ABSTRACT

This study was carried out during breeding season on 125 repeat breeding buffaloes to evaluate the therapeutic efficacy of various hormonal and non-hormonal drugs in improving their reproductive efficiency. Repeat breeding buffaloes (112) were treated parenterally with 5 different drugs, keeping 13 animals as untreated control, and results were compared with 22 normal cyclic buffaloes. The conception rates in treatment cycle and overall of 3 cycles post-treatment were compared between different groups. For 25 and 32 repeat breeding buffaloes treated with 0.02 mg Gonadotropin Releasing Hormone (GnRH; Receptal 5 ml i/m), just after artificial insemination (AI) and 500 mg of hydroxy-progesterone caproate (Duraprogen 2 ml i/m) on day 4th or 5th post-AI, the conception rates (CRs) in the treatment cycle were 60.00 and 43.75% and overall CRs within 3 cycles were 76.00 and 62.50% (P<0.05), respectively, with a mean treatment to fertile oestrus interval of 6.58±3.27 and 8.25±3.28 days. For 26, 23 and 6 repeating buffaloes treated with Enrofloxacin (Inj. Bayrocin single shot 30 ml) i/m at the time of AI, Ceftriaxone (Inj. Vetacef 2 g) intrauterine (i/ut) at 12-24 h post-AI, and Povidone plus Metranidazole (Ranvidone 20-40 ml) i/ut for 2-4 days (AI in next cycle), the first service conception rates were 23.08, 34.78 and 33.33%, respectively, and overall CRs 53.85, 65.22 and 83.33% (P<0.01), with the fertile oestrus intervals of 20.86±5.53, 12.20±4.49 and 17.20±8.17 days, respectively. The results of ceftriaxone were better as compared to enrofloxacin. The overall CRs for the GnRH, progesterone and antibacterial therapies were 76.00, 62.85 and 53.85 to 83.33% (P<0.01), respectively (normal cyclic group 81.82%, repeat breeding control 38.46%), with significantly (P<0.05) shorter fertile oestrus interval in GnRH and progesterone treated groups as compared to antibiotics treated one. Thus, all these regimes, and GnRH in particular, are recommended to the practitioners for their use in the field to ameliorate the problem of repeat breeding in buffaloes.

Keywords: repeat breeding, buffaloes, hormonal/ non-hormonal therapy, conception rate
INTRODUCTION

The term repeat breeder or cyclic non-breeder describes the animal that has failed to conceive after 3 or 4 services of a fertile bull/artificial inseminations (AI). Repeat breeding is a major constraint in dairy farming. It is an important cause of low reproductive efficiency in buffaloes. The incidence of repeat breeding varies from 15-32% and seems to be lower in animals kept individually on small-holdings than in large herds. Endocrine imbalance, nutrition, faulty breeding management, early embryonic mortality and infectious agents leading to clinical and sub-clinical endometritis are amongst the major causes of repeat breeding in dairy animals (Zemjanis, 1980). Fertilization failure is rare in females but zygote does not survive and therefore subsequent oestrus follows normally. Luteal dysfunction leading to inadequate progesterone production post-breeding could be a cause of embryonic death. Gonadotropin Releasing Hormone (GnRH) / Human Chorionic Gonadotropin (hCG) and/or progesterone analogues have been successful to sustain early pregnancy and improve conception rate in repeat breeding bovines (Sreenan and Diskin, 1983; Dhami et al., 2009; Patel et al., 2014). Similarly post-insemination antibiotics therapy intrauterine is beneficial in enhancing conception rate in repeat breeders of unknown etiology and particularly with low grade genital infection (Mahto et al., 2006; Dhami et al., 2009). However, in most of the reports only one protocol has been tested at a time, and the literature on comparative efficacy of hormonal and antibacterial approach in the same condition is scarce. Hence the present study was planned to evaluate the comparative therapeutic efficacy of GnRH, Progesterone and Antibiotics/Antiseptics at a time in repeat breeding buffaloes under field conditions.

MATERIALS AND METHODS

This study was conducted under field conditions in villages of Anand district in Gujarat (India). The buffaloes managed by the farmers individually at their door-step and brought to the AI Centers of the concerned village co-operative societies for AI, pregnancy diagnosis and sexual health control camps were initially screened through gynaeco-clinical examinations. In all 125 repeat breeding buffaloes that had taken more than 3 infertile services, even with good quality frozen-thawed semen, beyond 6 months to 1 year postpartum and confirmed by rectal palpation twice 10 days apart, were selected and subjected to different therapeutic regimes (112), keeping 13 as untreated control. Moreover, 22 buffaloes exhibiting spontaneous estrus within 90 days postpartum and inseminated without any treatment served as normal cyclic controls. Buffaloes in estrus were inseminated by the concerned lay inseminator of the society.

