Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes


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Abstract- This investigation was aimed to evaluate the fertility response in 55 postpartum (>90 days) anoestrus rural buffaloes treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated control (n=10), and the findings were compared with a group of normal cyclic control buffaloes (n=10). All the 15 (100 %) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from PGF2α injection. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 66.67, 73.33 and 60.00 per cent.

Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

GJMR-G Classification : NLMC Code: WC 900

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Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes


Abstract - This investigation was aimed to evaluate the fertility response in 55 postpartum (>90 days) anoestrus rural buffaloes treated with three standard hormonal protocols (CIDR, Ovsynch and Crestar, n=15 each), keeping a group of untreated control (n=10), and the findings were compared with a group of normal cyclic control buffaloes (n=10). All the 15 (100%) buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from PGF2α injection. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding overall conception rates of three cycles were 66.67, 73.33 and 60.00 per cent. These were achieved with the mean intervals from PGF2α injection of 11.40±4.65, 12.70±5.13 and 10.88±3.84 days among treated conceived buffaloes in three groups, respectively. In untreated anoestrus control (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived giving first service and overall pregnancy rates of 50.00 and 10.00 per cent, respectively. In normal cyclic control (n=10), the conception rates at first and overall of 3 cycles were 40.00 and 70.00 per cent, respectively, and the service period was of 105.67±7.44 days among conceived ones. The overall mean plasma progesterone concentrations in true anoestrus buffaloes on day 0, 7, 9 (AI) of treatment and on day 21 post-AI were 1.13±0.66, 4.97±1.68, 0.73±0.67 and 3.47±1.89 ng/ml, respectively, in CIDR protocol; 1.09±0.15, 3.75±0.47, 0.58±0.10 and 4.06±0.47 ng/ml in Ovsynch and 1.12±0.36, 1.28±0.15, 0.66±0.10 and 2.44±0.44 ng/ml in Crestar protocol, respectively. The values on day 7 and 21 were significantly (P<0.01) higher than other two periods in all three groups. The mean plasma progesterone concentrations were non-significantly (P>0.05) higher in conceived than non-conceived group on day 21 post-AI in CIDR (4.41±1.62 vs. 2.66±1.80 ng/ml), Ovsynch (4.14±0.57 and 2.96±0.82 ng/ml) and Crestar (3.21±0.30 vs. 2.05±0.61 ng/ml), but no differences were observed on day 0, 7 or 9 of treatment in any group. The high plasma progesterone in non-pregnant animals on day 21 post-AI was attributed to early embryonic mortality and irregular short or long cycle in them. However in normal Cyclic Control group, the mean plasma P4 values on day 21 post-AI was significantly (P<0.05) higher in conceived buffaloes than in non-conceived ones (3.86±0.47 vs. 1.18±0.52 ng/ml). Thus, Ovsynch and/or CIDR protocols can be conveniently used to improve fertility in anoestrus rural buffaloes by the practicing veterinarians with results similar or even better than in normal cyclic buffaloes. Keywords: buffalo, anoestrus, treatment protocols, oestrus induction, conception rate, fertile oestrus induction interval.

I. INTRODUCTION

The postpartum anoestrus is the most prevalent reproductive problem in dairy animals, for which several hormonal preparations and protocols are being practised by the field veterinarian, but with inconsistent results. Hormonal therapies have good therapeutic value to enhance reproductive efficacy in infertile animals with good nutritional status (Ghuman et al., 2009; Malik et al., 2010, 2011; Chaudhari et al., 2012, Bhoraniya et al., 2012; Parmar, 2013; Savalia et al., 2014). The variable results obtained following hormonal treatments by different workers may be largely due to nutritional status, faulty management, ovarian changes, endocrine events and even uterine infection. Use of hormonal protocols like Ovsynch, CIDR and Crestar during breeding season can be helpful in inducing and synchronizing oestrus and getting better conception rate in them with lesser number of services per conception and making acyclic buffaloes to cycle normally, thereby achieving ideal inter-calving interval. Plasma progesterone levels denote either the presence or absence of CL and its functional competency which is directly related with fertility of the female. The progesterone hormone is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. Hence, this study was planned under field conditions to evaluate the comparative efficacy of CIDR, Ovsynch and Crestar protocols in anoestrus buffaloes for fertility enhancement and their influence on plasma progesterone profile.
II. Materials and Methods

