

# Evaluation of Reproductive Status in Romanian Buffalo Farms Using Ultrasonographic Monitoring

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**Abstract:** The aim of this study was to evaluate the reproductive status of buffaloes from two farms in Romania by means of transrectal ultrasonography, with the intent to provide practical insights for both farmers and veterinary professionals. A total of 336–352 buffalo cows were examined at Farm A (Arad County, Romania), and 178–183 at Farm B (Brașov County, Romania), during the spring and autumn of 2024. Farm A implemented artificial insemination (AI) based on natural estrus detection, while Farm B relied on natural mating, with an approximate bull to female ratio of 1:25. The results indicated superior reproductive performance in Farm B, demonstrated by a shorter calving interval (428–431 days) and a reduced service period (125–129 days), in comparison with Farm A (445–448 days and 136–142 days, respectively;  $p < 0.05$ ). The average number of inseminations per conception in Farm A was 1.6–1.7, and the incidence of postpartum endometritis was slightly higher (1.0–1.2%) than in the naturally mated group (0.3–0.5%). Transrectal ultrasonography proved indispensable for the accurate diagnosis of gestational stages, ovarian structures, and uterine pathologies, enabling the classification of buffaloes into well defined reproductive groups. These findings suggest that, under local management conditions, well structured natural mating can surpass the efficiency of standalone AI, highlighting the importance of accurate estrus detection, strict hygiene protocols, and periodic reproductive monitoring in optimizing fertility and overall herd productivity.

**Keywords:** buffalo cows, reproduction, transrectal ultrasonography, artificial insemination, natural mating

## 1. Introduction

Buffalo husbandry in Romania has a longstanding tradition, particularly in lowland and hilly regions, where buffaloes are recognized for their robustness, adaptability, and extended productive lifespan. According to historical sources, water buffaloes were introduced into the territory of present day Romania as early as the 5th century AD, via the Balkan Peninsula. Owing to their high quality milk and considerable draft power, they quickly became an integral component of traditional rural households [1-3].

Despite their genetic potential and rustic hardiness, buffalo populations in Romania have significantly declined in recent decades. One of the major challenges currently facing buffalo farmers is the optimization of reproductive performance. Reproduction is a key determinant of farm profitability; fertility disorders lead directly to prolonged calving intervals, reduced milk yield, increased costs due to repeated inseminations, and economic losses through the premature culling of infertile females [4-6].

The Romanian buffalo exhibits distinctive reproductive characteristics, including relatively subtle estrus expression, with signs such as restlessness, reduced feed intake, and occasional vocalization, lasting on average 5–27 hours, with ovulation occurring roughly 24–48 hours after estrus onset [7,8].

Ovarian activity typically involves two or three follicular waves per estrous cycle, most commonly two, with the estrous cycle averaging around 21 days. Ovary size and follicle number vary throughout the cycle, reflecting ongoing follicular growth, while the corpus luteum develops from the ruptured follicle after ovulation, with its size and

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function being critical for pregnancy maintenance. Hormonal profiles show lower peak progesterone and estradiol-17 $\beta$  levels compared to other ruminants, and variations in luteal phase duration influence overall cycle length, all of which are important for effective reproductive management in this breed [9].

Recent studies have highlighted the main reproductive pathologies affecting buffaloes. Ovarian hypofunction remains the predominant cause of infertility, with reported incidence rates reaching 70–80% in certain groups [10]. Other commonly observed dysfunctions include prolonged postpartum anestrus, subestrus, characterized by weak or undetectable signs of estrus, and early embryonic mortality [11,12].

These conditions are influenced by seasonal factors, inadequate nutrition, and heat stress during the summer months. Structural abnormalities such as ovarian hypoplasia, cystic ovaries, and persistent corpus luteum have a direct negative impact on conception rates. In addition, infectious diseases like endometritis and pyometra, although less prevalent than functional disorders, can compromise the fertility of the entire herd [13,14].

In Romania, reproductive parameters in native buffalo breeds remain relatively modest compared to cattle. The average age at first calving is 38–40 months, service periods frequently exceed 130–170 days, and calving intervals often range between 440 and 480 days. Such extended intervals are indicative of suboptimal fertility, with direct repercussions on the productivity and sustainability of buffalo farms [4,5].

