Repeat Breeding Syndrome of cows in Bangladesh- A review

A Clinical Report Presented in Partial Fulfillment of the Requirement

for the Degree of

Doctor of Veterinary Medicine

A Report Submitted by

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Session: 2014-2015

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September, 2020

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September, 2020

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List of Abbreviations

Abbreviation	Elaboration
RBS	Repeat Breeding Syndrome
BCS	Body Condition Score
RB	Repeat Breeding
DLS	District Livestock Services
GnRH	Gonadotropic Releasing Hormone
LH	Lutinizing Hormone
PG	Prostaglandin
USG	Ultrasonography
PMNs	Polymorphonuclear leucocytes
CL	Corpus Luteum
hCG	Human Corionic Gonadotropin
AI	Artificial Insemination

ABSTRACT

Repeat Breeding Syndrome is considered as one of the most emerging and frustrated reproductive disorders among dairy herds that hinders favorable productivity and causes heavy economic losses to the livelihood of the farmers. Repeating breeding (RB) means a cow not to conceive after three regular artificial insemination (AI) services by an inseminator or natural services by a breeding bull. The aim of this study were to review the status of Repeat Breeding Syndrome (RBS) includes the factors that increases the risk of RBS, the different etiological factors, the diagnostic & treatment approaches of RBS that are commonly practice in Bangladesh. Its incidence varies among different management systems, environments and regions. It can be diagnosed by using different techniques like recto-vaginal palpation, vaginoscopy, uterine cytology & the in-vivo imaging technique of ultrasonography. The incidence of repeat breeding in dairy cattle can be reduced by improving their conception rate through careful handling of genitalia during insemination to avoid acquired abnormalities, appropriate treatment of reproductive tract infections, administering hormone therapy to improve fertilization success and lower embryonic mortality, and ensuring insemination of dairy cattle after proper clinical examination by a skilled inseminator. Therefore, it is essential to determine the prevalence and associate risk factors for occurrence of repeat breeding in cows and also to evaluate the efficacy of the drugs or interventions used for treatment of repeat breeder cows in Bangladesh.

Keywords: Repeat Breeding, Conception rate, Artificial insemination, Cytology, Hormone therapy.

CHAPTER 1: INTRODUCTION

Sixty-three percent (63%) of the total population of Bangladesh lives in rural areas and their main source of income comes from agriculture and livestock (https, 2018). Livestock plays an important role in agricultural development and the economy of the country contributing around 13.46% of agricultural Gross Domestic Product (GDP) and 1.47% to the national GDP of Bangladesh (Updated, 2018-2019). Cattle (*Bos tauras*) and water buffalo (*Bubalus bubalis*) has been considered as the important dairy animals in Bangladesh. About 20% of the peoples directly & about 45% of the people indirectly are in animal husbandry practices in which cow (24.09 Million), and buffalo (1.49 Million) has a major contribution in GDP (Updated, 2018-2019). Farmer's rear about 98% crossbred dairy cattle for milk production contributing two-third milk production of the country. Economy of dairy farming mainly depends on good conception rates after insemination. Reproductive efficiency is necessary for profitable dairy farms (Nebel and Jobst, 1998). Poor conception rate and delayed conception have been identified as major constraints of profitable dairy farming in Bangladesh (Alam and Gosh, 1988; Shamsuddin *et al*., 2001). Reproductive disorders have also been responsible for noticeable economic loss to the farmers and dairy industry in Bangladesh (Mia and Islam, 1967). Repeat breeding syndrome (RBS) is one of them among these reproductive disorders. Normally cow's conception rate is 50 to 60 percent within 1-3 consequent insemination. A cow with repeat breeding syndrome (RBS) is a cow that seems healthy and has a regular estrous cycle but she will not conceive after three or more sequential insemination without any clinical manifestation of pathological disease (Noakes *et al*., 2001; Gustafsson and Emanuelson, 2002; Moss *et al.*, 2002). Repeat breeding is a major reproductive disorder causes a great economic loss in dairy herds (Katagiri and Takahashi, 2004) by increasing the cost of production like AI, treatment, feed, labor and other management cost. It also increases calving interval, culling rates, decreased calf and milk production etc. (Lafi *et al.*, 1992) As a result, repeat breeding has been made a major concern to dairy farmers. There are several studies for finding out the pathogenesis and consequences of RBS in dairy cattle, their main common point is in the complex and plurifactorial etiology of this syndrome (Beam and Buttler, 1999; LeBlanc *et al.*, 2002; Spicer, 2004; Ferreira *et al.*, 2011) Prolonged duration of estrus, extended follicular phase, delayed luteinizing hormone (LH) surge and thus delayed ovulation, late postovulatory rise in plasma progesterone considered to be most distinguished factors responsible for repeat breeding (Bage *et al*., 2002). Other risk factors include

reproductive tract obstructions & abnormalities, early or latent embryonic abnormalities, poor breeding, hormonal dysfunction which causes about 40.1% of repeat breeding (Maurer & Echternakamp,1985), management techniques including genetic, nutritional and infectious factors etc. Its incidence can also be increased due to frequent heat stress incidence in dairy cows (Macmillan *et al*., 1996; Roche *et al*., 2000; Royal *et al*., 2000; Lucy, 2001). Failure of fertilization is considered as a prominent factors which causes mainly due to poor heat detection by farmers, improper estimation of fixed-time artificial insemination, poor semen quality. Failure of fertilization or early embryonic death is considered to be major pathogenesis of repeat breeding animals (Wodaje & Mekuria, 2016). Failure of fertilization is considered as a prominent factors which causes mainly due to poor heat detection by farmers, improper estimation of fixed-time artificial insemination, poor semen quality. Usually repeat breeder cows are diagnosed and treated by the veterinarians on the basis of history of the previous services and clinical examination of the cows. The alternative way of different hormonal therapy could be applied in dairy herds to increase the reproductive efficiency and minimize the reproductive problem related to anestrous and failure of fertilization and conception (Savalia *et al*., 2014). It is crying need to solve the RB problems for producing more milk to achieve the vision 2021 of Bangladesh & to make profitable & sustainable dairy farming in Bangladesh.