This study was carried out during breeding season from November, 2013 to March, 2014 on 55 postpartum (>90 days) anoestrus buffaloes and 10 normal cyclic buffaloes of average BCS selected from tribal areas of Gujarat. The buffaloes were initially screened gynaecologically for their reproductive status as cyclic, anoestrous and detailed history and rectal palpation findings were recorded. Anoestrous buffaloes were confirmed by rectal palpation of small smooth inactive ovaries twice 10 days apart. All the selected buffaloes were initially dewormed using Ivermectin, 100 mg s/c. Owners of the ear-marked animals were supplied with multi-mineral boluses (Bolus-Minotas, Intas Pharma) for oral supplementing to their animals @ one bolus daily for 7 days. The anoestrus buffaloes were then randomly subjected to different standard estrus induction/synchronization protocols (viz., CIDR, Ovsynch and Crestar, n=15 each) with fix timed AI (Ghuman et al., 2009; Naikoo et al., 2010; Savalia et al., 2014). Another 10 anoestrous animals were kept as anoestrous control and 10 normal cyclic buffaloes served as normal cyclic control group. Buffaloes in spontaneous or induced estrus were inseminated using good quality frozen-thawed semen. Buffaloes detected in oestrus subsequent to FTAI were re-inseminated up to 3 cycles and in non-return cases pregnancy was confirmed per rectum 60 days of last AI. The hormonally treated/untreated true anoestrous and normal cyclic buffaloes were studied for their reproductive status and plasma progesterone profile. For this, jugular blood samples were collected in heparinized vacutainers four times from true anoestrous animals, i.e., on day 0 - just before treatment (on diagnosis), on day 7 - at the time of PGF2α administration, on day 9 - induced oestrus/FTA (FTA1 done twice 24 hrs apart, i.e. on day 9 and 10 after initiation of treatment) and on day 21 post-AI. Blood sampling for two control groups of animals was done on the day of spontaneous oestrus if any, and on day 21 post-AI. The samples were centrifuged at 3000 rpm for 15 min. and plasma separated out was stored deep frozen at -20°C with a drop of merthiolate (0.1%) until analyzed. Plasma progesterone profile was estimated by using standard Radio-Immuno-Assay (RIA) technique of Kubasic et al. (1984). Labelled antigen (l125), antibody coated tubes and standards were procured from Immunotech, France. The sensitivity of assay was 0.1ng/ml. The intra- and inter-assay coefficients of variation were 5.4 and 9.1 per cent, respectively.

The data on oestrus response, conception rate (by Chi square test) and plasma profile of progesterone (ANOVA) were analyzed statistically (Snedecor and Cochran, 1994) using online SAS software version 20.00.

III. Results and Discussion

a) Estrus Induction and Conception Rates

The oestrus induction response and conception rates at induced oestrus and overall of 3 cycles in animals under different hormonal treatment protocols are presented in Table 1. The cent per cent buffaloes in each group under CIDR, Ovsynch and Crestar protocols exhibited induced oestrus with prominent, moderate or weak oestrus signs within mean intervals of 65.00±1.55, 69.46±1.04 and 46.00±1.37 hrs, respectively, from the time of PGF2α injection. The occurrence of prominent oestrus signs was observed in 66.67, 60.00, and 73.33 per cent of buffaloes in three groups, respectively, and it was statistically similar to the normal cyclic control group. The conception rates obtained at induced oestrus in buffaloes under CIDR, Ovsynch and Crestar protocols were 46.67, 53.33 and 33.33 per cent, respectively. The corresponding CRs at second cycle were 25.00, 28.57 and 30.00 per cent and at third cycle, 16.67, 20.00 and 14.28 per cent. The overall conception (pregnancy) rates of all three cycles were observed to be 66.67, 73.33 and 60.00 per cent, respectively in CIDR, Ovsynch and Crestar protocols. These pregnancy rates were achieved with the mean time intervals from PGF2α injection of 11.40±4.65, 12.70±5.13 and 10.88±3.84 days among treated conceived buffaloes in three groups, respectively. In untreated Anoestrous Control group (n=10), only 2 buffaloes exhibited spontaneous oestrus within 90 days of follow up and one conceived at first AI (CR, 50.00 %) at 157 days postpartum giving overall pregnancy rate of only 10.00 (1/10) per cent. In normal Cyclic Control group (n=10), the conception rates at first, second, third cycle and overall of 3 cycles were 40.00, 33.33, 25.00 and 70.00 per cent, respectively, and the service period was of 105.67±7.44 days among conceived ones.