Against this background, the assessment of reproductive status in buffalo herds becomes essential. The present study aimed to monitor the reproductive condition of buffaloes from two Romanian farms through individual gynecological examinations carried out in both spring and autumn seasons, thereby providing a comprehensive overview of reproductive function throughout the year. The resulting data may serve as a practical reference model for both farmers and veterinary practitioners, offering clear insights into the prevalence of major pathologies, key fertility parameters, and evidence-based management strategies that can contribute to improving reproductive indices in Romanian buffalo farming systems.

## 2. Materials and Methods

### 2.1 Biological Material and Description of the Investigated Buffalo Farms

The study was conducted in two buffalo farms located in different counties of Romania (Arad County and Braşov County), selected as representative models for distinct breeding systems, nutritional management, and reproductive technologies. The purpose of this selection was to ensure a broad and comparative framework for evaluating reproductive performance under differing management conditions.

The first farm, situated in Arad County (Romania), is located in a lowland region and manages a herd of approximately 350 adult Romanian buffalo cows, aged between 1.5 and 12 years, all reproductively active. The animals are kept under a free housing system, with extensive grazing during the warm season and semi open shelters during the winter calving period. The on farm forage base consists of natural pastures, hayfields, and cultivated forage crops, supplemented with mineral and nutritional additives depending on the season. Reproduction in this farm is based exclusively on AI, applied 8-12h after the detection of natural estrus. This method is consistently used with the aim of optimizing reproductive indicators and reducing the risk of venereal disease transmission.

The second farm, located in Braşov County (Romania), operates in a hilly depression area and maintains a herd of 186 adult buffaloes, including both primiparous and multiparous females, all of which had initiated reproductive activity. The herd is managed under a semi intensive system, with access to mountain pastures during the warm season and structured housing throughout the winter. In contrast to the first farm, reproduction in this herd is carried out exclusively through natural mating, with a bull to female ratio of approximately 1:25, ensuring coverage of the entire reproductively capable population.

In both farms, the reproductive status evaluation included all females eligible for reproduction, without restrictive selection criteria, in order to obtain a representative picture of the herd's actual reproductive condition. The assessment protocol consisted of two complete gynecological examinations conducted in spring (April–May) and autumn (September–October) of 2024, aimed at capturing seasonal variations in ovarian and uterine function seasonality being a recognized factor influencing estrus expression in buffaloes.

By including both young females at first calving and multiparous cows, this study offers a comprehensive comparative perspective on reproductive parameters within two contrasting farming systems.

## 2.2 Animal Examination and Grouping Criteria

To ensure a comprehensive and accurate reproductive diagnosis, transrectal clinical examination was complemented, in both farms, by transrectal ultrasonography using the EasyScan Linear ultrasound device (BCF Technologies, Maravet), equipped with a linear probe and specialized imaging modes: Ovary, Early Pregnancy, Late Pregnancy, Pregnancy, and Detail. The application of these modes enabled real time visualization of ovarian and uterine structures, as well as pregnancy status, significantly improving diagnostic precision.

Based on the clinical and imaging findings, buffalo cows from both farms were assigned to the following reproductive categories, each defined by specific clinical and ultrasonographic criteria:

- Pregnant (1–4 months): Diagnosis was established by identifying small amniotic vesicles, the characteristics of intrauterine fluids, and fetal size using the Early Pregnancy and Detail modes. These findings were corroborated by transrectal palpation (uterine fluctuation, presence of a functional corpus luteum, and asymmetry of the uterine horns).

- Pregnant (4–8 months): Diagnosis was based on direct visualization of the developing fetus, fetal positioning, amniotic fluid characteristics, and signs of fetal vitality using the Pregnancy and Late Pregnancy modes. These findings were confirmed by clinical examination (uterine size and tone of the pregnant horn).

- Pregnant (>8 months): This group included animals with advanced pregnancies, identified through ultrasound imaging of a near-term fetus, assessment of fetal presentation, and uterine expansion, in correlation with external clinical signs (enlargement of the abdominal volume, pelvic ligament relaxation).

- Reproductively Active: Diagnosis was based on the detection of either a functional corpus luteum or a dominant mature follicle with typical morphology and dimensions, visualized via the Ovary mode. These findings confirmed the presence of active ovarian cyclicity.