CHAPTER 2: METHODOLOGY

A scientific literature review was done from different articles, published authenticate reports, & reliable information from seniors & supervisors of my faculty on the present status (risk factors, etiological factors, diagnostic & treatment approaches) of repeat breeding syndrome (RBS) of dairy herds in Bangladesh. I have summarized the relevant information in systemic way and also some are summarized in tabulated form.

CHAPTER 3: RISK FACTORS

A large number of risk factors that increases the prevalence of repeat breeding have been described for cows including regional areas, age, BCS, parity, herd size, milk yield etc. Some are described below-

1. Region: The overall prevalence of repeat breeding in cows in Bangladesh is 11% (Boettcher and Perera, 2007) but it varies from different regions of Bangladesh due to weather, nutrition, management etc. The prevalence of repeat breeding in cows according to different areas (Asaduzzaman *et al.*, 2016) is presented in Fig 1:

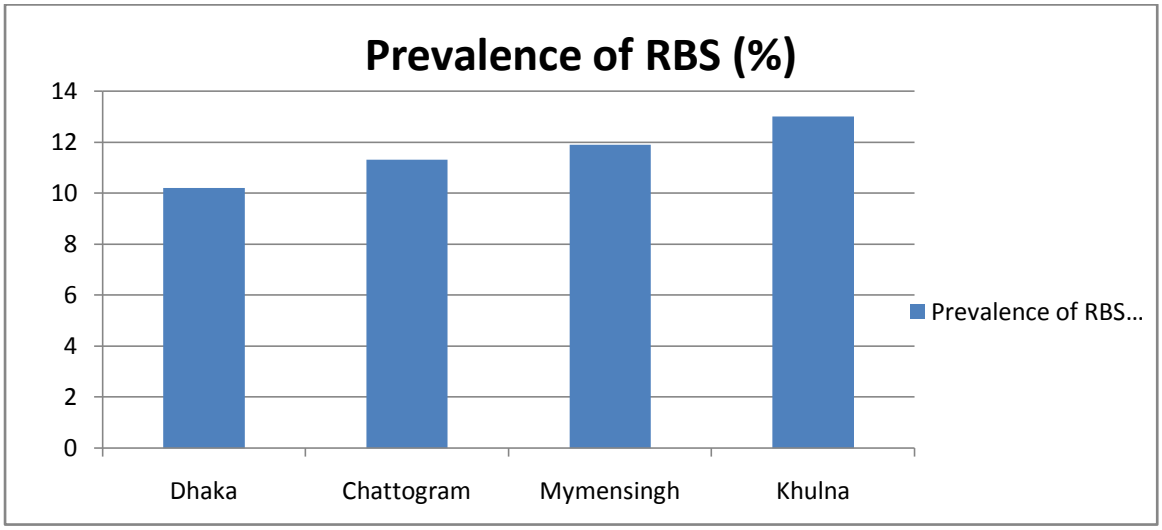

Fig 1: Fluctuation of Prevalence of RBS in different regions of Bangladesh

2. **Breed**: Higher prevalence of repeat breeding has been reported in cross breed cows than those of local breed (Mandefro and Negash, 2014). It seems for the reasons of lower occurrence of repeat breeding in local cows that local cows are more tolerant to prevailing environment of Bangladesh than that of the Friesian cross cows. The prevalence of repeat breeding in cows in respect to breed (Asaduzzaman *et al.*,2016) is showed in Fig 2:

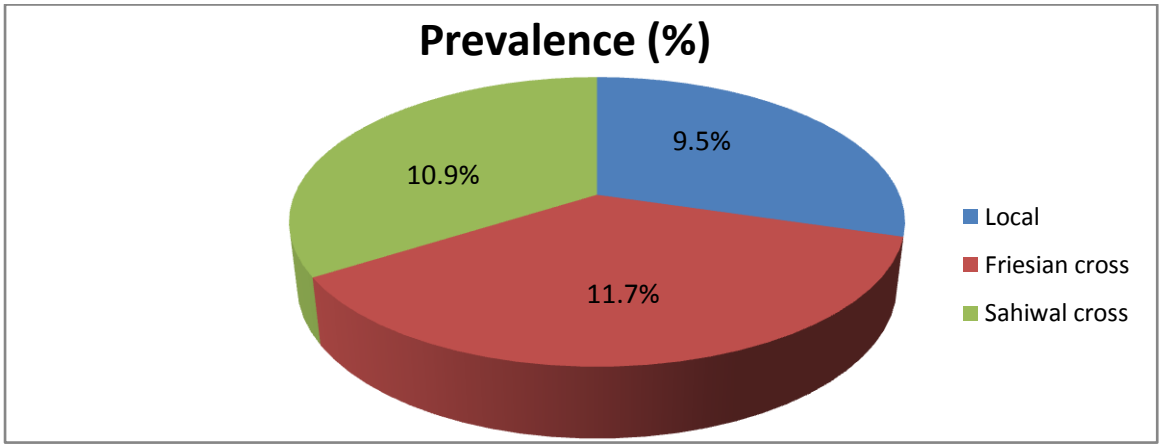

Fig 2: Fluctuations of prevalence of RBS in different breeds

3. **Parity**: A higher conception rate is observed in multiparous cows than that of uniparous cows (Coleman *et al.*,1985). Moreover, Boyd and Reed (1961) reported that an increased conception rate is showed from parity 2 up to 6, and then declined at parities 7 and 8.

