

## Observations on Repeat Breeding in Farm Animals with Emphasis on its Control

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**Abstract:** Repeat breeding (RB) is an important reproductive disorder which causes great economic losses in farm animals. The repeat breeder animal is usually defined as sub-fertile animal which served three or more times and becomes not pregnant and continually return to service in the absence of any obvious pathological disorder in the genital tract. Rectal palpation and ultrasonography with endometrial culture can be included as a fundamental part of genital examination for diagnosis of such case. The causes of this type of subfertility can be divided into two major categories; fertilization failure and early embryonic death. To reduce the incidence of RB syndrome in farm animals, a successful diagnosis and treatment using recent approach should be applied. Recent trends such as presynch- protocol and fixed-time embryo transfer (FTET) have proven to reduce the incidence of RB. Miscellaneous trials including administration of insulin, immunomodulators as well as broad spectrum antibiotics have been used.

**Key words:** Repeat breeding • Etiology • Diagnosis • Treatment and Farm animals

### INTRODUCTION

Ovarian inactivity, silent heat, endometritis and repeat breeding (RB) are the main reproductive disorders in buffaloes in Egypt [1]. In spite of all the advances in veterinary medicine, RB remains the most economically important type of infertility in domestic animals. The condition contributes to longer calving interval, increasing culling and replacement costs, wasting semen and insemination costs and losing genetic gain through increased generation intervals [2] and reducing fertility [3]. Repeat breeder animal is usually defined as sub-fertile animal which mated three or more times during the proper period and does not become pregnant and continually return to service in the absence of any obvious pathological disorder in the genital tract [4] and has normal estrous cycles [5].

The incidence of typical RB ranged between 0.7 and 8.33% in buffaloes [1, 6], 5.5 -33.33 % in cattle [7] and 8.6 - 11.3% sows [8]. The variable incidences may be attributed to the heterogeneity or multifactorial causes of RB syndrome as well as the effect of locality and season [9].

The causes of this type of subfertility can be divided into two major categories; fertilization failure and early embryonic death [10]. Embryonic mortality and conception rate were estimated to be 65 and 17- 28 % in

RB cows as compared to 10 - 40 and 35 - 45% in first-service normal herds, respectively [11].

In this article, pertinent literature on the etiology, diagnosis and treatment of RB syndrome in farm animals were reviewed.

### The Etiology of Repeat Breeding

#### Non Infectious Factors

**Genetic:** Chromosomal aberrations as well as autosomal recessive genes are the major causes of early pregnancy failure in animals and may account for 20% of total embryonic and fetal losses [12]. 1/29 Robertsonian translocation is chromosomal abnormality found in various animal breeds and result in lower conception rates or high abortion rates after field insemination or lower cleavage and blastocyst rates *in vitro* [13]. Coagulation Factor XI (FXI) deficiency, complex vertebral malformation (CVM) and deficiency of uridine-5-monophosphate synthase (DUMPS) are hereditary disorders observed in various mammalian species with history of RB and cause intrauterine mortality through gestation period [14-16].

**Anatomical Defects of the Reproductive Tract:** The most common congenital abnormalities of the uterus, cervix and vagina in Camelidae are infantilism, segmental aplasia, uterus unicornis, double cervixes, vaginal constriction

and septum. These anomalies should be suspected when there is difficulty in penile intromission. Traumatic injuries of the vagina can lead to the formation of complete adhesions between the vaginal wall and development of pyometra [17].

**Hormonal Imbalance:** An abnormal endocrine status during folliculogenesis and ovulation failure has been reported to be major causes of RB in cows [18] and Camelidae due to inadequate LH release in response to copulation [17]. This insufficient LH release could be due to a hypothalamo-pituitary function disturbance or reduced stimulatory effect of copulation or reduced concentration of a GnRH-like factor present in semen that stimulate ovulation. It was reported that ovulation of very small follicles ( $11.5 \pm 0.2$  mm) in lactating cows resulted in smaller CL, lower serum progesterone concentrations and lower conception rates compared with ovulation of larger follicles ( $14.5 \pm 0.2$  mm) [19]. The reduction in conception rate might have been due to a reduction in interferon-tau (IFN- $\tau$ ) production by the conceptus since maternal progesterone concentrations were positively correlated with IFN- $\tau$  production. Alternatively, the lower conception rate in cows with lower plasma progesterone concentrations might have resulted from the development of a stronger luteolytic signal [20].