- Anestrus: Buffaloes in this category exhibited no functional ovarian structures (absence of corpus luteum and mature follicles), as confirmed by the Ovary mode and the absence of external estrus signs. These findings were consistent with ovarian inactivity.

- Ovarian Cysts (Luteal or Follicular): Diagnosis was established by identifying cystic structures exceeding 2 cm in diameter, with thin walls and anechoic content (follicular cysts) or thickened walls and mixed echogenicity (luteal cysts), using the Ovary mode and confirmed by transrectal palpation.

- Uterine Infections (Endometritis): Animals with varying degrees of endometritis were diagnosed based on the presence of intrauterine echogenic fluid, increased uterine wall thickness, and intrauterine pathological material in the Detail mode, in conjunction with clinical signs such as vaginal discharge and altered uterine tone.

This integrative approach, employing advanced ultrasound equipment and standardized diagnostic protocols, allowed the classification of buffaloes into homogeneous reproductive groups. This provided a robust foundation for comparative analysis of reproductive parameters across farms, seasons, and reproductive management systems.

## 2.3 Statistical Analysis of Data

The data obtained from clinical and ultrasonographic examinations were compiled into an electronic database, structured by farm, control season (spring and autumn), and reproductive category. For comparative interpretation, the relative incidence of each reproductive status category, the proportion of fertile versus reproductively dysfunctional buffaloes, and the distribution of pathologies by age group and season were calculated.

Key reproductive parameters, such as calving interval, service period duration, and age at first calving, were statistically analyzed as arithmetic means accompanied by standard deviations, to capture both intra- and inter-group variability. Differences between farms and seasons were statistically tested using data processing software such as Microsoft Excel and SPSS version 25. Statistical significance of observed differences was assessed using frequency analysis and the independent samples *t*-test. Where relevant, correlations between management factors (housing, nutrition) and reproductive status were also explored.

A significance level of  $p < 0.05$  was established, ensuring that statistically significant results could be interpreted with an acceptable degree of confidence. All values were synthetically presented in tables and graphical formats, facilitating comparative analysis between the two reproductive and management systems investigated.

### 3. Results

#### 3.1. Reproductive Status in Farm A and Farm B

Following the clinical and ultrasonographic examinations conducted in spring and autumn 2024, the reproductive group structure showed significant variations between the two farms and across seasons, reflecting the influence of management practices, nutrition, and reproductive technologies applied.

In Farm A, the examined population consisted of 336 buffaloes in the spring and 352 in the autumn. The proportion of pregnant buffaloes remained relatively stable, with a slight increase towards autumn, indicating satisfactory reproductive efficiency within the context of artificial insemination performed at natural estrus. The distribution across gestational trimesters revealed a higher number of animals in the first and second trimesters, suggesting effective breeding planning and a relatively compact calving window.

Conversely, in Farm B, with 178 buffaloes examined in spring and 183 in autumn, the incidence of pregnant animals was somewhat lower. The reproductive group distribution highlighted a higher proportion of reproductively active buffaloes, alongside a slightly increased incidence of ovarian functional disorders, particularly anestrus and follicular cysts.

Comparatively, the incidence of uterine infections (endometritis of varying severity) remained low in both farms, with a statistically higher prevalence observed in Farm A ( $p > 0.05$ ). Summary data on the reproductive group structure are presented in Table 1, expressed as percentages of the total buffalo population examined, with comparisons drawn between farms and seasons.

**Table 1.** Reproductive Status in Farm A and Farm B

Reproductive Group	Farm A – Spring (n=336)	Farm A– Au- tumn (n=352)	Farm B – Spring (n=178)	Farm B – Autumn (n=183)
Pregnant – First tri- mester (1–4 mo)	31.0 <sup>a</sup>	28.4 <sup>a</sup>	21.9 <sup>b</sup>	23.0 <sup>b</sup>
Pregnant – Second tri- mester (4–8 mo)	27.1 <sup>a</sup>	29.5 <sup>a</sup>	20.2 <sup>b</sup>	21.3 <sup>b</sup>
Pregnant – Third tri- mester (>8 mo)	14.0	17.0	12.4	13.1
Reproductively active (CL/dominant follicle)	20.0 <sup>b</sup>	18.8 <sup>b</sup>	27.5 <sup>a</sup>	26.2 <sup>a</sup>
Anestrus	4.5 <sup>b</sup>	4.0 <sup>b</sup>	11.8 <sup>a</sup>	10.9 <sup>a</sup>
Ovarian cysts (lute- al/follicular)	2.2 <sup>b</sup>	1.8 <sup>b</sup>	4.5 <sup>a</sup>	4.4 <sup>a</sup>
Endometritis	1.2 <sup>a</sup>	1.0 <sup>a</sup>	0.5 <sup>b</sup>	0.3 <sup>b</sup>

