

ORIGINAL ARTICLE

Economic benefit in repeat breeder cows using intrauterine infusion of penicillin and estrus synchronization followed by timed artificial insemination

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ABSTRACT

Objective: Until recently, management of repeat breeding in cattle remains a major problem to dairy farmers. This study was carried out to improve the fertility in cows, those did not conceive after three or more consecutive services.

Materials and methods: Twenty-three repeat breeding cows were selected from the Central Cattle Breeding Station and Dairy Farm (CCBS & DF), Savar, Dhaka, Bangladesh. Data of another 23 repeat breeding cows that conceived after four or more services were collected from the same farm as controls. The repeat breeders were synchronized for estrus with two injections of PGF_{2α} 11 days apart and timed AI (TAI) was performed.

Results: Seventeen of 23 cows had uterine infections. Intrauterine infusion of penicillin daily for three successive days from the following day of the first PGF_{2α} injection resulted in 94.1% (16/17) recovery. Of 23 treated cows, four with estrus synchronization and TAI, and one with only AI after hormone treatment were detected pregnant by per rectal examination of the genital tract. By this pregnancy, the treated cows have advanced average of 131.6 days calving interval and days open, and thereby saved neat US \$3,045.3 in comparison to control cows. Control group cows incurred losses of US \$15,134.0 compared with average days open of the treated pregnant cows.

Conclusion: Intrauterine infusion of penicillin can successfully recover the uterine infections, and estrus synchronization followed by TAI resulted in pregnancy in a proportion of repeat breeder cows with economic benefit that had a uterine infection.

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Introduction

Profitability of dairy farms mainly depends on the optimum reproductive efficiency as well as higher milk yield. Certain reproductive goals must be achieved in a profitable dairy farming system. There are some constraints for achieving these goals. The main limitation of an efficient and profitable reproductive management of the dairy farm is repeat breeder cows [1,2]. Several factors are involved as causes of repeat breeder. Many authors ignore the term “repeat breeder” as worthless because, it also includes a number of healthy cows [3]. However, uterine infection,

errors in estrus detection accuracy, conception failure, mineral and vitamin deficiency, and early embryonic loss have been reported as important causes of repeat breeding in cattle [4,5].

Since uterine infection is one of the potential causes for repeat breeding syndrome in cows, antibiotics are commonly used for management of repeat breeder cows. However, the success of such antibiotic treatment for the management of repeat breeder cows is still controversial and results are variable. A variety of antibiotics such as cephalosporin [6,7], penicillin [1], oxytetracycline [7], cephalexin,

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gentamycin, and enrofloxacin [8] have been infused into the uterus for improvement of pregnancy rates in repeat breeder cows. Interestingly, intrauterine infusion of antiseptic solution, 1% Lugol's iodine has also been successfully used for management of repeat breeder cows [9]. On the other hand, systemic (intramuscular) injection of gentamycin and enrofloxacin has been demonstrated for the improvement of pregnancy rate in crossbred repeat breeder cows [10]. Penicillin as an intrauterine therapy can be used for the management of repeat breeder cows having uterine infections due to its rapid absorption in the uterus.

Elimination of causal factors and good management for repeat breeder cows should help getting rid of this reproductive problem. However, the management of repeat breeder cows is sometimes very difficult in particular situations. For example, the accuracy of estrus detection has still remained as one of the big problems and it is reported that 40% of cows remained undetected for estrus in Bangladesh [2]. Consequently, researchers have applied many approaches to overcome this problem. Hormone treatment with TAI is one of the helpful technologies developed in many countries [11,12,-13]. Hormonal regulation in repeat breeder cows is usually performed to synchronize their estrous cycles. Estrus synchronization controls both the function of the corpus luteum and the development of follicles [14]. Further, it overcomes the estrus detection errors in both efficiency, accuracy, and submission rate increases up to 100%. Therefore, the present work was designed with the aims (a) to identify the major cause of repeat breeding and effectiveness of intrauterine infusion of penicillin for removal of uterine infection in repeat breeders, (b) to increase the fertility of repeat breeder cows using estrus synchronization (hormone treatments) and timed AI, and (c) to evaluate the economic benefit of hormone treatments in repeat breeder cows.

