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# Current advances in management of repeat breeding syndrome in cattle and buffaloes

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## Abstract
Repeat breeding (RB), from a practical perspective, is a difficult issue for dairy farmers and veterinary professionals. The main etiological contributing causes of RB are maternal, genetic, uterine infections, genital tract abnormalities, hormonal imbalances, and other factors. Recto-genital palpation, vaginoscopy, uterine cytology, and ultrasound are all regarded as effective diagnostic and therapeutic methods. In many places, the only diagnostic methods accessible to clinicians for evaluating the most frequent causes of RB are vaginoscopy and palpation. However, when dealing with individual cows or buffaloes, ultrasonography can significantly improve diagnosis accuracy. The causes of pregnancy failure in dairy cows or buffalo are still difficult to pinpoint, even with the use of diagnostic procedures like hormone assays, colour Doppler ultrasound, and hysteroscopy, as some animals show ambiguous infertility. Depending on the potential cause of RB, a therapy plan is chosen. The use of immune-modulators like Escherichia coli lipopolysaccharide, eicosanoid PGF2α, and therapy with enzymes with or without antibiotic therapy are recent advancements in the treatment of endometritis. A variety of ovulatory disorders can be treated with regimens involving hCG, GnRH, prostaglandins, and their combinations.

**Keywords:** Advanced, buffalo, cattle, management, repeat breeding syndrome

## Introduction
Repeat breeding (RB) syndrome is still a significant issue in cattle and buffalo breeding, which costs dairy farmers a lot of money (Lafi *et al.*, 1992) [18]. According to some authors (Roy and Prakash 2007) [28], RB is overemphasized and modern high-producing Holstein cows have lower fertility as a result of intensive selection for high yields. However, other authors (O'Farrell *et al.*, 1983) [20] disagree with this theory. It has recently been determined that RB cows are a diverse group of sub-fertile cows with no structural abnormalities or illnesses who display a range of reproductive disorders across three or more successive heat cycles with a normal duration (17-25 days) According to Agarwal and Tomer (1998) [1], there is agreement for a similar definition of the water buffalo because cattle and buffalo are essential components of small holder mixed-crop-livestock farming systems in the emerging nations of the Asia-Pacific area (Boettcher *et al.*, 2007) [3]. With the obvious exception of the equatorial regions, where the reproductive function is primarily determined by the availability of feed rather than the length of daylight hours, buffaloes are considered tangentially seasonal animals and their reproductive efficiency is typically negatively affected by increasing the length of daylight (De Rensis *et al.*, 2007) [7]. In essence, therefore, RB in the buffalo must only be taken into account during the mating season due to these and other minute differences between cattle and buffalo (fertility in buffaloes is considered lower than in cattle). Repeat breeding was significantly more common among crossbred cows (17.57%) than among buffaloes (12.74%) and native cows (8.64%) (Khosa *et al.*, 2020) [15]. Early embryonic deaths or failure to fertilize have long been cited as the main causes of the RB syndrome, with the latter accounting for the majority of reproductive wastage in dairy cattle (Gabor *et al.*, 2007) [9]. Although there are fewer of these accounts for the buffalo (Campanile *et al.*, 2005) [4], the phenomenon is typically thought to occur on a par with it. More recently, it has been thought that RB is caused by the cow, the bull, and a variety of environmental and handling conditions, many of which overlap, and that it is frequently difficult to pinpoint the fundamental reason (Perez-Marin and Espana, 2007) [22]. When studies on a herd must be conducted instead of investigations on a farmer's cows or buffaloes from a variety of management approaches, such problems are considerably more challenging to trace. Contrary to artificial insemination, natural service is generally associated

with higher conception rates (Overton and Sischo 2005) [21]. As a result, RB must also be seen from this aspect. In general, veterinarians in many poor nations struggle to treat similar issues in cattle and buffaloes since there are few diagnoses and no set therapeutic protocol. The focus of this review is on potential diagnostic techniques and treatment plans for RB cows and buffaloes.

**Definition of Repeat breeding condition (RBC)**

A repeat breeding condition is generally defined as any cattle that have not conceived after three or more services associated with true estrus. A repeat breeding animal has normal or nearly normal estrus; estrus cycles as well as reproductive tract and though has been bred three or more times by fertile bull semen but had failed to conceive.

The classical definition of repeat breeder is "The cow should have had three or more unsuccessful services, has normal estrus cycles with approximately 21 days intervals between services, is free from palpable abnormalities, Show no abnormal vaginal discharges, have calved at least once before and, is less than 10 years old" In herds of normal fertility, where conception rates are commonly at 50-55%, about 9-12% of the cows are expected to be repeat breeders. As the conception rate decreases, the number of cows requiring additional services increases. As a result, breeding rapidly becomes significant.

