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Posted Date: 8 April 2025

doi: [10.20944/preprints202504.0643.v1](https://doi.org/10.20944/preprints202504.0643.v1)

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Article

Flaws in Estrus Synchronization Protocols Increase Vaginal Prolapse and Hydrometra Risk in Sheep

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Abstract: This study examines the reproductive outcomes of Lacaune crossbred ewes and hoggets under intensive production systems, focusing on vaginal prolapse and hydrometra associated with flaws in estrus synchronization (E.S.) protocols. Data from multiple farms were combined for analysis due to the absence of significant variation at the farm level. The findings revealed a strong association between vaginal prolapse, parity, and litter size, with hoggets carrying multiple fetuses facing the highest risk ($p < 0.0001$). This highlights the need to reconsider equine chorionic gonadotropin (eCG) administration in hoggets, as it increases the likelihood of multiple pregnancies and, consequently, prolapse. Additionally, a progressive rise in hydrometra prevalence was observed with repeated synchronization cycles in ewes, increasing from 0.51% after the third treatment to 12.33% after the fourth ($p < 0.0001$). Notably, in this study, the four synchronization cycles were applied over a relatively short period (7.22 ± 1.64 months), further supporting concerns that excessive hormonal treatments within a compressed timeframe exacerbate reproductive dysfunction. This pattern confirms that prolonged progesterone exposure can impair uterine function, leading to fluid retention and hydrometra, as previously reported. To mitigate these risks, adjustments in synchronization protocols—such as extending the interval between successive treatments—are necessary to safeguard reproductive health and enhance animal welfare. These findings underscore the importance of optimizing estrus synchronization strategies to balance productivity with the well-being of sheep in intensive farming systems.

Keywords: Estrus synchronization; Vaginal prolapse; Hydrometra; Lacaune crossbred sheep; Equine chorionic gonadotropin (eCG); Reproductive management; Intensive sheep farming

1. Introduction

Sheep farming plays a crucial role in Greece's agricultural sector, with approximately 7.3 million animals spread across 83,000 holdings [1]. The sector is predominantly dairy-focused, with 93% of sheep raised for milk production, mainly to produce feta cheese—a Protected Designation of Origin (PDO) product and the largest export of Greece's agricultural industry [2].

Despite its economic and cultural significance, the Greek sheep population has declined by over 10% since 2010. This trend is accompanied by a shift from small and medium-sized farms to fewer, larger operations, often with flocks exceeding 500 ewes. These larger enterprises predominantly employ intensive indoor production systems, relying heavily on imported concentrate feeds, supplemented with locally produced hay and, occasionally, ensilage. The current sheep population

largely consists of crossbreeds between indigenous Greek breeds and foreign breeds, particularly Lacaune, which has shown strong adaptability to the intensive methods now prevalent in the Greek dairy sheep sector [3].

In response to the demands of modern dairy sheep farming, estrus synchronization protocols, such as the use of progestagen intravaginal sponges combined with intramuscular injections of equine chorionic gonadotropin (eCG), have become widespread. These protocols are extensively used in Greece to induce estrus and facilitate mating during the natural anestrous period from January to March, when reproductive activity is physiologically reduced.

Under natural conditions, sheep in Greece typically resume reproductive cyclicity in April–May after a brief period of reproductive inactivity from late January to late March. Advancing the breeding season into this infertile period aims to ensure early lambing and sustain year-round milk production, supporting the fresh yogurt industry, a major consumer of sheep milk [4]. Additionally, estrus synchronization helps to cluster lambings within specific ewe cohorts, thereby optimizing flock management and labor efficiency.

However, concerns have been raised by veterinarians and farmers regarding a potential link between eCG use in hoggets and an increased incidence of vaginal prolapse, as well as a higher prevalence of hydrometra in adult ewes subjected to repeated eCG administrations. Despite the clinical significance of these observations, no systematic studies have been conducted to quantify the occurrence of these reproductive disorders or assess their relationship with estrus synchronization protocols in large commercial flocks.

This study aims to systematically evaluate the prevalence of vaginal prolapse and hydrometra in a representative sample of modern Greek dairy sheep farms. It further investigates whether these reproductive disorders are associated with flaws in eCG-based estrus synchronization protocols, with the goal of providing insights into optimizing reproductive management while enhancing animal welfare in intensive sheep farming systems.