The mean oestrus induction intervals observed in buffaloes under CIDR and Ovsynch protocols under study compared favourably with the previous reports of Savalia et al. (2014) as 63.60±6.46 and 70.67±6.15 hrs and Dhami et al. (2014) as 66.00±4.23 and 87.27±3.53 hrs in anoestrous buffaloes, and Patel et al. (2013) as 66.00±3.22 and 86.67±3.33 hrs in anoestrous crossbred cows, respectively, using the same protocols. Kundalkar et al. (2014) however, reported these intervals to be much shorter as 44.00±2.92 and 44.99±2.50 hrs, while Azawi et al. (2012) reported comparatively longer oestrus induction intervals with Ovsynch as 122.8±6.3 hrs. The mean oestrus induction interval found in buffaloes under Crestar ear implant protocol compared favourably with the previous reports of Uitage et al. (2010) as 42.38±11.09 hrs and Dodamani et al. (2011) as 2.47±0.73 days, but Nath et al. (2004) observed it as 30.81±1.43 hrs only in anoestrous animals.
The first service conception rates of the present study are comparable with the earlier results of Bhoraniya et al. (2012) as 66.66 and 33.33 per cent in CIDR and Ovsynch protocols in anoestrus Kankrej cows, and Kundalkar et al. (2014) as 42.50 and 50.00 per cent with same two protocols in buffaloes, although comparatively lower first service conception rates of 40.00 and 30.00 per cent (Savalia et al., 2014), and 36.84 and 29.41 per cent (Dhami et al., 2014), respectively, are documented with CIDR and Ovsynch protocols in recent reports also. Further, Malik et al. (2010) recorded overall conception rates of 85.70, 75.00 and 86.67 per cent in CIDR, Ovsynch and Crestar group, respectively, in anoestrus buffaloes, which are very close to the present finding with Ovsynch protocol (73.33%), but far higher than in Crestar group (60.00%). Ozurtlu et al. (2003) reported overall conception rates of 44.00 and 53.85 per cent in Norgestomet and PRID groups, respectively, which are relatively lower than the present findings with Crestar and CIDR.

The overall conception rates in Ovsynch and CIDR protocol documented by Barucelli et al. (2003) as 52.50 and 28.20 per cent, Naikoo et al. (2010) as 50.00 and 50.00 per cent and Ali et al. (2012) as 60.00 and 33.33 per cent, respectively, are also lower than the present findings. Further, around 30 per cent conception obtained at second cycle in anoestrus buffaloes induced to cycle and even in normal cycling buffaloes, proved that all the oestrus induction and synchronization protocols induced oestrus and then established normal cyclicity in treated animals, resulting into conceptions in subsequent cycles like normal cycling/breeding buffaloes. These observations further supported the previous observations on use of similar protocols in anoestrus cows and buffaloes by many workers (Naikoo et al., 2010; Bhoraniya et al., 2012; Ammu et al., 2012a; Chaudhari et al., 2012; Patel et al., 2013; Savalia et al., 2013, 2014; Dhami et al., 2014).

The true anoestrus buffaloes thus could be induced to estrus within 2-3 days from the day of PG injection in each protocol and made pregnant within a period of 10-12 days in comparison to 125 days recorded in untreated control group, indicating a huge curtailment in the waiting period of 113 days for anoestrus animals to evince estrus and become pregnant, by putting them under such oestrus induction and synchronization protocols. The pooled conception rates obtained (66.67%) in the anoestrus buffaloes, irrespective of protocols used, indicated the positive contributory role of handling the problem of acyclicity in buffaloes for their induction of oestrus and making them pregnant to the levels, which is nearly at par with normal cyclic control buffaloes (70.00%). Based on the comparative conception rates obtained at induced/first oestrus, it can be surmised that Ovsynch and CIDR protocols could induce equally good fertile oestrus in anoestrus buffaloes. On the other hand, the frequency of induced fertile estrus was considerably low in Crestar protocol. The similar trend was also seen in overall pooled conception rates among the three protocols tested (Table 1).