**Causes of Repeat Breeding condition (RBC)**

**A. Maternal cause**

Age, genetic defects, genital tract infections, conformational defects, hormonal disorders, embryo mortality and nutritional defects have been reported. But there is no clear etiology and several concomitant causes often appear, which makes it difficult to characterize the problem.

**B. Influence of maternal age**

Age impacts negatively on fertility and higher RB rates have been described in old cows. It is attributed to alterations in hypothalamic or pituitary hormonal levels or the inability of the ovary response. It has also been demonstrated the relationship between old age and low oocytes viability, which explains the fertility decline. It is supported that fertility in dairy cattle improves after the 1st or 2nd parturition, and decreases from the 4th or 5th, but it should be taken into account the time required for uterine involution or problems associated with puerperium.

**C. Genetic factors in repeat breeder cows**

Individuals inherit their parent´s genetic merit, and then chromosomal or genetic abnormalities of the parent or those that occurred during the differentiation process may compromise fertility.

**D. Uterine infection and repeat estrous cycles**

The uterine environment promotes normal embryonic development. So, any disorder compromises the survival of the embryo and induces the RB syndrome. Reproductive failure appears after metritis, uterine infections (specific and nonspecific) will adversely affect the reproductive indexes by enlargement of the uterine and cervical postpartum involution, by alteration of follicular development, and by increased embryo mortality and repeat estrus rates. Subclinical endometritis should be considered when pregnancy failure or repeat estrus is observed. Cattle subclinical endometritis is thought to be a significant etiological element in RB diseases (Bedewy and Rahaway, 2019) [2]. In an another study, Thasmi *et al.* (2021) [33] noticed higher numbers of bacterial isolates in RB Aceh cows than fertile Aceh cows, with the most dominant bacterial isolate being Salmonella sp. (29.6%) followed by E. coli. However, clinical signs are difficult to detect; it is not easy to do the diagnoses by rectal palpation and the bacteriological analysis of cervical mucus does not reflect the endometrium status. Endometrial biopsies and uterine microbiological culture can enhance the diagnosis. Leukocyte infiltration in moderate degree with lymphocytes, neutrophils, plasmocits, eosinophils and macrophages are histopathological findings that have been reported in the endometrium of RBCs.

**Anatomical defects of the genital tract**

The reproductive tract of the cow provides a suitable environment for oocyte growth, as well as for sperm transport, fertilization and implantation. The uterus is a suitable habitat for embryonic and fetal development. Complex communication between hormones, proteins, etc. will be necessary for obtaining reproductive success. Oviductal abnormalities, that complicate and frequently inhibit reproduction, are present in 6-15% of adult cows and can reach up to 80% in those with a history of infertility or repeat breeding. Adhesions between the ovary, fallopian tubes or ovarian bursa, unilateral or bilateral obstructions, a moderate degree of hydro-salpinx and inflammation (peri-salpingitis, peritonitis) have been described in RBC syndrome. Acquired uterine alterations, as metritis, are critical to the resumption of the normal cyclicity during postpartum period, provoking RBCs (Shresta *et al.*, 2004) [30]. Other non-infectious abnormalities, such as uterine degeneration and neoplasia, could also be involved in this syndrome, although their incidence is low. Cervix is a defensive barrier and a sperm reservoir, and may undergo structural changes associated with inflammation. Cervical traumatic stenosis and obstruction, prolapse of cervical rings, adhesions or functional incompetence can be detected associated with RBCs. Vagina acts as receptacle for semen and is one of the uterine defensive barriers. Infectious disorders alter vaginal pH and bacterial flora, allowing the infection and reducing sperm vitality. Congenital anomalies, conformation defects (urovagina and pneumovagina) and infections (vaginitis) could be diagnosed. Vulvitis or vestibulitis may also modify the normal reproductive function.