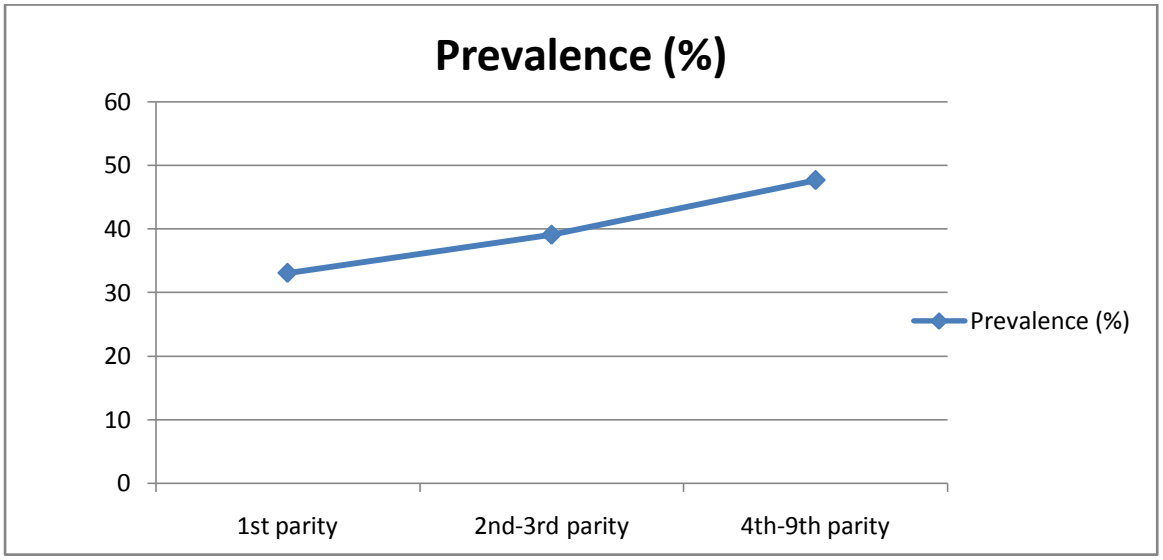

Fig 3: Fluctuations of Prevalence according to parity of cows

4**. BCS of herd**: Poor BCS causes low conception rate of cows in Bangladesh (Shamsuddin *et al*., 2001). It is thought that the cows of herds with low BCS suffer from more negative energy balance results in inadequate secretion of reproductive hormones that causes failure of fertilization or early embryonic death followed by repeat breeding.

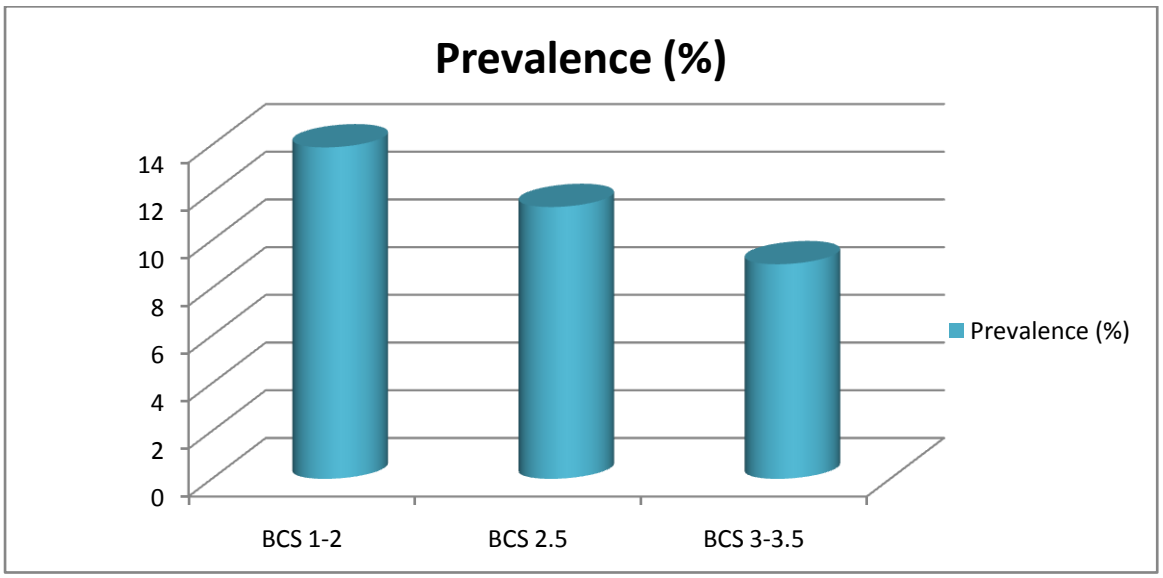

Fig 4: Fluctuations of Prevalence of RBS in respect with BCS of cows

CHAPTER 4: ETIOLOGY OF RBS

The etiology of repeat breeding has been multifactorial. The causes of repeat breeding have been classified in cows in various ways, yet failure of fertilization and early embryonic deaths had been one of the oldest classifications of the etiologies (Purohit,2008). It also may be related with external factors such as environmental stress and poor breeding management (Perez *et al*.,2012). The causes of repeat breeding in cows are discussed under the headings of failure of fertilization and early embryonic deaths-

1. **Failure of fertilization:**

It is observed that failure of fertilization accounts for a low proportion (10%–20%) of pregnancy losses during the first 21 days post insemination in cattle (Diskin & Moris,2008). Moreover, it is found that causes of fertilization failures are very few and suggest factors possible in the cow (ovulation failure, oviductal obstructions, abnormal ova and endometritis). The cause of fertilization failure can also lie with the bull and the technique and timing of insemination when using artificial insemination.

