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Repeat Breeding Syndrome (RBS) In Bovines: An Updated Comprehensive Overview

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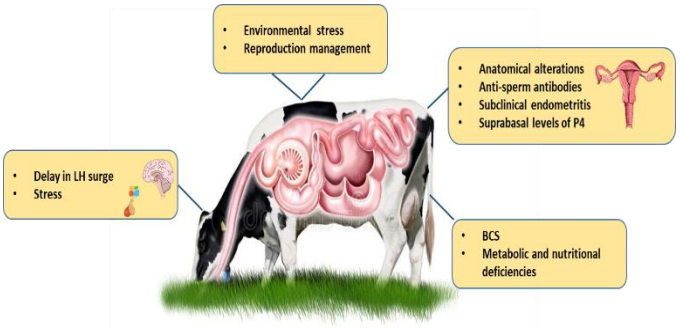
Abstract

Among the various infertility-related disorders, Repeat Breeding Syndrome (RBS) alone accounted for approximately 18–20%, highlighting its significant contribution to the overall infertility burden in dairy cows in India, with prevalence rates reported to be between 5.5% and 51.94%. Notably, 30.4% of cattle and buffaloes were culled, with infertility—including repeat breeding—identified as the primary reason for removal from the herd due to prolonged calving intervals, reduced milk production, and economic losses for dairy farmers.

Key words: Bovine, Immunomodulators, Infertility and Repeater.

INTRODUCTION



Repeat breeding Syndrome (RBS) is characterized by the failure of a sexually mature, cyclic, and clinically healthy female animal to conceive after three or more consecutive services with fertile semen or a fertile male, despite exhibiting normal oestrous cycles and the absence of detectable clinical abnormalities in the reproductive tract.

This syndrome accounts for more incidence in Crossbred cows at 17.57%, followed by buffaloes at 12.74%, and indigenous cows at 8.64%. Given its high prevalence and considerable economic impact, repeat breeding warrants focused attention for effective management and prevention strategies in both cattle and buffalo populations.

A **repeat breeder cow** is typically defined as a cow that:

- should have calved at least once
- should be **cyclic** and shows regular estrus behaviour,
- should be free from any anatomical and clinical abnormalities of the reproductive tract,
- not conceived even **after three or more breedings**, either by natural service or artificial insemination (AI),
- And **has not calved since the last parturition**.

ETIOLOGY

RBS is multifactorial, with several intrinsic and extrinsic factors contributing to its occurrence. However, all the major factors can be categorized into two:

1. Fertilization failure
2. Early Embryonic Death (EED)

1. FAILURE OF FERTILIZATION

Causes

Abnormal egg or oocytes

Several types of morphologic and functional abnormalities have been observed in unfertilized eggs, e.g., giant egg, oval shaped egg, lentil-shaped egg, and ruptured zona pellucida. Failure to undergo fertilization and normal embryonic development may be due to inherent abnormalities of the egg or to environmental factors. Successful fertilisation of the mature oocyte requires an adequate hormonal milieu. It is well known that a delay in LH peak, which is necessary for ovulation, is recognised as a frequently occurring event in cows with RBS (Bage *et al.,* 2002).

Abnormal Sperms

Certain forms of male infertility are related to structural defects of the DNA protein complex. Sperm aging and injury may cause alterations in the acrosomal cap that may prevent defective spermatozoa from fertilizing the egg. In bull, ram, and boar, a good correlation exists between fertility and acrosomal integrity. Leakage of vital intracellular constituents such as cyclic AMP or the formation of lipid peroxides from sperm plasmalogen when sperm are stored under anaerobic conditions. A gradual decrease in the fertilizing capacity of aging of spermatozoa in the female genital tract.

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Structural Barriers to Fertilization

Congenital defects are the result of arrested development of the different segments of the Mullerian ducts or of an incomplete fusion of these ducts caudally, which will interfere with transport of the gametes to the site of fertilization. A classic congenital anomaly associated with the gene for white coat color is "white heifer disease" in cattle, in which the prenatal, development of the Mullerian ducts is arrested, and the vaginal canal is obstructed by the presence of an abnormally developed hymen. It can be differentiated from the freemartin syndrome by the presence of normal ovaries, vulva, and labia. One of the main anatomical defects found in RBS is occlusion/ stenosis of the oviducts; this issue can occur bilaterally in 20% of RBS and unilaterally in 24% (Garrido *et al.,* 2019). These pathological conditions prevent gamete migration, thus preventing fertilisation of the oocyte (Pérez-Marín and Quintela, 2023). Common anatomical abnormalities are adhesions of the infundibulum to the ovary or uterine horns; this interferes with the pick-up of the egg or causes a mechanical obstruction of one part of the reproductive duct system. Bilateral or unilateral missing segments of the reproductive tract also cause anatomic sterility.

