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Repeat breeding in buffaloes: Prevalence, Risk factors, Diagnosis and Treatment

Dr. Abhay Kumar Meena [*1], Dr.Vikram Singh Gurjar[2], Dr. Virendra Kumar[3] and Dr. Arvind Kumar

[1,2,3]Assistant professor, Livestock Research Station, Kodamdesar
Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334001 (Rajasthan)

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Introduction

Failure of pregnancy establishment referred to as repeat breeding (RB) is one of the biggest reproductive problems in female buffaloes, however compared to cattle the incidence of repeat breeding in the buffalo is low (8.68% vs. 18.79%). Pregnancy failure in buffaloes is common during the hot summer months. The problem is exacerbated by poor nutrition, poor management and poor housing. Failure of buffaloes to conceive with 3 consecutive artificial inseminations (AI) or natural matings during the breeding season, is considered as repeat breeding. The etiology of RB appears to be multifactorial and has been recently described under the headings of failure of fertilization and early embryonic deaths. Clinical evaluations mention that endometritis is the most frequent cause of failure of fertilization in RB buffaloes, whereas improper insemination and poor semen quality are the bull-side factors for fertilization failure in RB buffaloes . Early embryonic deaths in buffaloes originate due to low luteal progesterone and seasonal influence on corpus luteum (CL) development.

Prevalence

The overall incidence of RB in buffalo seems to be lower than in cattle. According to several reports, the incidence of RB in buffaloes ranged from 0.32% to 55.4% in one study and from 0.70% to 30.0% in another**.**

Risk Factors

First parity, periparturient illness, lactation, and spring and winter calving were all regarded as major risk factors for RB in buffaloes. The highest prevalence was found in Murrah buffaloes during the autumn. The 2nd and 3rd parities had the highest incidence of RB. According

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to Shahzada et al., buffalo heifers and buffalo with third parity had the lowest incidence of RB, whereas those with sixth parity had the greatest incidence.

Etiology.

Recently the etiologies for RB have been classified under two headings: failure of fertilization and early embryonic deaths. Failure of fertilization can occur due to problems with either the female or the male and poor breeding management. Female factors for fertilization failure include anatomic defects of the genital tract, infections of the genital tract, ovulatory disturbances and improper timing of insemination. The male factors include poor quality of semen, improper semen handling, infectious diseases and poor breeding management. Early embryonic deaths can result from defective function of the CL, infectious disease affecting the embryo and stress.

1. Fertilization Failure

Causes of failure of fertilization have been described in buffaloes recently, and include problems with the female, the bull (or the semen used in AI) and the breeding management often with overlapping effects.

Female Buffaloes

Failure of fertilization in female buffaloes can occur on account of congenital or acquired anatomic defects, ovulatory disturbances, genital infections such as endometritis and defects of ova.

Table 1. Anatomic Defects Found in RB Buffaloes

Anatomical Abnormalities	Incidence Range (%)
Poor genital development	0.54- 17.73%
Ovariobursal adhesions (OBA)	0.04-13.4%
Uterine tumor/abscess	0.3-0.72%
Abnormal cervix	0.28-6.45%
Oviductal abnormalities	25.2%

The most common genital affection in buffalo that leads to failure of fertilization is endometritis. Endometritis was significantly higher in 2nd parity buffaloes. Endometritis is associated with accumulation of inflammatory debris in the uterus, increased oxidative stress and increased poly morphonuclear leucocytes in the uterine lumen. Endometritis results in a uterine environment unfavorable to the spermatozoa and the embryo. The most common predisposing factor for endometritis in buffaloes was retained placenta and the most prevalent bacteria in the uterine lumen of affected buffaloes were E. coli (13%), Arcanobacter pyogenes (13%) and Staphylococcus aureus (10%).

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Buffalo Bull

Buffalo sires are known to affect pregnancy rates both in natural service and AI. The CR with frozen semen AI from different buffalo bulls varied from 17% to 84%. Moreover the age of the bull and the season when semen was collected, affects the fertility with adult buffalo bulls producing the best quality semen during cooler months.

Breeding Management

Poor overt estrus expression in buffaloes poses logistic problems for timing AI thus resulting in poor conception and RB. While this has been difficult to correct, other related problems such as time of insemination need attention. Recent reviews suggested that buffaloes should be inseminated 18 to 24 h after the onset of estrus as ovulation occurs relatively late (30 h)

2. Early Embryonic Death

Early embryonic deaths (2-3 weeks) account for 45% of pregnancy failures in dairy cows. Luteal insufficiency (lower production of progesterone by the CL) appears to be the most common cause of early embryonic death in dairy cows and buffaloes. Optimal progesterone appears necessary for endometrial and embryonic signal exchanges necessary for maternal recognition of pregnancy and implantation. High producing dairy cows have an increased metabolic clearance of progesterone with less availability for pregnancy. Environmental or shipping stress and presence of pathogenic microbes in the uterus are other causes of early embryonic death.In buffaloes, embryonic death has been mentioned to occur between Day 25 to Day 50 of gestation.