Materials and Methods

The present experiment was performed at the Central Cattle Breeding Station and Dairy Farm (CCBS & DF), Savar, Dhaka, Bangladesh during the period from August to September 2005.

Ethics statement

Cows were used according to the guideline and rules for the care of the animals approved by the authority of CCBS & DF, Savar, Dhaka, Bangladesh (Approval No. Section 1/E1912/2005/170).

Animals

Twenty-three cows that failed to conceive after three or more consecutive inseminations were selected as repeat

breeders irrespective of their body condition score (BCS), age, breed, parity, milk yield, lactation length, days open, calving interval, and disease history. The nutritional status of cows was estimated by scoring body condition using 1–5 scales (0.5 fractions between two scores) on the basis of bony prominence and subcutaneous fat deposition according to a previous protocol [15]. The animals were kept for 24 h in sheds with natural ventilation. Cows were milked twice a day. Cows were fed with green grass, straw, and concentrate; total feed and forages were given in splits immediately before milking. The reproductive tracts of the treatment group cows were evaluated by rectal palpation of the ovary and uterus, and the findings were recorded to diagnose uterine infection.

Twenty-three repeat breeder cows that conceived after four or more inseminations were randomly selected, in the same way, from the farm records irrespective of BCS, age, breed, parity, milk yield, lactation length, days open, calving interval, and reproductive diseases.

Estrus synchronization and breeding cows

Individual cows had received an intramuscular injection of 25 mg PGF_{2α} analog (Dinoprost®, 5 ml, 5 mg Dinoprost BP as Trometamol/ml, Techno Drugs Ltd., Dhaka, Bangladesh). A second intramuscular injection of 25 mg PGF_{2α} analog was given 11 days later. Fixed time AI was done at 70 and 90 h following second PGF_{2α} injection. A single shot of 500 µg GnRH analog (Fertilon®, 5 ml, 0.1 mg Gonadorelin/ml, Techno Drugs Ltd., Dhaka, Bangladesh) was given to individual cows during first AI.

Treatment of cows with uterine infections

Cows with uterine infections, such as purulent vaginal discharge, cervicitis, cloudy to purulent genital discharge, and hydrometra were treated with intrauterine infusion of 1.5 million I.U. procaine penicillin and 0.5 million I.U. benzylpenicillin (Pronapen® 40 Lac I.U. Renata Ltd., Dhaka, Bangladesh) per cow daily, for three successive days, started from the following day after the first PGF_{2α} injection.

Pregnancy diagnosis

All cows were evaluated by the rectal palpation technique to confirm pregnancy on Day 45–60 after insemination. Average days open after being repeat breeder of pregnant cows, parity at which cows became repeat breeder, and average service required for the conception of both the treated and control groups were recorded.

Statistical analysis

Descriptive statistical analysis was performed using SPSS software version 11.5 (SPSS, Chicago, IL). The economic

Table 1. Incidence of repeat breeding in different parities and average number of services required for conception.

| Cows | Incidence of repeat breeding in different parities | | | | Total | Avg. services required for conception |
|------|--|--------------|---------------|-----------------------|-------|---------------------------------------|
| | Second parity | Third parity | Fourth parity | Fifth and more parity | | |
| TC* | 8 | 6 | 2 | 7 | 23 | 4.7 + 2.0** = 6.7 |
| CC* | 11 | 5 | 3 | 4 | 23 | 5.0 |

* TC = Treated cows; CC = Control cows; **Two services given to all cows at TAI.

benefit of the hormone treatments in pregnant cows was analyzed on the basis of their average days open (DO) and calving interval in respect to those of the control cows. Cost of drugs, veterinary services, labor, AI service, and semen was also considered for economic analysis.

Results

Recovery of repeat breeder cows following antibiotic and hormonal treatment

Of the 23 repeat breeding cows, on clinical examination, 17 (73.91%) were identified with uterine infections. After intrauterine penicillin infusion, 16 cows (94.11%) had clear genital discharge and apparently recovered cervicitis and hydrometra. However, 45–60 days after TAI, four cows were diagnosed pregnant by rectal palpation. Further, one cow, which had uterine infection became pregnant with AI at detected estrus after estrus synchronization and TAI.