- ***Female cows*:**
    - **Ovulatory disturbances:** Delayed ovulation, anovulation and luteal insufficiency with the incidence of delayed ovulation can cause repeat breeding in dairy cattle. In fact, considered that there is a correlation of follicle diameter with pregnancy outcome in dairy cattle (Kapse *et al*., 2017). The diameter (11.53±0.32 mm) of ovarian follicle ovulating at the time of AI were more likely to become pregnant than the lesser diameter.
    - **Oviductal obstructions and adhesions**: Obstructions in the oviduct such as hydrosalpinx, pyosalpinx, salpingitis and stenosis or growths in the oviduct may prevent fertilization (Vala *et al*.,2011). Ovarobursal adhesions have been shown as a lower incidence varying from 0.04% to 6.4% (Vala *et al*.,2011) yet abattoir studies reflect a higher incidence which affects fertility as they interfere with tubal motility.
    - **Endometritis**: Endometritis has effect on fertility that may be mediated directly by bacterial endotoxins or indirectly by inflammatory mediators such as cytokines, nitric oxide and oxidative stress affecting sperm, ovarian, uterine and embryonic function (Gilbert,2011). Endometritis can affect sperm motility and function and results in increased sperm

phagocytosis (Gilbert,2011) which may be due to the increased influx of polymorphonuclear leucocytes in the lumen or poor development of resultant zygotes. Oocytes exposed to bacterial toxins are less likely to develop to the blastocyst stage. Denudation of endometrial epithelium, edema of connective tissue and scanty uterine glands in the endometrium is found in histological studies (Babu,2013). An altered endometrial morphology may consider for fertilization/pregnancy failures and an altered ovarian function which can cause repeat breeding of animals.

- ***Bull Factors*:** Repeat breeding may also be due to bull & semen factors (Perez *et al*.,2012). The age of bull affects the semen volume and the proportion of abnormal spermatozoa where adult bulls produce the highest volume and lowest abnormal sperms (Khawaskar *et al*.,2012). The bull breed, semen type (liquid or frozen), quality and source have marked effects on the conception rates (Andrabi, 2009). Season is also a very important consideration for semen collection because semen collected during hot summer months has been shown to have lower fertility (Khawaskar *et al*.,2012) which can contribute to repeat breeding. Disease infected bulls like Campylobacter can be a probable reason for early embryonic deaths and repeat breeding in cows (Perumal,2013).
- ***Breeding management***: As the use of AI in cattle is increasing day by day, so some factors like insemination technique, time of insemination and site of semen deposition, skills of inseminator etc is to be considered for optimum fertility. A problem with AI is the poor estrus expression and lack of efficiency to detect estrus. Insemination relative to ovulation also appears important to achieve high fertility. A survey suggested that 14.75% dairy cattle as repeat breeder due to managemental causes (Singh *et al.,* 2008). Among these, 6.78% were malnourished whereas 5.63% were repeat breeder due to incorrect time of artificial insemination (Singh and Pant, 1998b). In spite of all the developments in semen handling, AI procedures and training of veterinarians, the average post-AI conception rate remains below 30% in the field, which make a serious challenge to the future of dairying because of inseminator skill & increasing the incidence of endometritis, cervical fibrosis, ovarian adhesions, cervicitis, perforated rectum and lacerated uterus etc which causes fertilization failure. Another major managemental cause is deterioration of semen quality during transportation from semen laboratory to field institutions due to not maintaining

cool chain properly. The success of AI program is linked with the prolongation of fertile life of spermatozoa under *in vitro* storage conditions.

2. Early Embryonic death: Embryonic mortality occurs in cattle mostly within the first 2–3 weeks of gestation (Diskin & Moris,2008) and accounts for 45% of pregnancy failures during this time. Low concentrations of circulating progesterone appear to be the cause of embryonic deaths in cattle due to failure of endometrial and embryonic signal exchanges (Bajaj & Sharma,2011). Besides this, some other possible reasons also found like genetic selection of cows for high production (Diskin & Moris,2008), environmental stress, shipping and heat stress and uterine infections with pathogenic microbes (Bajaj & Sharma,2011). Rearing systems that provide adequate cooling during the hot summer months resulted in significantly lower embryonic mortalities. Other studies revealed that the proportion of embryonic mortalities was not affected by age, parity or lactation stage. Infectious agents were mentioned only for 2%–8% of the embryonic losses in cattle (Campanile *et al.*,2005).

CHAPTER 5: DIAGNOSIS OF RBS

The diagnostic procedures for repeat breeding in the present review have been classified into the following groups:

1. Record analysis
2. Visual
3. Recto-genital palpation
4. Vaginoscopy
5. Tests to evaluate uterine health:
    (a) Uterine pH
    (b) Uterine microbiology
    (c) Uterine biopsy and cytology
6. Metabolic profiles
7. In vivo imaging techniques
8. Immunological tests
9. Endoscopy
10. Tubal patency testing
11. Hormone assays

1. Record Analysis: Records of previous actual number of inseminations & peri-parturient diseases should be analyzed. Insights into poor fertility can be traced in individual cows by record analysis.