Diagnostic Approaches

When RB is a herd problem, breeding management should be reviewed, as well as the buffalo bull and check for infectious diseases. Before evaluating pregnancy failures in individual animals, the semen and the AI technique used should be evaluated. Fertilization failures often occur on account of pathologies in the genital tract and dysfunction of the ovaries.

A combination of visual estimates, transrectal palpation and transrectal ultrasonography are suggested for such evaluations. More specialized techniques such as hysteroscopy, metabolic profiles, uterine biopsies and tubal patency testing suggested previously should be reserved for pathologies in the genital tract not easily traceable. Ovarian and oviductal pathologies such as tumors, pyosalpinx and oviductal obstructions are often not detectable with routine techniques. Failure to regain fertility following medical or surgical therapy often limits their use and the value of the affected animal.

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Evaluating Genital Health

Visual inspection of cervico-vaginal mucus is often the first diagnostic method for finding infections in the genital tract of cows, however, due to the lower quantity of mucus secretions during estrus in buffaloes and the tendency of buffaloes to show estrus during the night hours, visual appraisal of cervico-vaginal mucus appears difficult in the buffalo.

vaginoscopic examination is helpful in evaluating the health of the vagina and cervix and also of the uterine secretions accumulating in the vagina . Such an examination can also help in diagnosing cervicitis or vaginitis or changes in cervical morphology which could be a possible cause of RB especially under natural mating conditions.

Trans rectal palpation of the genital tract can determine gross enlargements, strictures and adhesions of the genital tract or ovarobursal adhesions and the findings can be evaluated further by using trans rectal ultrasonography.

A wide variety of microbes have been isolated from RB buffaloes including Salmonella, Staphylococcus, Corynebacteria, Pseudomonas and E. coli, however, their significance is important only when samples have been collected properly. Often these organisms are present collectively with anaerobic bacteria like Fusoformis necrophorus and Bacteroids species.

Evaluating Ovarian Function

The precise evaluation of ovarian function such as ovulation and CL formation is only possible with the use of trans rectal ultrasonography because of the smaller dimensions of these structures in buffalo. To monitor ovulations, frequent examinations (6-12 h after estrus) are required.

Embryonic Deaths

Evaluating embryonic death in buffaloes is possible through two approaches; the decline in progesterone concentrations and the disappearance of the previously visualized fetus and its annexes as examined by transrectal ultrasonography. Since most bubaline embryonic deaths occur after Day 25, weekly evaluations starting from Day 23 have been suggested. Diagnosing embryonic deaths is helpful in rebreeding the non-pregnant animals as soon as possible.

Serum Biochemical Profile

The uptake and utilization of minerals and energy decrease with the season in buffaloes. The breeding season was associated with a decrease in crude protein and mineral intake. RB buffaloes had significantly lower serum calcium, phosphorous, magnesium, copper, iron and zinc. Variations in circulating levels of biochemicals, macro and micro minerals were recorded in RB buffaloes (Table 2), however, the data cannot be used to predict potential fertility. These results could be used to predict deficiencies in representative samples from large herds.

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Table 2. Serum Biochemicals, Macro and Micro Minerals in RB Buffaloes from Different Studies			
Biochemical	**Normal Cycling Female**	**RB Female**	**Reference**
Glucose (mg/dL)	63.48±1.61	38.05±0.67	Sabasthin et al. [2012]
Total protein (g/dL)	11.05±0.30	9.06±0.21	Butani et al. [2011]
Cholesterol (mg/dL)	183.09±11.70	143.97±7.49	
Serum calcium (mg/dL)	9.22±0.33 to 9.30±0.29	7.46±0.29 to 8.86±0.38	Chaurasia et al.[2010]; Akhtar et al.[2014]
Serum phosphorous (mg/dL)	5.39±0.14 to 5.56±0.11	3.71±0.19 to 4.50±0.16	
Serum magnesium (mg/dL)	2.18±0.04 to 2.86±0.06	2.13±0.03 to 2.77±0.08	
Serum sodium (mg/dL)	340.2±1.49	340.5±1.62	
Serum potassium (mg/dL)	16.20±1.11	15.30±1.95	
Zinc (µg/mL)	1.0±0.04	0.88±0.15	Singh et al. [2006]
Copper (µg/mL)	0.88±0.03	0.62±0.03	
Cobalt (µg/mL)	0.022±0.002	0.016±0.001	
Iron (µg/mL)	367.49±1.31	353.81±1.96	Akhtar et al. [2014]

Therapeutic Approaches

Therapies in a herd with suboptimal fertility include corrective measures to prevent/combat disease and/or deficiency and reducing stress. Perhaps the easiest point to improve or correct is management. The temporary replacement/change of the bull may take care of infertility due to the bull. When using frozen semen, the storage and handling should be evaluated. Strategies to control RB in individual buffaloes can be attempted using one or a combination of the following approaches.