Incidence of repeat breeding was observed highest in the second and lowest in the fourth parity in both groups compared with the third, fifth, or more parity; average services required for conception were more in treated cows than that in control cows (Table 1).

Economic benefits of antibiotic and hormonal treatment

Hormone treatment advanced average days open and calving interval in treated pregnant cows after being repeat breeder by 131.6 days, and thereby saved about total gross US \$3,290.0 for the cows of treated group compared with that of control cows (Table 2). On the other hands, control cows lost total gross US \$15,134.0 compared with average

days open and calving interval of the pregnant treated cows (Table 2).

Total costs incurred for drugs used, veterinary services and labors were US \$244.7 for hormone treatment of 23 cows (Table 3). Therefore, in these 23 cows, the neat benefit for hormone treatments was US \$3,045.3 due to the advancement of days open and calving interval of the treated pregnant cows.

Discussion

Seventeen of 23 cows (73.91%) presented by the farm as repeat breeder that had uterine infections of varying degrees. Sixteen of 17 cows (94.11%) had cleaned their abnormal genital discharge upon intrauterine antibiotic (penicillin) treatment. Only a few (4 of 23) repeat breeding cows conceived after fixed time AI at synchronized estrus and another cow became pregnant after the hormone treatment.

Majority of the cows had a uterine infection of varying degrees. High recovery rate with intrauterine penicillin infusion indicated that the treatment was effective to clean the uterine infection. However, the uterine infection has still considered as one of the major causes of bovine infertility [17]. Uterine environment is reported to be altered in repeat breeder cows and such improper uterine environment impairs normal embryo development in cows [18]. Therefore, apparently recovered cows from uterine infections might have uterine environment hostile to spermatozoa, fertilization process, and early embryo development.

Fertilization failure and early embryonic mortality are major contributors to the occurrence of repeat breeding in

Table 2. Data of cows after being repeat breeder and savings by the treated cows ($n = 23$) and losses by the control cows ($n = 23$). Columns with *italic* fonts have been put to relate to the reference of MacKay [16].

| No. of TPC* | Avg. DO_1 * of TPC | Avg. DO_1 of CC* | Avg. CI* & DO_2 * of TPC | Avg. CI & DO_2 of CC | Longer CI & DO_2 of CC than TPC | Savings by the GTC* for HT* (US\$) | Losses by the GCC* (US\$) |
|-------------------|----------------------|--------------------|----------------------------|------------------------|-----------------------------------|------------------------------------|---------------------------|
| By ES* & TAI* = 4 | 398 days | 529.6 days | CI: 398 + 280 = | CI: 529.6 + 280 = | CI: 809.6 – 678 = | 131.6 × 5.0† = | 131.6 × 5.0 = |
| N-AI* = 1 | | | 678 days DO_2 ; | 809.6 days DO_2 ; | 131.6 days DO_2 ; | 658.0 × 5 = | 658.0 × 23 = |
| Total = 5 | | | 678 – 365 = | 809.6 – 365 = | 444.6 – 313 = | 3,290.0 | 15,134.0 |
| | | | 313 days | 444.6 days | 131.6 days | | |

* TPC = Treated pregnant cows; ES = Estrus synchronization; TAI = Timed-AI; N-AI = Normal AI; DO_1 = Days open; CC = Control cows; CI = Calving interval; DO_2 = Days open after 1-year calving interval; GTC = Group of treated cows; HT = Hormone treatment; GCC = Group of control cows.

†Losses of up to US \$5.0 per head per day are incurred by cows that do not yearly calved [16].