All the animals identified were dewormed using Albandazole 3000 mg (Helmiguard 3000, Vetcare India Ltd.) and were also treated for ectoparasites, if any, by using Flumethrin (Flupor, Vetnex-RFCL India Ltd). Owners of the ear-marked animals were supplied with mineral mixtures (Amul brand) for supplementing to their animals 50-55 g per day for 15 days. The following were the treatment protocols used (Table 1).

Animals of all seven groups once inseminated were followed for 1 to 3 cycles post-treatment, and overall as well as cycle-wise conception rates and fertile oestrus intervals were compared between groups by Chi-square test,
RESULTS AND DISCUSSION

Effect of gonadotropin releasing hormone (GnRH)

Twenty five repeat breeding buffaloes were treated with 20 µg GnRH intramuscularly (i/m), just after AI. The conception rates obtained were 60 and 76% in the treatment cycle and overall of 3 cycles post-treatment, respectively, with a mean treatment to fertile oestrus interval of 6.58±3.27 days. Maximum buffaloes conceived in the treatment cycle itself indicating beneficial effect of GnRH in inducing fertile ovulation and CL growth (Table 2, Figure 1). The present findings of 76 and 60% conception rate using GnRH as against 38.46 and 14.29% in untreated control group coincided well with the reports of Ghulam et al. (2002); Vijayarajan et al. (2007); Sharma and Dhami

Table 1. Different approaches used in the treatment of repeat breeding (Gr. 1 to 6) in buffaloes under field conditions.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment Groups</th>
<th>No. of Buffaloes</th>
<th>Status of Repeat Breeding / Normal Cyclic Animals</th>
<th>Treatment Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GnRH</td>
<td>25</td>
<td>Long oestrus, free from visible genital infection</td>
<td>Buserelin acetate-GnRH 20 µg i/m simultaneous to AI (Receptal, 5 ml)</td>
</tr>
<tr>
<td>2</td>
<td>Progesterone</td>
<td>32</td>
<td>Normal oestrus, apparently free from visible genital infection</td>
<td>Hydroxyprogesterone 500 mg i/m 4th or 5th day post-AI (Duraprogen, 2 ml)</td>
</tr>
<tr>
<td>3</td>
<td>Enrofolxacin</td>
<td>26</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>Enrofloxacin 3 g i/m (Bayrocin 1 shot, 30 ml), AI simultaneously or in next cycle</td>
</tr>
<tr>
<td>4</td>
<td>Ceftriaxone</td>
<td>23</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>Ceftriaxone 2 g i/uterine in 20 ml DW (Vetaceph 2 g) 12-24 h post-AI</td>
</tr>
<tr>
<td>5</td>
<td>Ranvidone</td>
<td>06</td>
<td>Repeat breeding with clear unhealthy discharge</td>
<td>Povidone + Metronidazole 20-30 ml i/uterine for 2-3 days, AI in next cycle</td>
</tr>
<tr>
<td>6</td>
<td>Untreated Control</td>
<td>13</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>No any treatment, only AI and follow up</td>
</tr>
<tr>
<td>7</td>
<td>Normal Cyclic Control</td>
<td>22</td>
<td>Normal first oestrus within 90 days postpartum, free from visible genital infection</td>
<td>No any treatment, only AI and follow up</td>
</tr>
</tbody>
</table>
(2008); Dhami et al. (2009); Savalia et al. (2013) in repeat breeding buffaloes and Patel et al. (2014) in crossbred cows. Stevenson et al. (1990) found overall conception rate of 32.1 vs 41.6% (P<0.01) following AI alone and AI+100 µg GnRH i/m among hundreds of repeat breeding cows. Morgan and Lean (1993) recorded 12.5% increase in overall conception rate with the use of GnRH (250 µg) in normal cows and up to 22.5% in repeat breeding cows, while in other studies the conception rates of 60 vs 40% were found for GnRH treated (0.02 mg i/m) vs untreated repeat breeding cows (Ata and Tekin, 2001; Shelar et al., 2002). Further, Mandal et al. (2004) found first service conception rates of 50.0 vs 37.5% and overall conception rates 87.5 vs 75.0% in GnRH (2.5 ml Receptal) treated vs untreated repeat breeders. The beneficial results with GnRH injection at AI could be due to induction of timely ovulation with improved CL function.