2. Visual: Its importnace in diagnosing an animal that subsequently repeated to service or artificial insemination (AI) may be cause of improper oestrus detection or insemination asynchrony may many times contribute to the failure of pregnancy establishment in an individual animal. Visual observations that need the attention of the inseminators include the colour, consistency and clarity of the cervico-vaginal mucus, vulvar oedema and vaginal congestion. A cow showing a cervico-vaginal mucus discharge that is watery, thin or copious should be viewed as one with suspicious subsequent fertility.

3. Recto-Vaginal Palpation: Recto-genital palpation of both uterine horns, cervical os and the ovaries is considered as the commonest diagnostic method used for cows nowadays in Bangladesh repeating to natural services/inseminations. Animals with poor uterine tone at palpation often have poor conception. Pleuriparous cows, especially those

which have had some periparturient problem, have irregularly shaped cervices, creating difficulty in the insetion of the insemination pipettes. Most of the loss of offspring in cattle is observed during the first 42 days after breeding (Inskeep, Fields *et al*.,2002) and in particular 6–20 days after breeding (Ayalon,1978), when it is quite possible by rectal palpation to evaluate pregnancy.

4. Vaginoscopy: A common clinical method to estimate uterine infection is seems that of Examining the vagina and cervix by vaginoscopy to determine the presence or absence of small quantities of infected material in inside. The presence of discharge in the vagina may be influenced by the severity of the infection, myometrial contraction, uterine clearance mechanisms, perineal conformation, body condition, postural changes, exercise etc. Discharges may not be detected in cows in which the cervix is closed, although these cows may harbour infection.

5. Test to evaluate uterine health:

(a) ***Uterine pH:*** The pH of vaginal mucus was found 8.5+1.16 in repeat breeder cows compared with 7.2+1.10 in normal breeding cows (Gupta *et al.*,1981). But high protein diets alters the uterine environment by reducing concentrations of magnesium, potassium and phosphorus in uterine secretions and by reducing uterine pH (Jordan *et al*.,1983).

(b) ***Uterine Microbiology:*** A wide variety of microbes normally harbour the uterine lumen but when their numbers are high, purulent mucus flakes of pus and changes in odour of the genital discharges are observed. Many studies explained that mostly mixed infections are present in the uterus of RB cows (Javed & Khan,1991). Different tests are used such as 'white side test', uses cervical mucus of suspected cows (with metritis/endometritis) heated with sodium hydroxide solution up to boiling point. If the color turns yellow, its considered as positive. A correlation between the number of leucocytes present in the mucus and the intensity of yellow color is the basis of this test (Javed & Khan,1991). Other recent developments to evaluate endometritis include the novel intravaginal device 'Metricheck', which is known to be more sensitive in detecting endometritis than vaginoscopy (McDougall *et al*.,2007). The device is inserted through cleaned vulvar lips, advanced to the cranial extent of the vaginal fornix and then retracted back. Purulent material may be visualized within the concave surface, or adherent to the convex surface, of the device if infection is present.

(c) ***Uterine biopsy & Cytology:*** Uterine biopsies can reveal the changes in the endometrium and the extent of cellular infiltration and/or cellular morphology (Brus,1955). In RB cows the glandular secretions and supranuclear vacuolations are

observed in histological sections prepared from collected biopsies (Ohtani *et al*.,1995). Changes observed in cows with endometritis include denudation of epithelial lining and infiltration of lymphocyte and neutrophils (Javed & Khan,1991). The endometrial EGF concentration is altered in RB cows and can serve as a potential marker for the identification of cows that would turn out to be repeat breeder (Katagiri *et al*.,2004). The sensitivity and specificity of uterine biopsy for pregnancy was found to be 92 and 77% (Bonnett *et al*.,1991). Cytology mainly helps to diagnose subclinical uterine inflammation. Different procedures have been described for obtaining the uterine cells and performing a count, and include flushing the uterus with small amounts (2–5 ml) of fluid or using a commercially available cytobrush (Kasimanickam *et al*.,2005). The threshold for defining subclinical endometritis was finding of Polymorphonuclear leucocytes(PMNs) >18% at 20–33 days postpartum, whereas the respective threshold at 39–47 days postpartum was >10%.

6. Metabolic Profile:

Table 1: Blood biochemical constituents in normal & repeat breeding cows-

Parametre	Normal cows	Repeat Breeding cows
Glucose (mg%)	47.16–84.54	45.6–97.73
Cholesterol (mg%)	83.0–249.22	77.8–182.37
Hb (gm%)	9.06–11.74	8.98–9.71
Ca (mg%)	6.17–10.73	6.60–69.65
P (mg%)	22–8.19	3.37–8.03
Fe (mg/dl)	1.9–2.4	2.47–11.3
Blood Urea (mg%)	18.80	28.88
Vitamin A (mg/dl)	41.216	37.14
Co (microg/ml)	2.18–9.67	0.85
Na (meq/l)	133.7	140
K (meq/l)	4.44	4.27
Cl (meq/l)	96	96.1
Mg (mg%)	3.19-22.57	2.56-9.82

7. In vivo imaging technique: The most important diagnostic modality for reproductive diagnosis is ultrasonography (USG). Ovarian dysfunction like ovarian cysts, ovulation defects, luteal dysfunction and a prolonged life span of pre-ovulatory follicle is common

in RB cows(Bage *et al.*,2002). USG examination must be done at AI to determine the presence of an ovulatory follicle and then repeated at 12 h intervals to find out if ovulation has occurred, and subsequent examinations have to be done at 4 day intervals to observe CL formation (Perez *et al*.,2007). A uterine lumen with a diameter of 0.2 cm and presence of echogenic content in the uterus is considered as endometritis. Improvements in the in vivo imaging technique include computer-assisted image analysis of USG, three-dimensional USG, color Doppler USG and magnetic resonance imaging (MRI) can also be used to detect repeat breeding of animals (Sarty *et al*.,1996).