Table 3. Costs for drugs used, veterinary services and labor ($n = 23$).

| Drugs used | Total doses of drugs used | Price/dose (US\$*) | Costs incurred for | | | | Total costs |
|---------------------------------|---------------------------|--------------------|--------------------|---------------------------------|---|---|-------------|
| | | | Drug costs (US\$) | Vet. services (5.0 US\$ /visit) | Contractual Labor (2.0 US\$/day for particular works) | AI service & semen (0.5 US\$/AI) | |
| Dinoprost® (PGF _{2α}) | 23 × 2 = 46 | 2.0 | (46 × 2.0) = 92.0 | 4 Vet. visits × 5.0 = 20.0 US\$ | 4 labor × 2.0 = 8.0 US\$ | (0.5 × 23 × 2) + (0.5 × 1 × 1*) = 23.5 US\$ | 244.7 US\$ |
| Fertilon® (GnRH) | 23 × 1 = 23 | 3.5 | (23 × 3.5) = 80.5 | | | | |
| Pronapen® 40 lac (Penicillin) | 23 × 3 = 69 | 0.3 | (69 × 0.3) = 20.7 | | | | |
| | Total (US\$) | | 193.2 | 20.0 | 8.0 | 23.5 | 244.7 |

*One US\$ equal to 80 Taka, Bangladeshi local currency; one additional AI service required for pregnancy in another cow.

cattle [19]. Since embryo carries half of its paternal genes, thus it is considered as semi-allogene by the local immunity of the bovine female reproductive tract [20]. Abnormal immune response of the oviduct and uterus may disrupt the fertilization and further embryo development, which negatively affect the pregnancy outcome in cattle. It has been estimated that major (20%–50%) pregnancy loss in high producing dairy cows occurs during the first 2–3 weeks of pregnancy [21]. Several causes are responsible for pregnancy loss in cattle, among which the rejection of semi-allogenic embryo by the maternal immune system could be one of the main reasons [22]. Thus, embryonic mortality due to abnormal maternal immune response could be one of the major causes of conception failure in repeat breeder cows.

During this experiment, from August to September, the maximum ambient temperature was as high as 35°C to 38°C. Heat stress in cows between Day 8 and 17 of pregnancy period can change the uterine microenvironment and thereby hampers the development of the conceptus [23]. Furthermore, heat stress antagonizes the inhibitory effects of the embryo on the secretion of PGF_{2α} from the uterus [24]. Normally, 10%–16% of pregnant cows lose their embryos by 56 days after TAI [25]. In the present research study, a higher proportion of embryonic loss from TAI to pregnancy diagnosis (at 45–60 days after TAI) in cows might have associated with high ambient temperature.

It is reported that deficiency of P, Mg, and Mn can cause conception failure in cows [3]. Lower pregnancy rate in cows in this research study might be due to the deficiency of such minerals, which requires further investigation. Incidence of the highest repeat breeding in the second parity was not clearly understood but may be due to management factors like nutrition management.

Ovulation occurred in 85% of the cows treated with GnRH and 100% cows began a new follicular wave [26]. However, the conception of a cow after the hormone treatment was thought to be due to the positive effect of GnRH. Losses of up to US \$5.0 per head per day are incurred by

cows that do not yearly calved [16]. However, hormone treatment advanced average days open and calving interval in pregnant cows of the treated group after being repeat breeder by 131.6 days. Conception of only five cows resulted in neat savings of US \$3,045.3 for the treatment group cows with an investment of only US \$244.7, which was an impressive finding. Losses about US \$15,134.0 was incurred by the compared cows were also surprising in respect to the pregnant treated cows.

Conclusion

Results of this research can be concluded as (a) uterine infection was a major cause of repeat breeding in dairy cows and intrauterine infusion of penicillin can successfully clear the infections; (b) estrus synchronization followed by timed AI resulted pregnancy in a proportion of repeat breeder cows that had uterine infection before prostaglandin treatment; and (c) finally, intrauterine infusion of penicillin and hormonal treatment lead to economic benefit in repeat breeder cows. Further study is recommended to isolate specific bacteria from uterine and/or vaginal mucus of repeat breeder cows and to test their sensitivity against different types of antibiotics for identification of the most effective and suitable antibiotic.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contribution

Abul Khair conceived, designed, performed the experiments, and wrote the paper. Md. Asaduzzaman and Zinat

Sultana performed the experiments. Anup Kumar Talukder and Ziban Chandra Das performed analysis and edited the paper. Md. Golam Shahi Alam and Mohammed Shamsuddin provided guidance and critically reviewed the final paper.

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