**Bad Management:** Heat stress, poor nutrition and improper insemination technique are reported to be factors causing RB in farm animals. Heat stress turns the buffaloes into repeat breeders or anoestrus during summer season [21]. Cows under heat stress have reduced duration and intensity of estrus, altered follicular development and impaired embryonic development [22] due to endocrine changes reducing follicular activity, altering the ovulatory mechanism, leading to inferior oocyte and embryo quality and modified uterine environment which reduce the likelihood of embryo implantation. Poor nutrition or underfeeding occurs most frequently and can result in weak estrous expression and reduced ovulations, extended periods of anoestrus following calving, low conception and fertilization rates [23]. Cows in negative energy balance (NEB) have lower blood concentrations of insulin and insulin like growth factor (IGF-I). These endocrine hormones can influence GnRH secretion by acting on GnRH neurons, or their neuronal pathways or on the pituitary gonadotrophins [24]. Problems related to protein deficiency (below 8 % of dietary crude protein) are weak

expression of estrus, anoestrus, RB, fetal resorption and weak offspring at birth. On the other hand, feeding of excessive protein (more than 13% crude protein) can result in lowered first service conception, increased services per conception and increased number of days open due to the possible alteration in blood urea concentrations and uterine pH as well as hormone secretion such as progesterone leading to embryo mortality [25].

Repeat breeder dairy cows had lower levels of serum Zn, Cu, P and inorganic I as compared to normal animals [26]. This was confirmed by improvement of recovery and conception rates in RB buffaloes and cattle following supplementation with mineral mixture [1, 27, 28]. Vitamin A deficiency can cause problems in females ranging from anestrus, RB, abortions, weak offspring at birth and retained placenta. Feeding RBs with  $\beta$ -carotene improved the pregnancy rate by 33.3% compared with 27.2% in the control group which may be attributed to high oestradiol and progesterone levels resulting from increased follicle size and corpus luteum functionality [29].

**Infectious Factors:** It was reported that an increased incidence of RB may be due to subclinical endometritis which decreases reproductive performance by its negative impact on first service conception and pregnancy rates in cows [30]. *Arcanobacterium pyogenes*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* were isolated from uteri of she camels and cows with history of RB, retained placenta and metritis. Bovine virus diarrhea (BVDV) and Bovine herpes virus (BHV) [1, 4] were also isolated from caruncles and uterine contents in cows with acute postpartum SE and RB history [31]. A tight association is found between the occurrence of reproductive disorders such as repeat breeding, endometritis, retained placenta, abortions and instantaneous infections with BVD virus [32]. These viruses cause some pathological changes to the uterus and early embryonic death [33, 34].

**Diagnosis of Repeat Breeding Syndrome:** Accurate diagnosis of RB relies on the animal records, rectal palpation, ultrasonography, vaginal examination, uterine culture, uterine biopsy and uterine endometrial cytology [35].

- Ultrasonography (US) is used as a diagnostic tool for clinical endometritis and pyometra which is based on the well-defined signs of enlarged uterus, high volume of accumulated uterine content, closed cervix (i.e. no visible discharge) and CL on the ovary.

- Uterine endometrial cytology is used for diagnosis of SE. A cow with subclinical endometritis is defined by >18% neutrophils in uterine cytology samples collected 21-33 days post partum, or >10% neutrophils at 34-47 days [36].
- Uterine biopsy would be indicated, especially in the presence of repeated early embryo loss or abortion.
- Hormonal assay is used for determination of plasma p4 concentrations on a blood sample taken 7 to 8 days is done after mating for detection of ovulation and evaluation of luteal function. Low and high plasma progesterone levels are indicative of follicular and luteal cysts, respectively. Estrogen determination is indicated in follicular development which is not observed after a series of ultrasonographic examinations. The objective of the evaluation is to establish the normalcy of the genital tract, follicular development, ability to ovulate in response to breeding and development of a functional corpus luteum [17].