**Hormonal dysfunctions**

Hypo-functional CL provokes a decrease in progesterone and affects negatively fertility. CLs are small and poorly developed, with low progesterone production and LH peak asynchrony. Therefore, an inadequate uterine environment is formed and this increases the abnormalities and the loss of embryos. A delayed increase in progesterone levels may indicate late or insufficient corpus luteum formation following ovulation or the short luteal phase. Luteal inadequacy, due to a diminished response to circulating luteotrophic hormones, may contribute to embryo mortality in sub-fertile cows. A delayed and diminished post-ovulation progesterone curve has been associated with low conception rates in cattle, and a low progesterone curve is related to significantly reduced production of interferon-tau by bovine embryos recovered on Day 16 of pregnancy. Suprabasal progesterone level around estrus has been described in RBCs (Perez-Marin & Espana, 2007) [22]. It is associated with low gonadotrophin levels and with incomplete luteal regression after luteolysis, which prolongs the follicular growth and damages the oocyte. Perumal *et al.* (2021) [23] recorded RBS affected animals had significantly higher level of cortisol, prolactin and lower level of 17 β-estradiol (E2), progesterone (P4), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroxine (T4) and insulin like growth factor-1 (IGF-1) than the unaffected crossbred cows. A longer estrus-ovulation interval usually appears and premature insemination is then carried out. These levels reduce the number of blastomeres around day 3, accelerate the zygote progress through the oviduct and affect negatively fertility.

**Early Embryonic Death (EED) causes RB syndrome**

The bovine embryo releases a substance of trophoblastic origin (interferon tau) into the uterus around day 16-18 that prevents and maintains the luteal function and pregnancy. EED has been attributed to irregular LH and progesterone profiles that induce failures in the maintenance of CL. EED is associated with poor quality of gametes and zygotes, uterine alterations, hormonal imbalances and defects in the immunitary mechanisms. The EED occurs between days 8 and 16 post-mating, before cow returns to estrus. As a result, no variation at interestrus interval is observed and clinicians cannot differentiate between embryonic resorption and other pregnancy failures. The incidence of EED is highly variable, from 10.6 to 39.7%.

**Inadequate follicular growth**

Additionally, it has been suggested that RBCs' inability to conceive may be related to a problem with the recruitment of big follicles during the second half of the estrous cycle, which leads to absent or aberrant ovulation or early cow-embryo asynchrony, which can occur in both EED and RBC. The critical period in the follicular recruitment occurs 10 days before ovulation. Dominant follicle continues its growth when progesterone levels are subluteal (or suprabasal), in which case follicular function is compromised and oocyte quality is reduced, affecting negatively the fertility (Heidari *et al*., 2016) [11].

**Effect of nutrition on RBC syndrome**

The relationship between nutritional deficiencies and the increase in services provided per cow is due to the endocrine imbalances that result from decreased food intake, weight, and body condition rating. These imbalances affect reproduction and other organs or systems. By delaying uterine involution and lengthening the number of open days, the previously described nutritional shortage can also have an impact on the postpartum period. The main cause of delayed fertility in dairy animals that are lactating is a negative energy balance. When milk production reaches its peak, there is an increased demand for protein. This excess supply of protein, particularly rumen degradable protein (RDP), leads high yielders towards becoming infertile or breed again (Rajendran *et al.*, 2022) [26].

**Bull factors involved in the RBC syndrome**

When evaluating RBCs, the bull and sperm quality must also be taken into consideration. Evaluation of sperm function in both natural mating and artificial insemination is crucial. Semen straws that have been frozen need to be handled with care. Repeat pregnancy failure could be linked to the mentioned bull factors, in which estrus is repeated and interestrus interval has a normal duration (Washaya *et al.*, 2019) [40]. Some relevant aspects are mentioned below.

**Influence of bull fertility and semen quality on repeat breeding**

Optimal bull fertility (by natural breeding or AI) is necessary to achieve a high pregnancy rate and normal calving interval. Semen doses for AI must contain at least 6 million motile sperm after thawing, and fertility drops if sperm concentration is reduced. Currently, frozen semen doses are packed with 15-25 million motile sperm pre-freezing, because around 50% of spermatozoa recover motility after thawing. However, despite semen doses that may fulfil all requirements, there are donors of sperm with erratic fertility (Washaya *et al.*, 2019; Kumar *et al.* 2017) [40, 17].

**Site of semen deposition and estrus return**

The oviducts are where fertilization takes place for sperm that has recently been ejaculated into the vagina by the bull or implanted into the uterus by the inseminator. Within minutes of starting their upward journey through the female's tubular system, sperm reaches the fallopian tubes in search of the oocyte. It has been reported that the utero-tubal isthmus acts as a reservoir of sperm, making these cells go up gradually towards the ampulla, preventing polyspermy and ensuring that sperm finds the oocyte into the oviduct (Hunter, 2002; Washaya *et al.*, 2019) [13, 40].