8. Immunological tests: Antisperm antibodies are known to be present in serum and they result in sperm-immobilizing activity, leading to reduction of penetrating capability of sperm in cervical mucus and cause reduction of fertility (Kremer & Jager,1992).But its very rare in cows.

9. Endoscopy: Visualization of the morphology & live tissues of uterus and other genital organs is the main function of endoscopy. It has also been used to examine the ovaries by colpotomy or flank methods, particularly for follicular aspiration or oviductal transfer of in vitro produced embryos (Fayrer *et al*.,1989). But it is less practiced in cattle.

10. Tubal patency testing: The occlusion of the fallopian tubes can result in lowered fertility when it is unilateral and sterility when it is bilateral (Bruyas *et al*.,1993). This test is done by infusion of phenol sulpfopthalein (PSP) dye using a two-way catheter into the uterine horn and detection of the dye in urine. In animals with non-occluded fallopian tube, the dye is present in urine within 30 min; however, in cows with non-patent (occluded) fallopian tube, the dye is not visible in urine for up to 2 h (Kothari *et al*.,1978). When the same procedure is to be repeated in the other horn, a gap of at least 12–24 h must be provided or the dye must be changed.

11. Hormone assays: Among the reproductive hormones, especially LH and the ovarian steroids oestrogen and progesterone, can affect pregnancy establishment. A delay or deviation in the secretion of LH peak surge can affect ovulation and the development of CL. The levels of progesterone both at estrus and during the luteal phase appear to be critical in dairy cattle (Sreenan *et al*.,2001). Higher basal progesterone (the so called suprabasal (SB) progesterone) can be considered as a main tool for the identification of repeat breeder heifers (Waldmann *et at*.,2001), provided that heat detection and AI timing are optimal. It has been shown that as the progesterone level at AI rises, conception rate in cows declines (Ghanem *et al*.,2006).

CHAPTER 6: THERAPEUTIC APPROACHES OF RBS

The therapy of RB cows is supposed to be performed only when oestrus detection and breeding (natural or AI) protocols are optimal. Therapies in a herd or individual with suboptimal fertility constitute corrective measures to prevent or combat disease and/or deficiency and reducing stress.

A. Correction of endometritis: When clinical or subclinical endometritis is suspected, the clinician's first choice must be the administration of antimicrobials at the day after insemination to rid the uterus of organisms that might be detrimental to the survival of the conceptus. Alternatively, antibiotics may be infused in the uterus for 3–5 days during oestrous and insemination when more of pus is observed clinically or when therapy alone with antibiotics fails or in severe case that leads to high concentrations of the drug in the uterine cavity and endometrium, and a relatively small amount is absorbed into the systemic circulation (Masera *et al*.,1980). Systemic antibiotic administration should therefore be used in treatment of severe cases of metritis (Chenault *et al*.,2004). The new (third and fourth) generation cephalosporins have shown efficacy against most uterine pathogens at low MIC values and the first generation cephalosporin (cephapirin) is recommended for intrauterine use as the drug of choice for subclinical endometritis (Kaimanickam *et al*.,2005). It is suggested in most of the cases to combine an antibiotic with an imidazole derivative (metronidazole or tinidazole) to take care of anaerobic microbes and protozoa that may unusally be present (Purohit & Sharma,2007). Antiseptics such as dilute Lugol's iodine or povidone iodine have shown efficacy in intrauterine washing (Purohit,2005). Besides these, The most potent and safe approach seems to be the use of prostglandins (PG) from 5–10 days of oestrus alone or followed by a uterine lavage (Feldmann *et al*.,2005). In clinical practice it is sometimes recommended not to allow the animals for matings and this works in recovery from endometritis well, but if the animal suffers for a ling time, it is always better to use prostaglandins. Also the intrauterine infusion of immunomodulators such as E. coli lipopolysaccarides (endotoxin), oyster glycogen, infusion of serum, plasma or hyperimmune serum or leukotriene B4 has been reported widely (Saini *et al.*,1999). A single intrauterine infusion of 100 mg of E. coli LPS dissolved in 20 ml of phosphate-buffered saline (PBS) results in increase in the uterine neutrophils (of up to 80%) within 6 h, which remains for 72 h (Green *et al.*,2007). Likewise, 0.1–10% oyster glycogen (OG) (usually 500 mg) dissolved in 60 ml of vehicle or 30 nmol/l of leukotriene B4