Table 1: Effects of CIDR, Ovsynch and Crestar protocols on oestrus induction response, PG injection to induced oestrus and fertile oestrus intervals, and conception rates in anoestrus buffaloes

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>N.o.</th>
<th>Oestrus Induction Response (%)</th>
<th>PG Inj. to Oestrus Interval (hrs)</th>
<th>Conception Rate (%)</th>
<th>PG Inj. to Fertile Oestrus Interval (days) among conceived ones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Induced/First Oestrus</td>
<td>Second Cycle</td>
<td>Third Cycle</td>
</tr>
<tr>
<td>CIDR Protocol</td>
<td>15</td>
<td>100.00 (n=15)</td>
<td>65.00±1.55 (n=15)</td>
<td>46.67 (7/15)</td>
<td>25.00 (2/8)</td>
</tr>
<tr>
<td>Ovsynch Protocol</td>
<td>15</td>
<td>100.00 (n=15)</td>
<td>69.46±1.04 (n=15)</td>
<td>53.33 (8/15)</td>
<td>28.57 (2/7)</td>
</tr>
<tr>
<td>Crestar Protocol</td>
<td>15</td>
<td>100.00 (n=15)</td>
<td>46.00±1.37 (n=15)</td>
<td>33.33 (5/15)</td>
<td>30.00 (3/10)</td>
</tr>
<tr>
<td>Pooled</td>
<td>45</td>
<td>100.00 (n=45)</td>
<td>60.15±7.19 (n=45)</td>
<td>44.44 (20/45)</td>
<td>28.00 (7/25)</td>
</tr>
<tr>
<td>Untreated Anoestrus Control</td>
<td>10</td>
<td>20.00 (n=2)</td>
<td>--</td>
<td>50.00 (1/2)</td>
<td>--</td>
</tr>
<tr>
<td>Normal Cyclic Control</td>
<td>10</td>
<td>100.00 (n=10)</td>
<td>--</td>
<td>40.00 (4/10)</td>
<td>33.33 (2/6)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate number of animals/observations, * Service period/days open
Thus, the buffaloes waiting for spontaneous cyclicity beyond 100 days postpartum can be the most appropriate candidates to be subjected to any of the above oestrus induction and synchronization protocols, and CIDR or Ovsynch in particular, for saving their valuable days of reproductive life span at field level, and making them early pregnant and productive.

b) Plasma Progesterone Profile

The mean levels of plasma progesterone recorded on day 0, 7, 9 (AI) of treatment and on day 21 post-AI in buffaloes under CIDR, Ovsynch and Crestar protocols, and on day of AI and day 21 post-AI in control groups are presented in Table 2. The data show that the mean plasma progesterone (ng/ml) concentrations were low towards basal values on day 0, i.e., on the day of initiation of treatment in all three groups, suggesting that the animals were in anoestrous phase. These levels subsequently rose significantly (P<0.01) to the peak values on day 7 (4.97±1.68, 3.75±0.47 and 1.28±0.15 ng/ml), particularly in animals under CIDR and Ovsynch protocols, i.e. just before implants were removed and PG was injected. Thereafter the mean progesterone levels dropped suddenly and significantly within 48 hrs of PG injection and/or implant removal to the basal values coincident to induced oestrus, when FTAs were done. These levels again increased significantly (P<0.01) on day 21 post-AI in all the groups (3.47±1.89, 4.06±0.47 and 2.44±0.44 ng/ml) due to oestruses being ovulatory with development and maintenance of CL and establishment of pregnancy in some animals. In normal cyclic control group also the mean plasma progesterone levels were low towards basal values on day 0, i.e., on the day of initiation of treatment in all three groups, suggesting that the animals were in anoestrous phase. These levels subsequently rose significantly (P<0.01) to the peak values on day 7 of treatments in the present study with CIDR, Ovsynch protocols (4.97±1.62 ng/ml) recorded on day 7 in CIDR group might be due to the continuous release of the exogenous progesterone from the progesterone molded silastic coil inserted in the anterior vagina of the buffaloes. In the Ovsynch protocol the rise in mean progesterone level (3.75±0.47 ng/ml) noted on day 7 might be due to luteinization of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH, simulating diestrum phase, while in the Crestar protocol the mean progesterone level (1.28±0.15 ng/ml) did not rise much, probably due to presence of synthetic progestagen in that which is not detected by 17α-hydroxyprogesterone RIA, and the behavioural oestrus signs observed might also be attributed to i/m injection of oestradiol valerate simultaneous to norgestomet implant.