2. Materials and Methods

2.1. Study Design

A cross-sectional observational study was conducted from September 1, 2022, to February 29, 2024, in the counties of Karditsa, Trikala, and Larissa, located in the Thessaly region of Central Greece. This region is a major hub for intensive dairy sheep farming, representing typical conditions for large-scale, specialized milk production systems [1].

A total of 140 commercial dairy sheep farms participated in the study. The combined number of reproductive females (ewes and hoggets) across all farms was 97,215, with individual flock sizes ranging from 500 to 1,400 animals, consistent with the structural characteristics of modern Greek dairy sheep enterprises [4].

2.2. Farm Selection Criteria

Farms were selected based on the following inclusion criteria:

1. Housing system: Exclusive use of fully indoor intensive housing, characterized by permanent confinement and feeding with complete mixed rations composed of commercial concentrates and locally produced forages (hay and/or ensilage).
2. Flock size: A minimum of 500 reproductive females, reflecting the threshold for economically viable intensive dairy sheep production in Greece [1].
3. Estrus synchronization protocols: Routine application of estrus synchronization involving the insertion of progestagen-impregnated intravaginal sponges for 12–14 days, followed by an intramuscular injection of 500 IU of eCG at sponge withdrawal [4].
4. Ultrasonographic pregnancy diagnosis: Regular use of transabdominal ultrasonography 45–60 days post-mating as part of herd reproductive monitoring.

Farmers provided written informed consent prior to participation.

2.3. Data Collection

2.3.1. Reproductive History

Reproductive history was systematically recorded for each farm throughout the study period. Data collected included:

- Annual milk production.
- Age group (hoggets or ewes) of each reproductive female.
- Dates of reproductive management events, including estrus synchronization procedures and ram introduction for natural mating.

2.3.2. Clinical Examination

Biweekly farm visits were conducted throughout the study period. During each visit, all pregnant animals were visually examined for signs of vaginal prolapse, with manual palpation performed when necessary to confirm diagnosis. Vaginal prolapse was defined as the partial or complete protrusion of the vaginal wall through the vulva, with or without cervical displacement [5].

2.3.3. Ultrasonographic Examination

Ultrasonographic examinations were performed using a portable ultrasound device equipped with a 3.5–5.0 MHz convex transducer, following established protocols for pregnancy diagnosis in sheep [6]. Scans were conducted 45–70 days post-mating.

Fetal counts were initially determined through ultrasound examination and subsequently reassessed and corrected, if necessary, after parturition or post-mortem examination in cases of pregnancy loss.

Hydrometra was diagnosed based on the following ultrasonographic criteria [7]:

1. Visualization of anechoic or hypoechoic intrauterine fluid accumulation,
2. Absence of embryonic or fetal structures,
3. Presence of thin echogenic septations within the fluid, when present.

2.4. Data Management and Statistical Analysis

All collected data were entered into a digital database using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and verified for consistency and completeness.

The prevalence of vaginal prolapse and hydrometra was calculated for the entire study population. Associations between categorical variables (e.g., hoggets vs. ewes) were analyzed using the Chi-square (χ^2) test, while differences among multiple groups (e.g., number of estrus synchronization protocol applications, parity) were evaluated using one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

All statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA).

3. Results

The study population consisted of Lacaune crossbred ewes and hoggets, with farm breeding records indicating an estimated Karagouniko breed ancestry of less than 20%. The adult females of these crossbred animals had an average body weight of approximately 70 kg and exhibited the morphological characteristics of the Lacaune breed. Their mean annual milk production during an 8-month lactation period was 420 ± 42 kg ($n = 85,403$).

Hoggets were introduced to intravaginal sponges for the first time at an average age of 8.5 ± 1.05 months ($n = 14,494$).

Both ewes and hoggets followed the same estrus synchronization protocol, which involved the insertion of intravaginal estrogen-impregnated sponges for 14 days, followed by an intramuscular administration of 500 IU of eCG at sponge removal.

The study population included hoggets as well as ewes in their second, third, and fourth parity, with one lambing per year. Under the intensive management systems examined, ewes were typically retained until their fourth lambing and culled at the end of the subsequent lactation period.

