes observed in the combined treatment group.

Neonatal viability and metabolic efficiency

Vitamin supplementation also had a profound impact on lamb survival and growth. The elimination of neonatal mortality, coupled with improved birth weights, suggests that vitamins AD_3E enhance maternal-offspring metabolic efficiency. Several mechanisms can be proposed: improved colostrum quality and immunoglobulin transfer (Reyes, 2023; Hurlburt, 2025), enhanced antioxidant defence capacity (Borges et al., 2018; Nikolova et al., 2023), and optimized neuromuscular coordination essential for suckling (Stenhouse et al., 2022). These effects are particularly critical in resource-constrained systems, where early neonatal losses remain a major bottleneck to productivity (Dwyer et al., 2016; Samuel and Shortnacy, 2025). The results confirm that beyond fertility enhancement, nutritional support contributes directly to offspring viability and long-term flock sustainability.

Comparative efficacy and economic perspectives

The reduced fertility observed in Group 1 (80%), which relied solely on hormonal synchronization, highlights the limitations of hormonal interventions when nutritional constraints are not addressed. This observation aligns with evidence that metabolic status significantly modulates ovarian responsiveness to exogenous hormones (Larsen, 2021; Martin et al., 2004; Bacha et al., 2017). From an economic standpoint, although the combined protocol produced the most favourable reproductive outcomes, vitamin supplementation alone (Group 3) also yielded substantial improvements at a lower cost. This approach may, therefore, represent a practical and scalable solution for smallholder or resource-limited farmers. The relative cost-benefit of each strategy must be carefully weighed against production objectives, market dynamics, and management capacities.

Practical and ethical implications

In practice, the choice between combined protocols and vitamin supplementation alone depends on the production system and socio-economic context. While semi-intensive farms with structured breeding programmes may justify the higher investment in combined treatments, extensive systems may prioritize the cost-effective benefits of vitamin supplementation alone (Meziane et al., 2024). Ethical considerations also play a role, particularly regarding the use of PMSG and the necessity of sustainable reproductive management strategies (Veterinary Medical Ethics, 2022). Tailored recommendations for North African semi-arid regions should thus balance biological efficacy, economic feasibility, and ethical acceptability, ensuring that reproductive technologies remain both effective and accessible for livestock keepers.

Conclusion

This study successfully addresses its fundamental research objective by demonstrating that the integration of hormonal synchronization protocols with vitamin AD_3E supplementation significantly enhances reproductive outcomes in Rembi ewes during seasonal anoestrus under semi-arid conditions. The experimental results directly respond to the initial scientific problem of declining reproductive efficiency in North African sheep production systems by providing two evidence-based solutions: a combined protocol achieving optimal reproductive performance (100% fertility, 160% fecundity, 0% neonatal mortality), and a vitamin-only approach offering substantial improvements in lamb viability and birth weights.

The findings establish clear connections between our research aims and outcomes by:

1. validating the synergistic potential of combining reproductive biotechnologies with nutritional management;
2. demonstrating context-appropriate strategies for different production systems;
3. providing scientific justification for targeted nutritional interventions in reproductive management.

These results offer practical solutions to enhance production resilience in semi-arid regions while addressing both economic constraints and animal welfare considerations. Future investigations should focus on elucidating the molecular mechanisms underlying the observed vitamin-hormone interactions and conducting economic analyses to facilitate the implementation of these strategies in traditional farming systems.

This comprehensive approach effectively bridges the gap between experimental research and practical application, providing sustainable solutions to improve reproductive efficiency in challenging agricultural environments while maintaining scientific rigor and practical relevance.

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