2. EARLY EMBRYONIC MORTALITY

Embryonic mortality denotes the death of fertilized ova and embryos up to the end of implantation. About 25 to 40% of embryos are normally lost in farm species. It is also noted in large litters of swine and during multiple pregnancies in cattle and sheep. In the past it was believed that the bovine conceptus was resorbed but transrectal ultrasound examination has demonstrated that the conceptus and its breakdown products apparently are eliminated by expulsion through the cervix, which either goes unnoticed or appears as a vulval discharge of clear mucus (Kastelic *et al.,* 1991). Embryonic mortality after natural breeding or artificial insemination accounts for the majority of reproductive failures in the cattle, with a mortality rate of up to 40% of all fertilized eggs. In cattle, most embryonic deaths occur between days 8 and 16 during hatching of the blastocyst and implantation without affecting cycle lengths. Nutritional causes such as B-carotene, selenium, phosphorus and copper deficiencies have all been implicated in embryonic loss, but unequivocal data are not available. High intakes of crude protein, in particular rumen – degradable protein have been associated with reduced fertility. This is said to be due to the toxic effects of blood urea or ammonia on the embryo. Stress, e.g. heat stress, has also been shown to result in embryonic loss. A high rate of increase in milk yield and high milk yield per se in early lactation are negatively correlated with fertility and this could be considered a metabolic stress.

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Sequel to Embryonic or Fetal Death

Following early embryonic death, the embryonic tissue are usually resorbed, and the animal returns to estrus if there is no other conceptus in the uterus. If death occurs before the maternal recognition of pregnancy has taken place, the estrous cycle will not prolonged. If it occurs after recognition, it will be prolonged. If the death is due to an infection, then even though the embryonic material may be absorbed, a pyometra may follow. In cattle this condition is characterized by persistence of the corpus luteum, closed cervix and pus accumulation in the uterine body and horns. It is a particular characteristic of infection with *Tritrichomonas fetus*. If fetal death occurs after ossification of the bones has begun, complete resorption of fetal material cannot take place, instead, fetal mummification occurs.

Causes for early embryonic death

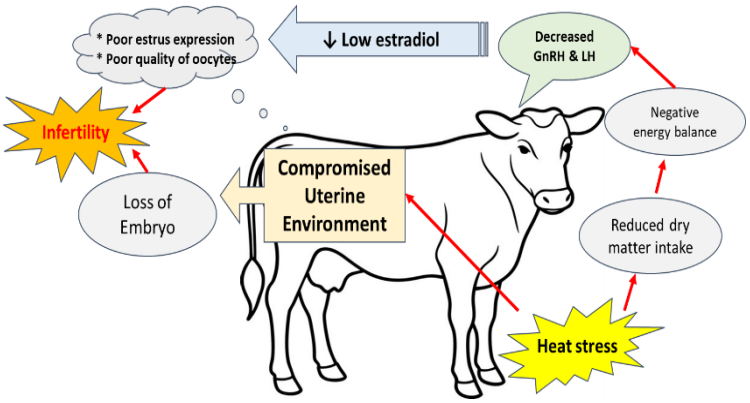
Embryonic mortality can be due to maternal factors, embryonic factors, or to embryonic-maternal interactions. Maternal failure tends to affect an entire litter, resulting in complete loss of pregnancy. In contrast, embryonic failure affects embryos individually, often leaving others in litter unharmed. In other cases, the maternal environment may be insufficient, allowing the support of only a few strong embryos.

Endocrine Factors

Accelerated or delayed transport of the egg, as a result of estrogen – progesterone imbalance, leads to preimplantation death. An abnormally undersized conceptus might not be able to counteract the uterine luteolytic effect, with consequent regression of the CL and termination of pregnancy. A critical period of embryonic survival is the late blastocyst stage. Normally, the developing CL secretes progesterone, which acts on the female tract in close synchrony with the development of the embryos. The cause-and-effect relationship between luteolysis and embryonic deaths is controversial. Apparently, embryonic mortality in cattle is not caused by a progesterone deficiency during the luteal phase of the cycle; luteal regression follows rather than precedes embryonic mortality. However, a diminished response to circulating luteotrophic hormones may contribute to embryo mortality in subfertile cows.