Further, the mean plasma progesterone concentrations in conceived and non-conceived groups in all three treatment protocols and in normal cyclic control group were found to be statistically similar on day 0, 7 and even on day 9 (AI), but on day 21 post-AI, the conceived buffaloes had non-significantly higher mean plasma progesterone concentrations as compared to non-conceived ones in all the three groups, but differed significantly only in normal cyclic Control group (3.86±0.47 vs 1.18±0.52 ng/ml, P<0.05) (Table 2). These findings on plasma progesterone profile with respect to effect of CIDR and Ovsynch protocols and/or in normal cyclic group closely corroborated with the observations of Raghorte et al. (2009), Naikoo et al. (2010) and Savalia et al. (2013, 2014) in anoestrous buffaloes, and of Bhoraniya et al. (2012) and Patel et al. (2013) in anoestrous cows under such protocols.
Influence of Controlled Breeding Techniques on Estrus Induction Response, Conception Rate and Plasma Progesterone Profile in Anoestrus Buffaloes

Table 2: Plasma progesterone concentrations (ng/ml) in anoestrus and cyclic buffaloes on different days of treatment/AI under various oestrus induction protocols

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Pregnancy Status</th>
<th>No.</th>
<th>D-0</th>
<th>D-7</th>
<th>D-9 (AI)</th>
<th>D-21 post-Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDR</td>
<td>Conceived</td>
<td>7</td>
<td>1.10±0.51</td>
<td>4.87±1.58</td>
<td>0.53±0.33</td>
<td>4.41±1.62</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>8</td>
<td>1.16±0.82</td>
<td>5.05±1.87</td>
<td>0.89±0.86</td>
<td>2.66±1.80</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>15</td>
<td>1.13±0.66*</td>
<td>4.97±1.68b</td>
<td>0.73±0.67*</td>
<td>3.47±1.89b</td>
</tr>
<tr>
<td>Ovsynch</td>
<td>Conceived</td>
<td>8</td>
<td>0.94±0.18</td>
<td>4.01±0.74</td>
<td>0.47±0.11</td>
<td>4.14±0.57</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>7</td>
<td>1.26±0.25</td>
<td>3.46±0.58</td>
<td>0.70±0.17</td>
<td>2.96±0.82</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>15</td>
<td>1.09±0.15*</td>
<td>3.75±0.47b</td>
<td>0.58±0.10*</td>
<td>4.06±0.47b</td>
</tr>
<tr>
<td>Crestar</td>
<td>Conceived</td>
<td>5</td>
<td>1.77±1.08</td>
<td>0.88±0.18</td>
<td>0.56±0.07</td>
<td>3.21±0.3</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>10</td>
<td>0.81±0.09</td>
<td>1.49±0.18</td>
<td>0.70±0.16</td>
<td>2.05±0.61</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>15</td>
<td>1.12±0.36a</td>
<td>1.28±0.15b</td>
<td>0.66±0.10a</td>
<td>2.44±0.44b</td>
</tr>
<tr>
<td>Untreated Anoestrus Control</td>
<td>Conceived</td>
<td>1</td>
<td>0.63±0.00</td>
<td>--</td>
<td>0.34±0.00</td>
<td>4.17±0.00</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>9</td>
<td>1.37±0.49</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>10</td>
<td>1.07±0.41</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Normal Cyclic Control</td>
<td>Conceived</td>
<td>4</td>
<td>--</td>
<td>--</td>
<td>0.16±0.03</td>
<td>3.86±0.47b</td>
</tr>
<tr>
<td></td>
<td>Non-conceived</td>
<td>6</td>
<td>--</td>
<td>--</td>
<td>0.61±0.18</td>
<td>1.18±0.52a</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>0.43±0.13a</td>
<td>2.26±0.56b</td>
</tr>
</tbody>
</table>

Means bearing uncommon superscripts within the row/column differ significantly (P < 0.05).

D-0 = Day of starting the treatment, D-7 = Administration of PG, D-9 = Fixed time AI, D-21 = Day 21 post-AI

The levels of plasma P₄ on the day of beginning of treatment protocol helped delineate the reproductive and endocrine status of the animals and thereby predicting the possible response to the therapy. The higher plasma P₄ recorded on day 21 post-AI in conceived buffaloes of all the treatment groups and even in normal cyclic control group was due to establishment of pregnancy and maintenance of CL function, while significantly low yet variable plasma P₄ noted on day 21 post-AI in non-conceived buffaloes could be due to their return to next oestrus at varying intervals on account of probable irregular or long cycle length, early embryonic mortality after day 17 or uncoordinated, unexplained hormonal changes in some of them.

Thus, it can be inferred that the hormonal protocols used, particularly Ovsynch & CIDR protocol, improved conception rates in anoestrus buffaloes under field condition, and also influenced the plasma progesterone profile significantly in a manner of normal cyclic animals, hence can be used by the practicing veterinarians in anoestrus field buffaloes to improve their reproductive efficiency and thereby the farmers economy.

IV. ACKNOWLEDGEMENTS

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REFERENCES