**Effect of progesterone supplementation**

For 32 repeat breeding buffaloes treated with 500 mg of hydroxy-progesterone caproate on day 4\textsuperscript{th} or 5\textsuperscript{th} post-AI, the conception rate obtained was 43.75% in the treatment cycle itself, and 62.50% overall, with a mean treatment to fertile oestrus interval of 8.25±3.28 days (Table 2, Figure 1). These findings of first service and overall conception rates obtained with progesterone supplementation, as against 14.29 and 38.46% in untreated control group, are closely comparable with the earlier reports of Kavani and Kodagali (1984); Awasthi et al. (2002); Kumar et al. (2003); Dhami et al. (2009) in cows and buffaloes. Sharma et al. (2004) and Patel et al. (2005) found conception rates of 66.7 vs 50.0% and 50.0 vs 33.3% for 4\textsuperscript{th} day post-AI progesterone treated vs untreated repeat breeding buffaloes and HF cows, respectively. Sharma and Dhami (2008) recorded 20 and 40% rise in conception rate over control group with 250 and 500 mg progesterone supplementation, respectively, on 4\textsuperscript{th} day post-AI in repeat breeding buffaloes suggesting beneficial role of higher dose of therapy. According to Das et al. (1992), the most critical period for the embryo survival was the late blastocyst. The failure of blastocyst to implant might be due to the gestational changes in the endometrium at the appropriate time. Kastelic (1994) stated that most embryonic losses occur during early pregnancy, and the cause is usually unknown. Embryonic loss before 125 days is usually preceded by, and may be caused by, luteal regression. Hence, hormonal treatment to increase plasma progesterone concentrations may improve pregnancy rates, particularly in repeat breeding cows.

**Efficacy of antibiotics/Antimicrobials**

Twenty six repeating buffaloes were treated with long acting enrofloxacin intramuscularly at the time of insemination. The conception rates in treatment cycle, and overall of 3 cycles were 23.08 and 53.85%, with the fertile oestrus interval of 20.86±5.53 days. Among 23 repeating buffaloes treated with intrauterine infusion of 2 g Ceftriaxone 12 to 24 h post-AI, 34.78% buffaloes conceived in treatment cycle with an overall conception rate of 65.22% after a mean treatment to fertile oestrus interval of 12.20±4.49 days. Among 6 repeat breeding buffaloes with clear endometritis treated with intrauterine infusion of Ranvidone 20-30 ml, 33.33% buffaloes conceived in post-treatment I cycle, with an overall conception rate of 83.33% after a mean treatment to fertile oestrus interval of 17.20±8.17 days. The results of ceftriaxone were better as compared to enrofloxacin treated group in terms of first service (34.78 vs 23.08%) and overall conception rates (65.22 vs 53.85%) and even for
fertile oestrus interval (12.20±4.49 vs 20.86±5.53 days). Further, the overall conception rate (83.33%) with Ranvidone was significantly higher than the antibiotics treated groups without significant difference in the time interval, but the number of animal included in this group was comparatively less (Table 2, Figure 1).

Present findings of varying conception rate in treatment cycle and overall with different modes of antibiotics therapy corroborated well or partly with the previous report of Sharma and Dhami (2008); Dhami et al. (2009), who obtained significantly higher overall conception rates within 3 cycle among repeat breeding buffaloes treated with cephalaxin 4 g and ceftriaxone 2 g intrauterine 24 h post-AI as compared to untreated control group, while Kumar et al. (2004) obtained 80% conception in repeat breeding cows treated with enrofloxacin intramuscularly as against only 20% in untreated control group and 45% in normal breeding group. Rane et al. (2003) obtained 71.67% conception rate within 33 days among 60 repeat breeding buffaloes treated with i/ut enrofloxacin at 1500 mg (15 ml) for 2 days at previous oestrus as per the sensitivity of isolates of cervical mucus. Mahto et al. (2006) achieved 50% CRs each with pre- and post-AI ceftriaxone treatment in repeat breeding cows as compared to

Table 2. Fertility response following various hormonal and antibacterial treatments in repeat breeding buffaloes under field condition.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Animals Treated</th>
<th>Conception Rate within 3 Cycles</th>
<th>Treatment to Fertile OI (Days)</th>
<th>Cycle-wise Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Treatment to Fertile OI</td>
<td>I Cycle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH (Receptal)</td>
<td>25</td>
<td>19</td>
<td>76.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58±3.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Progesterone (Duraprogen)</td>
<td>32</td>
<td>20</td>
<td>62.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.25±3.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic (Bayrocin)</td>
<td>26</td>
<td>14</td>
<td>53.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.86±5.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antibiotic (Vetacef)</td>
<td>23</td>
<td>15</td>
<td>65.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.20±4.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antiseptic* (Ranvidone)</td>
<td>6</td>
<td>5</td>
<td>83.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.20±8.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall Treated</td>
<td>112</td>
<td>73</td>
<td>65.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.66±2.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>13</td>
<td>5</td>
<td>38.46&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.40±5.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal Cyclic Control</td>
<td>22</td>
<td>18</td>
<td>81.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00±2.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