increases the PMN concentration within 12–24 h of administration (Zerbe *et al*.,1996). Within 72 h of administration of either LPS or OG, the denuded epithelial condition was recovered and the psuedostratification of uterine endometrium was completely cured. Addition of a small amount of autologous serum or plasma (50–100 ml for 2–3 days) to uterine secretions increases the opsonizing capacity and significantly enhances the phagocytic ability of PMNs (Hussain & Daniel,1991). Besides these, some other therapies suggested for resolving endometritis include the use of enzymes and antioxidants. Enzymes like trypsin, chymotrypsin and papain, Lysozyme when infused into the uterus resulted in a cure rate of 59.7% (Revealed by absence of vaginal discharge at re-examination); however, the conception rates were suboptimal (Drillich *et al.*,2005). Some medicaments like 4mM taurine and 50mM fructose in PBS and ascorbic acid (vitamin C) have been used for intrauterine infusion in order to change the uterine pH prior to insemination, and act as an antioxidant (Purohit,2005). Antioxidants such as vitamins C and E are known to modulate the oxidative stress and reduce the endometrial damage both at the biochemical and histological levels (Guney *et al*.,2007). Using these treatments the endometritis would usually be cured and cows can be inseminated at subsequent oestrus.

B. Correction of ovarian dysfunction*:* Ovulatory disturbances like anovulation, delayed ovulation, ovarian cysts etc. are observed in cows that causes poor fertility, pregnancy failure and hence RB. Unovulation seems to be due to a lack of a preovulatory surge in response to the high oestradiol concentrations, presumably because of lack of progesterone or may be the presence of suprabasal progesterone concentrations during oestrus, which has an inhibitory effect on the positive feedback of high oestradiol concentrations on the hypothalamus, resulting in high LH pulse frequency and effects on follicular growth (Stock & Fortune,1993). GnRH treatment can stimulate ovulation and, in some cases, result in increased pregnancy rates & administration of either hCG (1500–5000 IU, intravenous, or 5000–10 000 IU, intramuscularly) or 100 mg of GnRH or hMG (Leslie,1983). Alternatively, may also be administered of glucose and insulin, prostaglandins, metformin, antiprolactins and clomifene. It has been reported that intramuscular (IM) administration of 0.2 IU/kg bovine insulin to dairy cows on days 8, 9 and 10 of oestrus resulted in increased concentrations of progesterone & administration of 500 ml of 25% dextrose at oestrus along with insulin (5 ml of bovine insulin) (Selvaraju *et al*.,2002). Clomiphene can also be used & thus started preferably 1 day before oestrous (300 mg orally after copper sulphate at 12 h intervals) until the onset of

oestrous by up-regulation of receptors that follows a down-regulation would facilitate LH release and ovulation. The most common ovulatory disturbance in dairy cattle is the cystic ovarian disease with signs of nymphomania, mucometra, and frequent oestrous. Its suggested for the administration of a single IM 200 mg injection of progesterone in oil or insertion of intravaginal progestagen implants , 100 mg of GnRH , hCG followed by prostaglandin treatments after 8–10 days (Verma & Debas,1994). Ovsynch protocol suggested and used widely utilizes the administration of GnRH (100 mg) on day 0, followed by prostaglandin on day 7, GnRH (100 mg) on day 9 and AI 16–20 h later (Vasconcelos *et al*.,1999). The second dose of GnRH make sure of ovulation of the newly formed follicle. Regimens suggested to resolve mucometra include oral (3–10 g of potassium iodide for 5–10 days) or injectable administration of elemental iodine or uterine lavage otherwise it may cause the regeneration of cysts (Rajan *et al*.,1991). Probable preventive measure to reduce suprabasal progesterone will be possible by monitoring the diet and reducing stress.

C. Correction of luteal insufficiency*:* Luteal inadequacy causes may be due to decreased response to the circulating luteotrophic hormones leads to insufficient progesterone production during the luteal phase after breeding which can be the cause of embryonic death (Kimura *et al*.,1987). Early in the luteal phase, the progesterone down-regulates the oxytocin receptors (OTRs) for at least 10 days, thus preventing premature luteolysis. Therapeutic approaches include administration of one of the following:

a. GnRH at the time of insemination or day 11–13 post AI (Gonzalez *et al*.,1999);

b. hCG on day 4–7 or day 15–16 post AI (Rajamahendran & Slanangama,1992).

c. Progesterone supplemented as a single IM injection (500 mg) on day 5 post AI, chlormadinone daily oral feeding (10 mg) from day 14–23 or progestagen vaginal implants from day 5–12 of AI (Devanathan *et al*.,1999)

d. Recombinant bovine somatotropin (500 mg SC) at the time of oestrus and 10 days later.

Management Strategies:

The overall management of dairy cows is important as it affects the fertility. Among these, nutrition, timing of insemination, periparturient diseases etc should be considered.

A. Improving Nutritional Imbalances: The effects of nutrition on fertility in dairy cattle have been extensively reviewed recently particularly on the effects of macro- and

microminerals during the periparturient period (wilde,2006), the impact of controlled nutrition during the dry period (Beever,2006), the effect of rumen degradable proteins (Tamminga,2006), and embryo survival in dairy cows managed under pastoral conditions. Poor nutrition during the dry and early postpartum period results in reduce glucose, insulin, insulin-like growth factor (IGF-1) and low LH pulse frequency with relatively increases in b-hydroxybutyrate, non-esterified fatty acids and negative energy balance all having negative effects on the probability of conception. However, it has been recommended that cows must not lose excessive body condition and should not be fed more than 10% of rumen degradable protein in postpartum period. A balanced feed during the dry period must therefore comprise a low-energy high-fibre ration containing high levels of chopped straw. Also recommended that, RB cows suffer from multiple deficiencies, especially those of glucose, vitamins such as A, E, and C and minerals like phosphorus, calcium and selenium and animals must be supplemented with these nutrients by oral or injectable supplementation if it is required.