3.1. Vaginal Prolapse

The incidence of vaginal prolapse was analyzed in ewes at their second, third, and fourth parity and compared to hoggets experiencing their first pregnancy (Table 1). The prevalence of vaginal prolapse was significantly higher in hoggets carrying twins or triplets compared to ewes with the same litter size ($p < 0.0001$). Furthermore, a progressive increase in vaginal prolapse occurrence was observed in both groups as litter size increased ($p < 0.0001$), with the highest prevalence recorded in triplet-bearing animals.

Table 1. Prevalence of Vaginal Prolapse in Hoggets and Ewes.

Category	Hoggets (n, %)	Vaginal Prolapse in Hoggets (n, %)	Ewes (n, %)	Vaginal Prolapse in Ewes (n, %)
Total Pregnant Animals	14,494 (100%)	135 (0.93† ^a)	71,445 (100%)	243 (0.34§ ^a)
Pregnancy with Singleton	3,154 (21.76%)	5 (0.16† ^b)	14,245 (19.94%)	24 (0.17§ ^b)
Pregnancy with Twins	6,275 (43.29%)	69 (1.10† ^c)	31,740 (44.43%)	105 (0.33§ ^a)
Pregnancy with Triplets	5,065 (34.95%)	61 (1.20† ^c)	25,460 (35.64%)	114 (0.45§ ^c)

†, §: Prevalences between hoggets and ewes followed by different symbols are significantly different (Chi-square test, $p < 0.0001$). ^{abc}: Prevalences in the same column followed by different letters differ significantly within hoggets or ewes (ANOVA, $p < 0.0001$).

3.2. Hydrometra

The analysis of hydrometra cases was limited to ewes with one or two previous parturitions (Table 2). According to standard farm management practices, ewes that had undergone three parturitions and failed to conceive after a single estrus synchronization attempt were culled and sent to slaughter. Similarly, hoggets that failed to conceive after two consecutive synchronization attempts were removed from the herd.

Table 2 presents the prevalence of hydrometra in ewes subjected to consecutive estrus synchronizations. A total of 146 ewes underwent four consecutive synchronization treatments over an average period of 7.22 ± 1.64 months. The analysis revealed a significant association between repeated estrus synchronization and hydrometra development. No cases of hydrometra were recorded following the first two synchronization attempts. However, the prevalence increased to 0.51% after the third synchronization and rose sharply to 12.33% following the fourth synchronization ($p < 0.0001$).

Table 2. Prevalence of Hydrometra in Ewes.

Category	1st E.S. (n, %)	2nd E.S. (n, %)	3rd E.S. (n, %)	4th E.S. (n, %)
Treated	80,250 (100%)	5,542 (100%)	585 (100%)	146 (100%)
Pregnant	74,408 (92.72 ^a)	4,865 (87.78 ^b)	403 (68.89 ^c)	62 (42.47 ^d)
Non-Pregnant †	5,842 (7.28 ^a)	677 (12.22 ^b)	182 (31.11 ^c)	84 (57.53 ^d)
Hydrometra Cases	0 (0%)	0 (0%)	3 (0.51 ^a)	18 (12.33 ^b)

†: Includes cases of hydrometra. ^{abcd}: Prevalences in the same row followed by different letters are significantly different (ANOVA for pregnancy and non-pregnancy; Chi-square test for hydrometra, $p < 0.0001$).

4. Discussion

This study evaluated the reproductive outcomes of Lacaune crossbred ewes and hoggets managed under intensive production systems, with a focus on vaginal prolapse and hydrometra associated with estrus synchronization protocols. The findings highlight important reproductive challenges that must be addressed when optimizing breeding strategies in dairy sheep populations.

Data from multiple farms were combined due to the lack of additional variation at the farm level in previous pre-trials, ensuring a more comprehensive and robust assessment. The results revealed a significant association between vaginal prolapse and both parity and litter size, with hoggets carrying multiple fetuses exhibiting the highest risk ($p < 0.0001$). These findings align with previous studies suggesting that increased fetal burden, particularly in younger animals, exerts excessive pressure on pelvic structures, making them more susceptible to prolapse. The higher incidence in hoggets may also be linked to their still-developing musculoskeletal support structures, which may be less capable of withstanding the mechanical strain associated with twin or triplet pregnancies [8,9].

In Greece, it is recommended that hoggets be mated when they have reached at least two-thirds of the adult ewe weight (approximately 70 kg) or when they are at least nine months old. However, in practice, these guidelines are often not followed. This may contribute to the increased incidence of vaginal prolapse observed in this study, as younger or underweight hoggets may experience reproductive complications, including prolapse, due to insufficient pelvic and musculoskeletal development [5,8].