**Time of semen deposition**

The AM/PM rule, which involves insemination in the morning if the cow was in heat the previous evening or in the afternoon if the heat was discovered in the morning, is the most widely used procedure on dairy cattle farms. If the technician palpates ovaries before insemination and considers that the follicle texture is far from ovulation, double insemination is recommended separated by 12 hours. Similar pregnancy rates have been reported after one or two inseminations per day (Wahome *et al.*, 1985) [36]. The most common problems in RBCs, in which any change of pH, endometrial stroma and glands, endometrial flora, etc., could affect reproduction (Kumar *et al.* 2017) [17].

**Environmental and management factors affecting the RBC syndrome**

Calving interval, milk production, repeat estrus cows in larger animals due to size, lactational stress, or livestock size (a greater number of RBCs are detected in autumn calves). The first insemination during the winter, reproductive abnormalities prior to the first insemination, high milk output, or a clinical history of recurring estrus are risk factors for RBC syndrome. Other risk factors include initial lactation, dystocia or calving difficulties, high milk output, and recurring estrus in the past. Therefore, environmental and animal management factors should be considered to reduce the incidence of RBC syndrome.

**Diagnosis of RB syndrome**

First of all, a complete clinical history should be obtained at the herd and individual levels. Age, parity, milk yield, previous diseases, reproductive indexes, estrous cycles characteristics, insemination schedule, bulls, estrus detection, hormones, food and farm hygiene should be registered. Now, the anatomy, morphology and function of cows should be inspected. The reproductive status of animals must be according to their production. Sexual behaviour must be evaluated to detect disorders, such as muscle or claw lameness. Similarly, it is necessary to examine the behavior of bull and bull-cow interactions when natural breeding is carried out. Vulva, vagina, cervix, uterus, fallopian tubes and ovaries must be evaluated to diagnose reproductive defects.

**External inspection and vaginal evaluation**

External inspection can identify congenital or acquired anatomical defects such as pneumovagina, vulvar defects, tumors or injuries. The anatomy of the area, secretions around vulva or tail, and vulvar and vaginal coloration should be evaluated. Vaginoscopy is helpful to visualize the vaginal cavity and cervix. Faces are removed from the rectum before the perineal area; vestibule and clitoral fossa were cleaned and finally dried. The speculum -which must be clean, dry, sterile, and lubricated is inserted in direction of the pelvic canal. Once crossed the vestibule, the negative pressure inside allows air to penetrate. Hyperemia may result in 30-60 sec, which hampers the assessment of the vaginal mucosa colouration. The vestibule acts as a defensive barrier of the female genital tract and hinders the entry of the speculum. If this does not occur, dysfunctions can be present (e.g. in pneumovagina). Similarly, if the material is accumulated into the vagina, speculum helps to localize its origin. The examination of internal organs (uterus, oviducts and ovaries) demands other techniques, such as manual palpation and ultrasound.

**Rectal palpation in cow**

Rectal palpation is a widely used diagnostic method in cattle with high accuracy, easy to be implemented and at low cost in comparison with other sophisticated techniques. Plastic gloves are lubricated and then faeces are withdrawn. Air should not be present in the rectum to get a more relaxed mucosa and easily manipulate the structures beneath. The cervix is presented as a solid structure, tubular, fibrous, with 3-4 folds projected inside and localized on the pelvis floor in normal non-pregnant cows. It is cylindrical, with a length of 5-10 cm and a diameter of 1, 5-7, 0 cm. Cranially the uterus can be palpated. At heat, uterus is turgid, erect and coiled. However, it is soft and flaccid during luteal phase and palpation is a bit more difficult; it is a consequence of the progesterone action, released from CL. Palpation helps to diagnose anomalies such as uterine infections. After that, it is interesting to palpate the ovaries. They are located ventro-laterally to the pelvis floor and sometimes placed under the bone. During anestrus, ovary size ranges from 2 to 3 cm approximately. Follicles (at different stages of growth) and CLs (hemorrhagic, mature or/and albicans) are developed at the ovaries and their size could suggest some diseases.

**Ultrasonography (US)**

Trans-rectal ultrasound diagnosis has improved our ability to assess the reproductive organs in cattle and to follow the dynamic interactions between ovarian follicular cohorts. Even 2-3 mm follicles can be seen, quantified and sequentially monitored, allowing the development of superovulation regimens, an essential practice for the embryo transfer industry. Routine assessment of follicular and luteal development, and differential diagnosis of cystic ovaries, ovarian abscesses and tumours, can be considered factors associated with RBC syndrome. It is also useful for detecting pathological conditions such as metritis, pyometra, maceration or mummification, and it is an important tool for diagnosing ovarian cystic disease (Raja mahendran, 1994) [25].