- Improving uterine health
- Correcting ovarian malfunction
- Maintaining luteal support
- Improving management.

When applied correctly, alone or in concert, these therapeutic approaches should result in a decrease in RB in buffaloes. Altered uterine health, ovarian malfunction and poor management can have overlapping effects both on the fertilization and pregnancy maintenance.

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Improving Uterine Health

Uterine infection that commonly occurs in RB buffaloes falls under the definition of subclinical endometritis with clinical signs showing at around 8 weeks postpartum, with a complete absence of cervical discharge as was also mentioned for cows. When a microbial infection in the uterus is suspected, there are many therapeutic approaches including intra-uterine infusion of antibiotics or immunomodulators and administration of hormones.

Intrauterine Infusion of Antimicrobial Compounds

The direct intrauterine administration of oxytetracycline produce immediate therapeutic concentrations in the caruncles of both healthy and affected cows and oxy tetracycline has been shown to be an effective therapy for endometritis in buffalo. However, studies showed that the massive irritation of the uterine mucosa produced by oxy tetracycline might have significant negative effects on uterine defense mechanism and on self-healing ability of the uterus. The antibiotics commonly used for intrauterine infusion in therapy of bubaline endometritis have been recently summarized and include penicillins, aminoglycosides and cephalosporins. However, the routine intrauterine infusions of antibiotics suggested previously such as gentamicin, ciprofloxacin and tinidazole or oxytetracycline plus tylosin cannot be considered as optimal therapy for endometritis, particularly because of antibiotic resistance and the possible residues in milk.

Use of Prostaglandins (PG) and Estradiol Benzoate.

Postpartum buffaloes treated with PG on Day 7 had less endometritis than non-treated buffaloes. Mid cycle administration of PG to RB buffaloes resulted in 1st service CR of 50% in treated animals. Similarly, the administration of PG on Day 6 of estrus or 2 PG injections 11 days apart starting from Day 6 of estrus in RB buffalo heifers resulted in 50% and 100% animals showing estrus and 80% and 100% CR respectively. Uterine lavage plus PG resulted in better CR in RB dairy cows compared to uterine lavage alone. In an attempt to enhance the uterine clearance of inflammatory exudates some researchers used antibiotics and estradiol benzoate to treat endometritis but the results demonstrated that there is no beneficial effect on uterine infection or reproductive performance in dairy animals. Injecting estradiol benzoate to lactating buffaloes could result in a sharp and irreversible decline in milk production, vaginal prolapse and the spread of the infection towards the oviducts and should thus be discouraged.

Immuno modulators

Recently, several therapy alternatives to the use of antibiotics and hormones have been suggested for the treatment of endometritis. The intrauterine infusion of immune modulators such as E. coli lipopolysaccharides (endotoxin, LPS) , oyster glycogen (OG), infusion of serum, plasma or hyper immune serum or leukotriene B4 has been reported widely. These immune

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modulators act as a chemo attractant to the PMNs through stimulation of interleukins produced by monocytes and macrophages.

Ovulation induction agents

- hCG Injection Pubergen/Chorulon 1500-3000 IU at AI
- GnRH
- PG at AI
- Antiprolactin Bromocryptine 10 mg orally
- Dextrose 500mL IV at AI plus Bovine insulin 0.2 IU/Kg IV or Metformin orally

Luteal support

- hCG injection at 4-5 days of AI
- Progesterone injection 500 mg at 5 days of AI
- Recombinant Bovine Somatotropin 500 mg SC at AI

Improving Nutritional Imbalances

The effects of nutrition on reproductive efficiency of buffaloes have been addressed in many studies with high energy diets improving fertility and mineral and vitamin supplements improving conception rates in RB buffaloes. Poor nutrition during the dry and early postpartum period results in reduced glucose, insulin, insulin-like growth factor (IGF-1) and low LH pulse frequency with concomitant increases in beta-hydroxy butyrate, non-esterified fatty acids and negative energy balance, all having negative effects on the probability of conception.

Reducing Stress

Stress appears to play an important role in the modulation of various biological events including reproduction. The role of various types of stress caused by disease, inadequate nutrition, high production, social factors and environment on reproduction has been explained. An important factor affecting buffaloes is heat stress that suppresses both male and female reproduction.

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