Our findings suggest that special attention should be given to hoggets during their first pregnancy, as parity is a significant risk factor for prolapse. In practice, many farmers administer the standard adult dose of eCG to underweight hoggets, resulting in a higher dose per kilogram. This practice could increase the likelihood of multiple pregnancies and, consequently, the risk of vaginal prolapse. To mitigate prolapse risk, it is crucial to reconsider the use of eCG in hoggets and adopt synchronization protocols that either reduce or eliminate eCG injections for this vulnerable group.

Regarding hydrometra, prevalence rates in the literature vary widely, typically ranging from 0.15% to 4.70%, with some studies reporting rates as high as 10% [10–15]. In this study, a clear correlation was observed between repeated estrus synchronization cycles and the development of hydrometra. No cases were detected after the first or second synchronization treatments, but prevalence significantly increased after the third treatment (0.51%) and escalated to 12.33% following the fourth treatment ($p < 0.0001$). It is worth noting that in our study, a total of 146 ewes underwent four consecutive synchronization treatments over a very short average period of 7.22 ± 1.64 months.

Our findings about hydrometra align with research suggesting that prolonged exposure to exogenous hormonal treatments can disrupt normal uterine function, leading to fluid accumulation and impaired embryo implantation [16]. Other studies indicate that hormonal treatments interfere with the luteolytic process, resulting in prolonged corpus luteum persistence and fluid retention within the uterus [17,18]. Hydrometra has also been associated with prolonged progesterone exposure, which suppresses uterine contractility and disrupts the clearance of uterine secretions [19].

Our results underscore the importance of re-evaluating the current estrus synchronization protocols in intensive dairy sheep farming systems. Although synchronization remains a useful tool for improving reproductive efficiency, there is growing evidence that adjustments are necessary to minimize the associated risks. A more sustainable approach may involve extending rest periods between synchronization cycles, reducing the frequency of hormonal treatments, and incorporating natural breeding methods or synchronization using prostaglandin sponges without the injection of eCG, particularly for vulnerable age groups such as hoggets. These strategies could help mitigate reproductive complications while preserving herd productivity and enhancing animal welfare.

5. Conclusions

This study highlights the reproductive challenges associated with estrus synchronization in Lacaune crossbred sheep. The findings demonstrate that hoggets carrying multiple fetuses are at a significantly higher risk of vaginal prolapse. Given that the administration of eCG increases the likelihood of multiple pregnancies, its use in hoggets should be carefully reconsidered to mitigate this risk. Additionally, the study reveals a notable increase in the incidence of hydrometra with repeated synchronization treatments in ewes, which may be related to the prolonged effects of progesterone on uterine function, as documented in previous research. These results underscore the importance of carefully selecting and adjusting estrus synchronization protocols to strike a balance between improving reproductive efficiency and prioritizing animal health and welfare.

Author Contributions: Conceptualization, N.T., and G.C.; methodology, N.T., D.G., and G.C.; software, M.A.B.C., and G.C.; validation, D.G., G.I.P., V.G.P., and G.C.; formal analysis, G.C.; investigation, N.T., I.T., C.K., and G.C.; resources, N.T., I.T., V.G.P., and G.C.; data curation, D.G., G.I.P., V.G.P., and G.C.; writing—original draft preparation, N.T., I.T., and G.C.; writing—review and editing, N.T., M.A.B.C., C.K., and G.C.; visualization, G.C.; supervision, D.G., G.I.P., V.G.P., and G.C.; project administration, G.C.; funding acquisition, N.T. and G.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Institutional Review Board Statement: All procedures involving the care and use of animals were reviewed and approved by the Institutional Ethics Committee of the University of Thessaly (Protocol Code 102, Date of Approval: 16 November 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the first author (N.T.) upon reasonable request.

Conflicts of Interest: Nikolaos Tsekouras is employed, as Small Ruminants Technical Manager, by MSD Animal Health Greece, which markets medications, including products for estrus synchronization for use in sheep. This may be perceived as a potential conflict of interest. The views expressed in this paper are those of the author, they are not influenced by his position and do not necessarily reflect the opinions or policies of MSD Animal Health Greece.

Abbreviations

The following abbreviations are used in this manuscript:

PDO	Protected Designation of Origin
ES	estrus synchronization
eCG	equine chorionic gonadotropin

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