**Hormonal function tests**

Progesterone can be considered as a sensor of reproductive capacity, both for its information about the estrous cycle and for its easy determination. Progesterone assay is an objective and accurate test to evaluate the ovarian function and to diagnose certain diseases that otherwise could not be correctly determined, such as delayed ovulation, persistent luteal activity, ovarian cysts or suprabasal progesterone levels (Waldmann *et al.,* 2000) [37].

**Endometrial cytology and uterine bacterial culture**

Numerous pathogens may be concentrated in the female reproductive system, which could have an impact on fertilization success. It is interesting to detect these conditions since infectious infections might cause endometritis, vulvitis, vaginitis, or cervicitis. The typical progression of uterine inflammatory disorders involves bacterial contamination of the uterine lumen, pathogen attachment to the mucosa, colonization or penetration of the epithelium, and/or endotoxin release. Even in the absence of an active bacterial infection, uterine inflammation can cause RBC syndrome and compromise embryonic viability. The endometrial bacteriological diagnosis is interesting to detect pathogens implicated in infertility. In cattle, especially due to the cervical anatomy, samples can be taken using a catheter connected to a syringe containing 30-60 ml of sterile saline. It is deposited into the uterus and then is removed and cultured. Clinical or subclinical endometritis could be diagnosed. The number of neutrophils indicates the type and grade of endometrial inflammation.

**Treatment**

**Nutritional treatments**

Nutritional deficiencies have been described as causes of RBC. Diets containing a higher concentration of inorganic iodine from 8-12 days before estrus improve the stimulation of the pituitary gland, reducing at the same time the RBC rate. Herds with problems of repeated estrus were supplemented with copper and magnesium, minimizing fertility problems (Ingraham *et al.,* 1987) [14]. However, Thasmi *et al.* (2020) [33] found out that the concentration of serum macro minerals do not affect the incidence of RB in Aceh cattle. Beta-carotene, the precursor of vitamin A, has recently been investigated for its involvement in the formation and function of CL. Beta-carotene improves the progesterone synthesis and reduces the luteal hypofunction (Wang *et al.,* 1988) [39].

**Assisted reproduction techniques**

RBC syndrome is brought on by abnormal gamete implantation and transport in combination with endometrial abnormalities. To treat this disease, some assisted reproductive technologies have been proposed, including intra-peritoneal insemination and *in vitro* fertilization (Sikka, 2022) [31].

**Intrauterine treatment**

Administration of 1% Lugol's iodine 24 h. after mating/AI (Tanaka *et al.,* 1994), although fertility results are poor. Antimicrobial treatments (chloramphenicol, gentamicin, enrofloxacin, tetracycline, or nitrofurantine) could improve the reproductive indexes.

**Alternative medicine**

Scientific contributions report the utility of some alternative medicines, such as acupuncture or moxibustion, to reduce the incidence of RBC syndrome. Moxibustion has been used to reduce reproductive failure in RBCs (Hosaha & Nakama, 2002) [12]. Moxas (balls of about 3 cm in diameter) containing 2 g of Artemisia spp. are applied on nine points of the skin and burned for 15 min. The treatments are applied for 3 consecutive days and then repeated during the heat. Authors describe an increase in blood flow at the uterine arteries, which is considered to improve fertility. A volume of 5-10 ml of glucose 50% was injected at certain points set by traditional acupuncture in cattle. This study reported a good fertility rate, although many gestations failed later (Lin *et al.,* 2002) [19].

**Hormonal treatments**

**Progesterone (P4)**

Progesterone is essential for the implantation and maintenance of pregnancy. CL dysfunction decreases P4 concentrations and then negatively affects fertility (Kimura *et al*., 1987) [16]. It has been noted that the administration of progesterone as of 3 to 5 days after insemination and for 2-3 weeks (or more) improves conception rates in RBCs. Due to the significance of low fertility in dairy cows, synchronising ovulation of the dominant follicle (DF) before fixed-time artificial insemination (FTAI) in dairy cows has been accomplished using a hormonal treatment using a short-term 5-day progesterone (P4) - prostaglandin F2 (PGF)-gonadotropin-releasing hormone (GnRH)-based protocol (Yama *et al.,* 2022; Bolivar *et al.,* 2000) [41, 29].