#### **Treatment of RB**

**Presynch- Protocols:** The effect of two types of controlled internal drug release (CIDR)-based timed artificial insemination (TAI) protocols was studied for treatment of RB dairy cows [37]. In the first type of CIDR, a device was inserted into cows and then the cows were administered an injection of 1 mg oestradiol benzoate (EB) plus 50 mg progesterone (P4) at Day 0. On Day 7, they were given an injection of PGF<sub>2</sub>α and the CIDR device was removed. The cows were given an injection of 1 mg EB on Day 8 and were subjected to TAI 30 h later. In the second type of CIDR, a device was inserted into the cows and then the cows were administered an injection of 250 µg gonadorelin (GnRH; Day 0). On Day 7, they were given an injection of PGF<sub>2</sub>α and the CIDR device was removed. The cows were given an injection of 250 µg GnRH on Day 9 and were subjected to TAI 17 h later on. In a second trial, 41 RB cows (20 treated -EB group and 21 GnRH -treated group) were randomly assigned to the same two treatments used in the first trial. The pregnancy rates following TAI in the first (18.5 vs. 32.1%) and second (40.0 vs. 38.1%) trials and the combined rates (27.7 vs. 34.7%) did not differ between the EB and GnRH groups. In conclusion, treatment with either EB or GnRH in a CIDR-based TAI protocol results in synchronous follicular wave emergence, follicular development, synchronous ovulation and similar pregnancy rates for TAI in RB cows.

**Fixed-Time Embryo Transfer (FTET):** RB cows are characterized by poor fertilization rates [38] and/or early embryonic losses [39]. Thus, embryo transfer (ET) can be potentially used to minimize the eventual effects of uterine environment and lactation on early embryonic development, promoting greater pregnancy rates and consequently, avoiding early embryonic death. In a retrospective study, it was reported that conception rates in RB Holstein cows were greater after ET (41.7%) than after AI (17.9%) indicating that ET may be an effective alternative to achieve satisfactory conception rates throughout the year, especially during periods of heat stress [40]. Higher pregnancy rates were achieved by ET following AI (with-AI group) than by ET alone (without-AI group) in both heifers (49.2 and 29.5%, respectively) and cows (41.5 and 20.4%, respectively) [5]. Embryos were transferred within 6-8 days after estrous detection in RB Holstein cows (PGF-Estrus group) treated with 150µg d-cloprostenol (PGF<sub>2</sub>α) [41]. According to the presence or absence of corpus luteum, cows received on Day 0 norgestomet ear implant plus 2mg EB and 50 mg P4 i.m. On Day 8, the implant was removed and 400 IU eCG, 150µg d-cloprostenol and 1mg oestradiol cypionate (i.m) were administered. Ultrasonographic exams were performed and only cows with a single CL or multiple CL received a fresh or frozen-thawed embryo on Day 17. Pregnancy was diagnosed by ultrasonography at 30 and 60 days of pregnancy. The proportion of cows receiving an embryo was greater in the FTET-CL (75.0%) than in PGF-Estrus (34.5%) treatment. Pregnancy rate (60 days) was also greater in FTET-CL (29.3%) compared to PGF-Estrus (16.2%). The pregnancy rate was increased in embryo transfer recipients cows receiving hCG on day 6 (67.5%) compared to control cows (45.0%) or cows receiving hCG on day 1 (42.5%) after estrus [42]. It appears that inducing accessory CL, thereby increasing progesterone, may improve fertility in RB dairy cows. However, body condition and parity may influence hCG response. The pregnancy rates were evaluated following either CIDR-based timed AI (TAI) or (FTET) protocol compared with that following a single PGF<sub>2</sub>α injection and AI after estrus (AIE) in lactating RB dairy cows [43]. In TAI group, cows at random stages of the estrous cycle, received a CIDR device and 2 mg EB at day 0, 25 mg PGF<sub>2</sub>α injection at the time of CIDR removal on Day 7 and 1 mg EB injection on Day 8. The cows then received TAI 30 h (Day 9) after the second EB injection using dairy semen. In FTET group, Cows at random stages of the estrous