OI = oestrus induction.
$ AIs were done in the next cycle following 2-4 days treatment with Ranvidone intrauterine.
Values bearing common superscript within the column do not differ significantly (P>0.05).
only 16% in untreated group. The usage of many other antibiotics and antiseptics have been reported with variable beneficial effects among repeat breeding and endometritic buffaloes and cows by earlier workers, viz. Dhabale et al. (1997) as 50.0 vs 16.7% conception with gentamicin and Singh et al. (2001) as 69.23% with cephalaxin and 55.56 to 64.28% with other antibiotics. Present results and many of the above researchers, thus, suggest that mild genital infection prevails in repeat breeders and it can be cleared with use of effective antibiotics, either locally in uterus or parenterally thereby improving conception rate, at par with the normal fertile animals, and reducing the calving interval to a desired goal.

**Comparison of treatment response in repeat breeders Vs. Normal cyclic group**

Among 112 repeat breeding buffaloes treated with hormonal and antibacterial drugs, 40.18% buffaloes conceived in treatment cycle, and another 18.75% and 6.25% conceived in post-treatment I and II cycles, respectively, with an overall conception rate of 65.18% after a mean interval from treatment to fertile oestrus as 11.60±2.00 days. Among 22 normal cyclic and 13 repeat breeding control buffaloes that were inseminated without any treatment on spontaneous oestrus, 54.55% and 15.38% buffaloes, respectively, conceived in I cycle. The overall conception rates for the two groups were 81.82% and 38.46%, with mean fertile oestrus intervals of 7.00±2.40 and 13.40±5.47 days, respectively, from the day of first AI. The differences between groups were significant for all the traits (Table 2).

As regards relative efficacy of different treatment protocols of repeat breeding problem, the conception rate in the first (treatment) cycle itself was the highest for GnRH treated group (60%), at par with normal cyclic group (54.55%), followed by progesterone treated (43.75%) and antibiotics/antiseptic treated groups (23.08 to 34.78%).
overall conception rates for the respective three protocols were 76.00, 62.85 and 53.85 to 83.33% (normal cyclic group 81.82%). The results with hormone therapy, particularly GnRH, were better and comparable with the normal cyclic group, followed by antibiotics treated group, and all these results were significantly better or superior than those of untreated control group. Moreover, cost-wise and looking to the period of response, use of GnRH was the most economic in repeat breeding buffaloes as compared to progesterone or antibiotics in the present study.

Studies on the comparative efficacies of different treatment protocols of repeat breeding cows or buffaloes on farm (Patel et al., 2005) or even field conditions (Sharma and Dhami, 2008; Dhami et al., 2009; Patel et al., 2014) are meagre. Patel et al. (2005) recorded conception rates of 66.66, 83.33 and 50.00% within two cycles following GnRH (0.02 mg), hCG (1500 IU) and Progesterone (500 mg) i/m treatments post-AI in repeat breeding HF cows as against 33.33% in untreated control. Sharma and Dhami (2008) also obtained significantly higher overall conception rates with i/m use of GnRH (90%), at par with normal cyclic group (88.2%) and hydroxy-progesterone caproate (80%), and i/ut cephalaxin (80%) and ceftriaxone (70%) in repeat breeding buffaloes than in untreated control group (40.00%). The present relatively better findings with GnRH and progesterone suggested that ovulatory problem, endocrine imbalance and luteal insufficiency leading to fertilization failure and/or embryonic mortality may be major causes of repeat breeding in buffaloes under the study area. Antibiotics therapy was also to some extent beneficial, but the relatively low results could be due to resistance developed by the genital microflora against them, since Ranvidone-an antiseptic gave the best results though in a limited number of animals.

CONCLUSIONS

Our findings clearly support that the use of GnRH at the time of insemination and of progesterone therapy 4th or 5th day post-insemination definitely improves conception rate by 10-20% depending upon the cause in repeat breeding buffaloes, and hence can be advocated to deal with this problem under field conditions. Present results with antimicrobials suggest that mild genital infection prevails in repeat breeders and it can be cleared with use of effective antibiotics, either infused locally in uterus or parenterally, thereby improving conception rates in repeat breeders and achieving the optimum calving interval.

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