**GnRH (gonadotrophin-releasing hormone)**

The administration of GnRH around the insemination time aims to accelerate and ensure ovulation in cows, acting directly on the pituitary, stimulating the secretion and release of gonadotropins, such as LH and FSH, and promoting the preovulatory LH peak, which is essential for follicular dehiscence. In many cases, RBC syndrome is associated with ovulatory defects, such as anovulation, delayed ovulation or gonadotrophin release failure. Due to the significance of ovarian structure, dairy cows with a functioning CL at the time of the first GnRH treatment, independent of their ovulatory response to the first GnRH treatment, had a higher pregnancy outcome than cows without a functional CL at the time of the first GnRH injection (Carvalho *et al.,* 2018) [5]. This suggests that a functional CL at the time of programme initiation has a bigger effect on pregnancy than ovulatory response to first GnRH treatment, even though ovulatory response to first GnRH treatment of the GnRH-based protocol can alter pregnancy to timed AI (Chaikol *et al.,* 2022) [6].

**Exogenous gonadotrophin**

These hormones have been used in RBCs to induce ovulation and exert the luteotrophic effect on the CL, administered intramuscularly pregnant mare serum (PMS) on day 15 or 16 of the estrous cycle, obtaining a conception rate of 73, 9% in RBCs compared with 44, 4% of control cows (Roussel *et al.,* 2005) [27].However, HCG is the most used exogenous gonadotrophin for treating RBC syndrome. Treatment with hCG on day 5 after insemination can achieve higher levels of progesterone for at least 2 weeks, due to the development of accessory CL (Walton *et al.,* 1995) [38]. When the Ovsynch protocol (GnRH-PGF2α-GnRH-TAI) was started in the middle of the diestrus, or days 5–12 of the estrus cycle, conception rates were often improved (Tiwari *et al.,* 2019) [35].

**Prostaglandins**

The luteolytic effect of prostaglandins has been used to treat RBCs. In this case, treatment aims to achieve better heat detection and to increase the number of cows in heat. Numerous protocols are used in cow, e.g. two PGF2α injections apart 12 days and insemination 80 hours later. Intravenous PGF2α (0, 2 ml cloprostenol) has also been reported in RBCs at AI time. However, the most frequent use of this hormone has been combined with other substances, serving as pretreatment and with the ultimate goal of improving reproductive management. From the second PGF2α administration till the insemination day, treating repeat breeding cows once day with 500 mg of resveratrol appears to marginally improve conception (Yildiz & Kiliç, 2022) [42].

**Reproductive management improvement**

Treatments are being used recently for fixed-timed artificial insemination, without heat detection. These protocols allow the treatment of cows with silent heats or ovulation problems. Ovsynch is a protocol based on the administration of GnRH, PGF2α and GnRH (Pursley *et al.,* 1995) [24] to schedule the insemination time. It is also essential to improve all aspects related to heat detection since it has been demonstrated that estrus detection mistakes involve very significant losses in the reproduction and production of cattle. Therefore, it could be interesting to implement different methods for estrus detection as Kamar or Bovine Heat-beacon devices and pedometers.

In anovulation, ovulation will not be evident and hence no CL will be felt on days of examination. The hormonal therapy includes hCG or LH preparations 1500 to 3000 IU I/V on the day of AI or GnRH i.e. Receptal or Fertagyl 500 µg I/M on the day of AI. Proluton depot 250 mg I/M at AI and 25% Dextrose 400 ml I/V + Inj. Vitamin-C 200 mg on the 5th and 17$^{th}$ can also be tried. Other treatments include Copper glycinate 10 ml. I/M on the day of AI as well as feeding carotene daily 400-600 mg/day per animal. Feeding of homoeopathy drugs such as Aurun Iodum, Thyroidinum, Aguns castus 10 pills of each; 3 times a day for 10 days may be beneficial. Feeding of the chelated mineral supplement daily especially containing Iodine, phosphorus, and manganese is recommended (Singh and Jerome, 2012) [32].

**Recommendation**

- After correcting a repeat breeding problem it is necessary to continually monitor conditions in the herd to ensure that the problem is not recurring.
- Consequently, the organized reproductive program and records must be kept up-to-date to allow for ongoing evaluation of the herd's reproductive performance.

- Small scale farmer needs to be diagnosed and corrected early in their development with the help of a veterinarian to diminish the loss by RBC.

## Conclusion

The key to success in detecting, treating, and preventing the problem of repeat breeding conditions is a thorough, continuous reproductive management programme requiring a collaborative effort from the producer, inseminator and the filed veterinarian.