cycle, received the same hormonal treatments as in the TAI group. The cows then received FTET on Day 16 using frozen-thawed blastocyst or morula embryos collected from Korean native cattle donors. In control group, cows during the luteal phase, received 25 mg injection of PGF<sub>2</sub> $\alpha$  and AIE using dairy semen. The ultrasonographic observations demonstrated that preovulatory follicles ovulated and concomitantly formed new corpora lutea in all cows of the FTET group. Accordingly, the mean serum P4 concentration remained constant between Day 0 and Day 7 of the luteal phase, decreased dramatically on Day 8 and subsequently increased by Day 16. The pregnancy rate was significantly higher in the FTET group (53.8%) than in the control (18.5%) or TAI (7.7%) groups. These data suggest that the CIDR-based FTET protocol can be used to increase effectively the pregnancy rate in lactating RB dairy cows.

**Administration of Insulin:** RB cows were injected s/c with a long acting purified form of bovine insulin at 0.2IU/kg body weight/day on days 8, 9 and 10 and then with 0.75 mg tiaprost (PGF<sub>2</sub> $\alpha$ ) i/m on day 12 of the oestrous cycle (oestrus = day 0). The overall pregnancy rate as well as progesterone concentrations were higher in RB cattle treated with insulin (63.64%, P4 2.9 $\pm$ 0.4 ng/ml) than in control (40%, 2.2 $\pm$ 0.3 ng/ml) group [44]. The results may indicate that there is beneficial effect of insulin on fertility in RB cattle as insulin and glucose concentrations were higher in animals, which subsequently became pregnant than in non-pregnant animals. A beneficial effect of insulin on fertility could be due to better follicular growth, oocyte quality and early embryonic development due to the presence of insulin receptors in the ovary, oocyte, embryo, oviduct and uterus [43, 46].

**Intrauterine Infusion of Immune Stimulants:** A single intrauterine infusion of 100  $\mu$ g *E. coli* LPS at estrus in RB cows cleared the bacterial infection from the uterine lumen within one estrous cycle. Following this treatment, the majority of RB cows conceived in a preliminary trial [47, 48]. The combined therapy of intrauterine infusion of 100 mg of LPS in 50 ml normal saline solution and two injections of 20 IU of oxytocin (IV) at 12 and 24-hours after infusion was effective for the elimination of persistent endometritis and improved reproductive performance of subfertile mares. The *E. coli* LPS serotype (026; B6) used in the current

experiment has previously been reported to stimulate the release of PMNs in uteri of dairy cattle, without producing any systemic effect [49]. A single intrauterine infusion of 500 mg oyster-glycogen (OG) enhanced uterine neutrophilic influx, cleared bacterial infections and improved conception rate in RB cows as they became pregnant with insemination at the first post treatment estrus [50].

**Antibiotic Therapy:** Although many repeat breeders give negative results after culturing for infectious organisms, broad spectrum drugs are used most frequently depending on identification of the infectious agent and drug sensitivity test. Some workers recommend diluted Lugol's solution (dilute iodine) as treatment when cultures and drug sensitivities are not done, while others recommend no treatment. Incorporation of a proper vaccination program aids in the prevention of diseases which can cause metritis. If uterine infections are excessive (greater than 20%) in a herd, management of dry and recently calved cows should be thoroughly examined. Adequate housing, nutritionally balanced and palatable rations for the dry and fresh cows, satisfactory calving facilities with optimal ventilation and sanitary conditions and avoidance of undue stress such as overcrowding and disease are absolute requirements for healthy fresh cows. The uterine lavage with saline plus 2 doses of PGF<sub>2</sub> $\alpha$  (Lutalyse) with intrauterine infusion of 500 mg benzathine cephapirin improved the conception rate in RB cows (70%) compared to (56.52%) in normal cows [51]. The results also showed that uterine lavage plus PGF<sub>2</sub> $\alpha$  without any antibiotic may be preferable in the treatment of RB cows as no milk waste or side effects on the endometrium occurred. PGF<sub>2</sub> $\alpha$  stimulates myometrial contraction by a mechanism that expels debris and microorganisms from contaminated uterine lumen. PGF<sub>2</sub> $\alpha$  may stimulate the phagocytic activity of uterine PMNs as well.

In conclusion, repeat breeding is an important syndrome which affects breeding animals and causing great economic losses. Recent techniques must be applied for diagnosis and treatment.

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