\* Values are expressed as percentages of the total buffaloes examined; different superscript letters within the same row indicate statistically significant differences between farms for the same season ( $p < 0.05$ ).

#### 3.2. Reproductive Performance Indicators in Farm A and Farm B

The data obtained highlight a generally superior reproductive efficiency in Farm B, where natural mating, organized with an optimal bull to female ratio and extensive grazing, contributed to a significant reduction in calving interval and service period compared to Farm A ( $p < 0.05$ ). In Farm A, where AI was applied based on the detection of natural estrus, the calving interval remained slightly longer. This may be attributed to the incomplete detection of estrus, which is characteristic of buffaloes with weak estrous expression, especially in the absence of strict hormonal synchronization protocols. The longer average service period in Farm A indicates a slower postpartum resumption of ovarian activity, a condition that could potentially be improved by implementing estrus synchronization protocols or more frequent ultrasound monitoring.

**Table 2.** Main reproductive performance indicators in Farm A (AI) and Farm B (natural mating), by season

Reproductive Indicator	Farm A – Spring	Farm A – Autumn	Farm B – Spring	Farm B – Autumn
Calving Interval (days)	448.6 ±	445.1 ±	428.2 ±	431.0 ±
Service Period (days)	142.5 ±	139.8 ±	125.2 ±	128.7 ±
AI per Gestation (mean number)	1.7 ± 0.4	1.6 ± 0.3	—	—

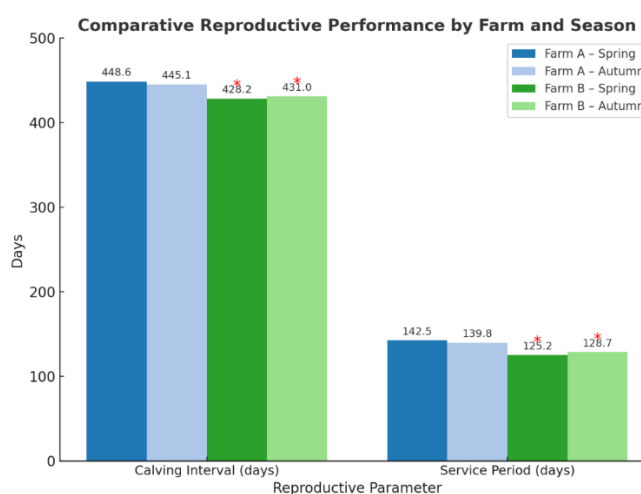
\*Values are expressed as arithmetic means ± standard deviation. Different superscript letters within the same row indicate statistically significant differences between farms within the same season ( $p < 0.05$ , t-test). The average number of inseminations per gestation applies only to Farm A, where AI was used.

The average number of inseminations per gestation in Farm A (1.7–1.6) remains reasonable for AI applied without synchronization but underscores the limitations of this method in species with subtle estrous signs. In contrast, natural mating in Farm B allows for more accurate detection of the optimal fertilization timing, which is directly reflected in more favorable reproductive parameters (Table 2).

#### 4. Discussion

The results obtained in this study provide a significant contribution to understanding the reproductive characteristics of buffaloes under the specific conditions of Romanian farms. Given the substantial decline in buffalo populations over recent decades, optimizing reproductive performance has become a crucial objective for maintaining the viability of this livestock sector and supporting the biodiversity of the Romanian buffalo breed [1].

The comparative analysis between the two farms reveals clear differences between the reproductive strategies used: natural mating (Farm B) versus AI performed on natural estrus (Farm A). The data indicate that in farms where an optimal male to female ratio and rigorous veterinary control are ensured, natural mating remains an efficient method capable of delivering favorable reproductive and productive outcomes. The significantly lower average calving interval and service period under 130 days observed in Farm B reflect effective detection of the silent estrus characteristic of buffaloes [15,16] (Fig. 1).