## References


1. Agarwal SK, Tomer OS. Reproductive disorders and their therapeutic management. Reproductive Technologies in Buffalo. Indian Veterinary Research Institute, Izatnagar, India; c1998. p. 50-65.
2. Bedewy RB, Rahaway MA. Comparative study for detection of subclinical endometritis in local cows. Adv. Anim. Vet. Sci. 2019;7(4):289-294.
3. Boettcher PJ, Perera BM. Improving the reproductive management of smallholder dairy cattle and the effectiveness of artificial insemination: a summary. Reproductive Management of Dairy Cattle Subjected to Artificial Insemination. 2007 May 15, 1.
4. Campanile G, Neglia G, Gasparrini B, Galiero G, Prandi A, Di Palo R, *et al*. Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in reproductive function. Theriogenology. 2005 May 1;63(8):2334-2340.
5. Carvalho PD, Santos VG, Giordano JO, Wiltbank MC, Fricke PM. Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. Theriogenology. 2018 Jul 1;114:165-172.
6. Chaikol W, Yadmak C, Yama P, Jitjumnong J, Sangkate M, Warittha U, *et al*. Ovarian luteal category at the time of exogenous progesterone treatment alters pre-ovulatory follicle size and pregnancy outcome but not initial GnRH treatment in repeat-breeder crossbred dairy heifers submitted to the 7-day fixed-time AI protocol. Veterinary and Animal Science. 2022 Sep 1;17:100257.
7. De Rensis F, Lopez-Gatius F. Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): A review. Theriogenology. 2007 Jan 15;67(2):209-216.
8. Dobson H, Smith RF, Royal MD, Knight CH, Sheldon IM. The high-producing dairy cow and its reproductive performance. Reproduction in domestic animals. 2007 Sep;42:17-23.
9. Gábor G, Tóth F, Ozsvári L, Abonyi-Tóth Z, Sasser RG. Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests. Reproduction in domestic animals. 2007 Dec;42(6):633-636.
10. Hafez ES, Hafez B. editors. Reproduction in farm animals. John Wiley & Sons, 2013 May 13.
11. Heidari M, Kafi M, Mirzaei A, Asaadi A, Mokhtari A. Effects of follicular fluid of preovulatory follicles of repeat breeder dairy cows with subclinical endometritis on oocyte developmental competence. Animal reproduction science. 2019 Jun 1;205:62-69.
12. Hosaha T, Nakama S. Effects of moxibustion for reproductive failure in dairy cattle. In XXII World Buiatrics Congress; c2002.
13. Hunter RH. Vital aspects of Fallopian tube physiology in pigs. Reproduction in Domestic Animals. 2002 Aug;37(4):186-190.
14. Ingraham RH, Kappel LC, Morgan EB, Srikandakumar A. Correction of subnormal fertility with copper and magnesium supplementation. Journal of Dairy Science. 1987 Jan 1;70(1):167-180.
15. Khosa BK. Repeat Breeding: A Dent to Dairy Farm Economy. Int J Curr Microbiol App Sci. 2020;9:4085-4091.
16. Kimura M, Nakao T, Moriyoshi M, Kawata K. Luteal phase deficiency as a possible cause of repeat breeding in dairy cows. British Veterinary Journal. 1987 Nov 1;143(6):560-566.
17. Kumar Patel G, Haque N, Madhavatar M, Kumar Chaudhari A, Kumar Patel D, Bhalakiya N, *et al*. Artificial insemination: A tool to improve livestock productivity. Journal of Pharmacognosy and Phytochemistry. 2017;6(6S):307-313.
18. Lafi SQ, Kaneene JB. Epidemiological and economic study of the repeat breeder syndrome in Michigan dairy cattle. I. Epidemiological modeling. Preventive Veterinary Medicine. 1992 Oct 1;14(1-2):87-98.
19. Lin JH, Wu LS, Wu YL, Lin CS, Yang NY. Aquapuncture therapy of repeat breeding in dairy cattle. The American journal of Chinese medicine. 2002;30(02n03):397-404.
20. O'Farrell KJ, Langley OH, Hartigan PJ, Sreenan JM. Fertilisation and embryonic survival rates in dairy cows culled as repeat breeders. The Veterinary Record. 1983 Jan 1;112(5):95-97.
21. Overton MW, Sischo WM. Comparison of reproductive performance by artificial insemination versus natural service sires in California dairies. Theriogenology. 2005 Aug 1;64(3):603-13.
22. Pérez-Marín CC, España F. Oestrus expression and ovarian function in repeat breeder cows, monitored by ultrasonography and progesterone assay. Reproduction in domestic animals. 2007 Oct;42(5):449-456.
23. Perumal P, Chaurasia D, De AK, Bhattacharya D, Bhowmick S, Kundu A, *et al*. Heamatological parameters, endocrinological profiles, antioxidant and oxidative stress profiles of repeat breeding crossbred cows in Andaman and Nicobar Islands. The Indian Journal of Animal Sciences. 2021 Aug 1;91(8).
24. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology. 1995 Nov 1;44(7):915-923.
25. Rajamahendran R, Ambrose DJ, Burton B. Clinical and research applications of real-time ultrasonography in bovine reproduction: a review. The Canadian Veterinary Journal. 1994 Sep;35(9):563.
26. Rajendran D, Gowda NK, Rao SB, Babu PE, Manimaran A, Kumaresan A. Nutritional Strategies to Improve Reproductive Efficiency in Cattle and Buffaloes. In Current Concepts in Bovine Reproduction. Singapore: Springer Nature Singapore; c2022. p. 47-67.
27. Roussel JD, Stallcup OT, Rakes JM, Ward RF. Treatment of repeat breeders with pregnant mare serum. Journal of Dairy Science. 1995;48:808.
28. Roy KS, Prakash BS. Seasonal variation and circadian rhythmicity of the prolactin profile during the summer months in repeat-breeding Murrah buffalo heifers. Reproduction, Fertility and Development. 2007 Jun