Lactation

Embryonic mortality occurs during lactation in cattle, sheep, and horses and is characterized by prolonged estrous cycles after breeding. Lactational stress can affect the



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quality of the egg (oocyte) released during ovulation, potentially reducing its ability to be fertilized or support early embryonic development.

Lactational stress can alter the uterine environment, making it less hospitable for the developing embryo. This can include changes in uterine lining, hormonal balance, and blood supply. Progesterone is crucial for maintaining a healthy uterine environment and supporting early embryonic development. Lactational stress can lead to reduced progesterone production or impaired luteal function, which can result in inadequate progesterone levels and early embryonic mortality.

Nutrition of the dam

Caloric intake and specific nutritional deficiencies affect ovulation rate and fertilization rate, as well as cause embryonic death. Also extremes in the level of feeding are detrimental to embryo survival, so too are extremes in the supply of specific dietary nutrients. In dairy cows, high intakes of rumen degradable protein may lead to embryonic mortality. This effect may be mediated through a reduction in the pH of the uterine environment during the luteal phases of the cycle in which the embryo must grow.

Age of the dam

A higher incidence of embryonic mortality is observed in gilts and in sows after the fifth gestation. In the ewe, the incidence of late embryonic loss is higher in ewe lambs and ewes over 6 years than it is in mature ewes, which is due to factors associated with the embryo rather than the uterine environment.

Overcrowding in Uterus

Because the degree of placental development is primarily influenced by the availability of space and vascular supply within the uterus, increasing the number of implantations decreases the vascular supply to each site and restricts placental development. This results in a high embryonic and fetal mortality rate and probably explains the higher incidence of embryonic mortality in cattle and sheep following twin rather than single ovulation. It should be noted, however, that uterine capacity does not limit the ability of the cow and ewe to carry twins, provided they are located in separate uterine horns. In cattle, embryo transfer experiments have shown a higher embryonic mortality rate in recipients which received two embryos in a single uterine horn. This loss may be due to overcrowding and intrauterine competition for nutrients. In cattle and sheep with multiple ovulations, the number of embryos

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surviving is reduced to a fairly constant number within the first 3 or 4 weeks of pregnancy, which implies that embryonic loss increases as the number of eggs shed increases.

Thermal Stress

Embryonic mortality increases in a number of species following exposure of the mother to elevated ambient temperatures, especially in tropical areas the effects of thermal stress on the early embryo are not apparent until the later stage of its development. Fertilized eggs of sheep and cattle, when subjected to high temperatures either in vitro or in vivo are damaged but continue to develop, only to die during the critical stages of implantation. Reduced fertility of summer heat-stressed dairy cows may result from decreased viability and developmental capacity of 6 day –old to 8-day-old embryos and may account for the well-documented seasonal reduction in the efficiency of artificial insemination during summer. Heat stress between days 8 and 17 of pregnancy may also alter the uterine environment as well as growth and secretory activity of the conceptus. Apparently heat stress antagonizes the inhibitory effects of the embryo on the uterine secretion of PGF2a.

Incompatibility

The inherited genotype of the male may include a variety of genetic factors that lead to incompatibility and early embryonic loss. There may be incompatibility between spermatozoa and mother, between spermatozoa and egg, or between zygote and mother. Immunologic incompatibilities may block fertilization or cause embryonic, fetal, or neonatal mortality. In cattle, homozygosity for certain blood groups and certain substances related to transferring and J-antigen in sera are associated with increased embryonic loss as well as decreased fertilization rate. Srivastava *et al.* (2017) highlighted that immunological reactions (anti-spermatozoa antibodies) against spermatozoa could be one of the possible causes of RBS, with an incidence of 58%.

DIAGNOSTIC APPROACHES

Accurate diagnosis is crucial for effective management of RBS, which can be done by using: Reproductive history, number of services, estrus pattern, previous illness, gynaecological examination of the reproductive tract for any anatomical abnormalities, vaginal examination to detect abnormal discharge or inflammation. USG assessment of ovulation, follicle size, and CL formation will help in assessment of cyclicity of animal, Harmonal profiling of progesterone or estrogen levels to detect luteal deficiency, Endometrial cytology- to diagnose subclinical endometritis (PMN count > 5%), microbiological cultures to detect infections and sensitivity. Advanced diagnostics include utilization of molecular techniques to identify specific pathogens or genetic predispositions.