**Figure 1.** Calving interval and Service Period for both farms (\* indicate significant differences between farms for the same parameter and season ( $p < 0.05$ , independent t-test).

In contrast, Farm A, despite theoretically benefiting from the advantages of strict AI control, showed longer calving intervals and service periods, particularly in the spring season. This may be explained by

difficulties in estrus detection in the absence of hormonal synchronization protocols, a well-known challenge in the literature. Buffaloes exhibit a discreet estrus, which can be weak or even absent under certain nutritional or stress conditions, limiting the efficiency of AI when applied without complementary monitoring methods [17,18].

Another important finding is the higher incidence of postpartum endometritis in Farm A compared to Farm B. Although absolute prevalence values did not exceed critical thresholds, the proportional increase highlights the iatrogenic risk associated with repeated manipulations during AI, especially when hygiene and disinfection protocols are not rigorously followed. Even minor contamination can lead to chronic uterine inflammation, prolonging the service period and increasing the risk of functional infertility [19].

From a seasonal perspective, the data confirm the biological seasonality of reproduction in buffaloes. In both farms, spring examinations revealed higher proportions of reproductively active animals and an increased number of buffaloes in the first two thirds of gestation, reflecting calvings concentrated in the previous warm season. In autumn, a slight increase in anestrus incidence was observed, especially in Farm A, where detecting the post gestational cycle is more challenging under prolonged housing conditions. Literature supports that summer grazing positively influences energy balance and ovarian cycle resumption, while housing periods may exacerbate the risk of nutritional or functional anestrus [12,18,19].

Thus, buffalo reproduction in Romania requires continuous adaptation to species specific biological factors such as estrus seasonality, thermal stress sensitivity, and nutritional particularities. Natural mating proves to be an effective reproductive option under optimal conditions, especially in farms with limited herds and resources for AI. On the other hand, larger commercial operations equipped with adequate infrastructure can leverage the benefits of AI combined with hormonal synchronization protocols, allowing enhanced reproductive control and reduced risk of venereal diseases [4,12].

Ultrasonographic transrectal monitoring plays a crucial role, proving indispensable for accurate diagnosis of pregnancy status, ovarian dysfunctions, and uterine pathologies. Grouping buffaloes into reproductive functional categories based on ultrasound provides veterinarians with an objective overview of herd reproductive efficiency, facilitating timely corrective actions [20-23].

This research proposes a practical model for farmers and veterinarians managing buffalo herds in Romania, providing relevant benchmarks regarding reproductive status incidence in two distinct technological systems. Interpreting these indicators seasonally allows for optimizing management strategies, improving calving planning, and reducing economic losses associated with functional infertility.

Reproductive success in buffalo farms depends on the careful correlation of species specific biological factors, estrus seasonality, feeding quality, reproductive hygiene, and the training level of personnel involved in reproductive technologies. By disseminating these results, this study contributes to establishing best practices adapted to current economic and technological conditions in Romanian buffalo farming [24,25].

## 5. Conclusions

The results showed that in Farm B, where reproduction was managed through natural mating, the calving interval was significantly shorter (428–431 days) and the service period was reduced (125–129 days) compared to Farm A, where artificial insemination at natural estrus detection was used (445–448 days and 136–142 days, respectively;  $p < 0.05$ ). The average number of inseminations per pregnancy in Farm A was 1.6–1.7, and the incidence of endometritis was slightly higher (1.0–1.2% compared to 0.3–0.5% in Farm B). These data confirm that, for moderate buffalo herds, well organized natural mating remains more reproductively efficient than unsynchronized AI, especially in species with silent estrus. Regular ultrasonographic monitoring and strict hygienic sanitary management remain essential for optimizing fertility and reducing economic losses.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, D.B. and S.C.; methodology, S.C.; software, S.C (Simona Ciupe).; validation, D.B.R.C and S.C.; formal analysis, R.C.; investigation, S.C.; resources, S.C (Simona Ciupe).; data curation, D.B.; writing—original draft preparation, S.C.; writing—review and editing, D.B.; visualization, R.C.; supervision, L.M.B; project administration, L.M.B.; funding acquisition, S.C. All authors have read and agreed to the published version of the manuscript”.

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