1;19(4):569-575.
29. Bolivar AO, Oba E. Concentración sérica de FSH, LH, Estradiol e Progesterona P4 em Femeas Bovinas Repetidoras de eio. ARA. Archivos de reproducción animal. 2000;12:26-32.
30. Shrestha HK, Nakao T, Higaki T, Suzuki T, Akita M. Resumption of postpartum ovarian cyclicity in high-producing Holstein cows. Theriogenology. 2004 Feb 1;61(4):637-649.
31. Sikka P. Future Assisted Reproductive Technologies in Diary Animals. UK Atheya, 2022 Dec 1.
32. Singh I, Jerome A. Buffalopedia Indian Council of Agricultural Research; c2012.
33. Thasmi CN, Husnurrizal H, Akmal M, Wahyuni S, Siregar TN. Detection of serum macro minerals concentration in repeat breeding Aceh cattle. Jurnal Kedokteran Hewan-Indonesian Journal of Veterinary Sciences. 2020 Jun 15;14(2):25-28.
34. Thasmi CN, Husnurrizal H, Wahyuni S, Hafizuddin H, Siregar TN. Identification of Bacteria in Aceh Cattle with Repeat Breeding. Veterinarija ir Zootechnika. 2021 Jul 1;79(2):32-36.
35. Tiwari I, Shah R, Kaphle K, Gautam M. Treatment approach of different hormonal therapy for repeat breeding dairy animals in Nepal. Archives of Veterinary Science and Medicine. 2019;2(3):28-40.
36. Wahome JN, Stuart MJ, Smith AE, Hearne WR, Fuquay JW. Insemination management for a one-injection prostaglandin F2α synchronization system. II. One versus two inseminations following detection of estrus. Theriogenology. 1985 Nov 1;24(5):501-507.
37. Waldmann A, Reksen O, Landsverk K, Ropstad E, Kommisrud E, Dahl E, *et al*. Progesterone at first insemination-effects on non-return and repeat-breeding. In14th International Congress on Animal Reproduction 2000;15:13-87.
38. Walton JS, Halbert GW, Robinson NA, Leslie KE. Effects of progesterone and human chorionic gonadotrophin administration five days postinsemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. Canadian Journal of Veterinary Research. 1990 Jun;54(3):305.
39. Wang JY, Owen FG, Larson LL. Effect of beta-carotene supplementation on reproductive performance of lactating Holstein cows. Journal of dairy science. 1988 Jan 1;71(1):181-186.
40. Washaya S, Tavirimirwa B, Dube S, Sisito G, Tambo G, Ncube S, *et al*. Reproductive efficiency in naturally serviced and artificially inseminated beef cows. Tropical animal health and production. 2019 Sep 1;51:1963-1968.
41. Yama P, Yadmak C, Sangkate M, Jitjumnong J, U-krit W, Promsao N, *et al*. *In vivo* follicular and uterine arterial indices as an indicator of successful hormonal stimulation for inactive ovaries in repeat-breeder crossbred dairy cows using a short-term progesterone-based programme. Animals. 2022 Jan 25;12(3):292.
42. Yıldız A, Kılıç A. The influence of resveratrol on conceiving of repeat breeder cows. Veterinary Sciences: Research and Reviews. 2022;8(2):105-109.