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TREATMENT STRATEGIES

Management of RBS involves addressing the underlying causes:

1. **Hormonal Therapies**
   - ✓ GnRH administration on th day of AI to induce ovulation and improve conception rates
   - ✓ hCG injections to induce ovulation and to support luteal function
   - ✓ Progesterone supplementation: Using CIDR devices for 7–10 days followed by PGF2α
   - ✓ OvSynch or PreSynch protocols: For timed AI and ovulation control
2. **Antimicrobial Treatments**
   - ✓ Targeted antibiotic therapy for uterine infections based on culture and sensitivity results.
   - ✓ Post insemination antibiotic therapy: If it is intra-uterine route, the interval between the antibiotic therapy and AI should be at an interval of 6– 8 hours. As far as possible, intra-uterine antibiotic therapy can be avoided and parental antibiotics can be administered at the time of AI, like Streptopenicillin, Ampicillin, Amoxicillin, Ceftriaxone, Ceftiofur, Cefuroxime + Sulbactam etc.,
3. **Nutritional Interventions**: Supplementation with minerals (Se, Zn, Cu) and vitamins (A, E), correction of negative energy balance in high-yielding dairy animals through dietary adjustments.
4. **Optimization of Reproductive Techniques**: Implementation of timed artificial insemination (TAI) protocols like double insemination or insemination based on ovulation timing, deep uterine insemination in specific cases and in advanced stages Embryo transfer to bypass certain fertility issues.
5. **Management Modifications:** Improving heat detection methods, ensuring optimal timing of insemination and enhancing overall animal welfare.

PREVENTIVE MEASURES

Preventing RBS requires a holistic approach:

- **Regular Monitoring**: Routine reproductive health checks to detect and address issues early.
- **Optimal Nutrition**: Providing balanced diets to meet the energy and mineral requirements of breeding animals.
- **Environmental Management**: Ensuring comfortable housing conditions to minimize stress.
- **Training and Education**: Equipping farm personnel with knowledge and skills for effective reproductive management.

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- **Record Keeping**: Maintaining detailed breeding and health records to identify patterns and make informed decisions.

RECENT ADVANCES IN TREATMENT OF RBS

Latest updates and advances in treatment of RBS:

- **Ozone therapy** is the therapeutic administration of a gas mixture made of ozone (O_3) and oxygen (O_2).
    - O_3has a standard redox potential of +2.07V which makes it one of the most powerful oxidants known which has anti-microbial, anti-inflammatory and immuno-modulatory activity, but even a paradoxically antioxidant action if used at lower concentration.
    - In a study by Mali *et al.* (2020), the use of intrauterine O_3 @ 60mL (55µg/mL) of O_3 gas on day 1 and 2 of the oestrus cycle through intrauterine route using glass syringe, the conception rate was improved in O_3 treated RBS cows compared to those treated with local antibiotics, suggesting that this type of therapy is effective in the treatment of subclinical endometritis. In addition, it was seen that the bacterial load and PMN percentage decreased more with O3 treatment, might be due to the positive effect of O3 in reducing spermicidal effect of inflammation and to provide a favourable uterine environment for the embryo.
- **Use of Immunomodulators** and **stem cell applications** are emerging as potential future interventions.
    - E. coli LPS, Granulocyte-macrophage colony-stimulating factor (GM-CSF) to enhance uterine immune function
- **Proteomic and genomic profiling** of follicular fluid and uterine environment is being explored for early diagnosis of subfertility by identifying molecular biomarkers associated with fertility, enabling more precise interventions (Ayantoye *et al.*, 2025).
- **Use of real-time ultrasonography** has become routine for precise ovulation detection and early pregnancy diagnosis. Confirmation of a day-28 pregnancy by re-examination using Ultrasonography on day-45 after insemination as well will be helpful to determine early embryonic mortality (Niyas *et al.*, 2020)
- **Advancements in TAI protocols** have improved synchronization and conception rates in dairy cattle.
- **Precision livestock farming (PLF)** tools using sensors and AI-based estrus detection systems are being increasingly used in progressive dairy herds to minimize managemental causes of repeat breeding.

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