B. Improving the Timing and Technique of Insemination: Improving the timing of insemination by appropriate oestrus detection can enhance the conception rate in cows. The use of pedometers and radio-telemetric devices (Lehrer *et al*.,1992) has been suggested to improve oestrus detection and, hence timing of insemination. It is suggested to repeat AI in twice at 12 h interval for optimum conception rates. Oestrus synchronization protocol with fixed time AI has been shown to improve fertility of dairy cattle. AI is usually scheduled 60–72 h of a PG injection which may improved fertility of cows suffering from heat stress (Rensis *et al*.,2002), as heat stress is known to decrease the intensity and duration of oestrus expression and increase the incidence of anoestrus and silent ovulation. Also suggested that, deposition of semen in the body of the uterus offers a distinct advantage in improving the conception rates, compared with when it is deposited in the mid cervix (Purohit,2010). Also if any errors occur in the preparation of the AI gun or those in the upkeep of frozen/liquid semen must be reviewed seriously cause it can contribute to conception failures.

C. Avoiding Periparturient diseases: Metabolic diseases such as ketosis and acidosis etc during postpartum period or parturient problems, such as hypocalcaemia, mastitis, retained placenta, dystocia, endometritis etc can be observed in cows (Roche,2006). The approaches suggested to reduce the incidence of these disorders include the feeding of anionic salts in combination with adequate calcium and magnesium (Wilde,2006) during

the dry period and feeding of high-fibre low-energy chopped straw during the dry period (Beever,2006). Many locations where animals are raised suffer from extremely poor hygiene. This is the fact here that farmers attending calving or manually removing placentas often handle animals without any sanitary measures. These practices are likely to occur low conception may be because of low grade infection or damaged genitalia. It is therefore important to educate farmers regarding the possible consequences of the poor hygiene at calving and postpartum.

D. Reducing stress: Stress appears to play an important role in various biological events including reproduction. Various types of stress may occur because of disease, inadequate nutrition, high production, social factors and environmental factors etc. To minimize these stress, cooling of cows during hot summer months by showering of water, give proper diet, not to expose repeated services for per conception etc those are known to improve fertility.

E. Imunoinfertility: It has since long been postulated that sperm, when deposited in the female tract, can act as an antigen and modulate the production of antibodies leading to immunoinfertility. Antisperm IgG and IgA have been isolated from bovine (Jones, 1979) which usually reduce the sperm penetration of cervical mucus with immobilizing activity but it is less proved in animal subjects. A few of the approaches include administration of vitamins C and E and dexamethasone (Wang *et al*., 1999) and intrauterine insemination of vitamin C with little success.

F. Miscellaneous Therapies: Despite all efforts of therapy, a proportion of RB cows remain as infertile for prolonged periods and they are described to have infertility of unknown origin (Bruyas *et al*.,1993); such an infertility known as 'idiopathic'. Some of the less common therapies described for cows suffering from the RB syndrome include acupuncture therapy (Lin *et al*.,2002), intraperitoneal insemination (Lopez-Gatius,1995), use of herbal drugs (Hegde *et al*.,2002) and embryo transfer at 7–8 days of oestrus with or without AI at oestrus (Dochi *et al*,2008). Such therapies however have little to offer in improvement of the condition. Cows that gain excess of body fat are a classic example of idiopathic infertility. Such cows, when made to lose weight by severe diet restriction, often conceive.

CONCLUSIONS & RECOMMENDATION

The present study gives an idea about the RBS, continues to be a big problem for dairy industry due to poor conception rate & embryonic death. It showed as a multifactorial problem & some factors also influence the prevalence of RBS which causes a great economic looses to farmers. Different methods (Uterine biopsy, Ultrasonography, MRI, endoscopy, tubal patency testing etc.) can be used to diagnose this condition & apply different therapeutic & managemental approaches to solve this problem. Yet, it is difficult to find the actual reasons. If it is possible to identify the actual causes of RBS, it'll be easy to treat & can improve our dairy industry.

LIMITATIONS

Repeat breeding is a plurifactorial condition of dairy animals. Some factors are diagnosed, but some are undiagnosed yet particularly the infectious cause of RB. To identify the uterine microbes, some modern techniques are already used. Recently, Department of Medicine and Surgery, CVASU conducting a research work on repeat breeding cows. In this research a modified human cytobrush is using for uterine biopsy sample collection to identify possible microorganisms under the supervision of Professor Dr. Azizunnesa. I was interested and involved in this research work. I also collected cow's uterine samples through human modified cytobrush from different areas of Chattogram district. But I couldn't complete my research (Lab work) due to this unavoidable circumstance COVID-19. Therefore, I turned out my decision with the permission of authority to this written review work on repeat breeding cows.

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ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest sense of gratitude to “The Almighty Allah”, who enables me to complete the research work and dissertation.

I would like to express the heartiest appreciation, gratitude and best regards to the supervisor **Dr. Azizunnesa**, Professor, Department of Medicine & Surgery, CVASU. I really deem it a proud to do a research work under her constructive, useful and effective supervision.

I feel much pleasure to convey thanks to the **Prof. Dr. Abdul Ahad**, Dean, Faculty of Veterinary Medicine; **Prof. Dr. A.K.M Saifuddin**, Director, External Affairs, CVASU for the valuable cordial co-operation and suggestion during the whole intern period.

Last but not least I would like to express my gratitude, cordial respect to my beloved family members for their immense sacrifice, blessings and encouragement.

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