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# Evaluation of different hormonal protocols in postpartum anestrous buffaloes under farm and field during breeding and low breeding seasons

**Chandra Prasad B, Venkata Naidu G, Srinivas M, Ragunath M and Ashwini Kumar**

**Abstract**
The present experiment was carried out to evaluate fertility response in postpartum lactating anestrous buffaloes in farm and field conditions during breeding and low breeding seasons by using synchronization protocols (Double prostaglandins, Presynch-Ovsynch, Ovsynch and CIDR-Ovsynch). The present study showed no significant difference ($P$>0.05) in estrus response between treatment groups, whereas significant difference ($P$<0.05) was observed between treatments and control group in farm and field, and significant difference ($P$<0.05) was observed in farm and field during breeding and low breeding seasons. Significantly ($P$<0.05) more number of buffaloes showed intermediate estrus during breeding and low breeding season under farm and field conditions. Overall conception rates were higher in Presynch-ovsynch (POVS) treated group during breeding and low breeding seasons under farm and field conditions, followed with Ovsynch (OVS), Double PG (DPG) and CIDR-Ovsynch (COVS) group. In conclusion, POVS hormonal protocol has been effectively improve the fertility response in sub-estrus buffaloes, whereas OVS hormonal protocol has been effectively improve the fertility response in true anestrous buffaloes in breeding and low breeding seasons.

**Keywords:** Buffalo, synchronization, estrus response, fertility response

## Introduction
India possesses 108.702 million buffaloes and contributes around 21.23% of the total livestock population as well as 51% of total milk production (19th Livestock census). Buffalo (*Bubalus bubalis*) is a premier milk producing animal contributing more than fifty percent of total milk production in the country. Clinical surveys in India have revealed higher incidences of postpartum anestrous due to inactive ovaries in buffaloes in comparison to cattle, and this affects production potential in the form of huge economic losses [1].
Indian buffaloes having better reproductive efficiency during winter compared to summer months attributed to environmental factors [2]. Hormonal induction of estrus and ovulation is an effective method for increasing conception rates in true anestrous and sub-estrus buffaloes in different seasons. Synchronization reduces the number of days spent visually appearing estrus, it also increase the accuracy of heat detection. Various estrus synchronization protocols have been tried among many other reproductive technologies for improving the fertility of buffaloes.
The current study was planned with an intention to augmentation of fertility in lactating postpartum anestrous buffaloes using different hormonal protocols.

## Material and methods
The lactating Graded Murrah buffaloes with varied parity (1 to 6) that experienced normal parturition, aged between 6-10 years, and had not exhibited estrus for the past 3 to 12 months or more were monitored and selected based on ovarian activity by per rectal examination, trans-rectal ultrasonography (TRUS).
The present study was carried out on Graded Murrah buffaloes during the period from September 2017 to August 2019 (includes breeding and low breeding season) which were maintained at buffalo research station (BRS) and ILFC Gannavaram, belonging to Sri Venkateswara Veterinary University, Andhra Pradesh (considered as farm) and buffaloes from the villages in and around Gannavaram within a radius of 25 KM (considered as field).

**Grouping of animals based on breeding and low breeding seasons**

Among the 392 postpartum lactating sub-estrus buffaloes 35 buffaloes reared under farm and 171 buffaloes reared under village conditions were treated during breeding and 25 in farm and 161 buffaloes of villages were synchronized during low breeding seasons.

The postpartum lactating anestrous buffaloes with no palpable structures on the ovaries were grouped under true anestrous (n=106). Among these 12 buffaloes belonging to the farm and 36 buffaloes of the field were treated during the breeding season, while 14 and 44 buffaloes belonging to farm and field conditions, respectively were treated during low breeding season.

The postpartum anestrous (n=61) buffaloes (farm, n=16 and field, n=45) which had exhibited estrus during breeding and low breeding season before induction of hormonal treatments were kept as control group.

**Treatment protocol for postpartum lactating sub-estrus and true anestrous buffaloes**

Treatment protocol for postpartum lactating sub-estrus and true anestrous buffaloes were recorded and depicted in Table 1.

**Group I (DPG): Double prostaglandin ($PGF_2\alpha$- $PGF_2\alpha$)**

Postpartum lactating sub-estrus buffaloes were administered with double dose of intramuscular injection of Cloprostenol sodium @ 500µg on day 0 and day 11 followed by fixed time artificial insemination (FTAI) at 72 and 96 hours or/and at observed estrus after the second dose of $PGF_2\alpha$.

**Group II (POVS): Presynch-Ovsynch ($PGF_2\alpha$- $PGF_2\alpha$- GnRH- $PGF_2\alpha$- GnRH)**

Postpartum lactating sub-estrus buffaloes which were subjected to Presynch-Ovsynch protocol which consisted of two intramuscular injections of Cloprostenol sodium @ 500µg on day 0 and day 14, followed by GPG protocol, similar to Group 2 and was initiated after a gap of 12 days after the second $PGF_2\alpha$ of the Presynch. Fixed time artificial insemination was performed (FTAI) at 16-18 hours or at observed estrus after the second dose of GnRH

**Group III (OVS): Ovsynch (GnRH- $PGF_2\alpha$- GnRH)**

Postpartum lactating true anestrous buffaloes were administered with intramuscular injection of 10µg of GnRH analogue (Buserelin acetate) day 0 (treatment initiation day), seven days later intramuscular injection of 500 µg of Cloprostenol sodium followed by intramuscular injection of 10µg of GnRH analogue (Buserelin acetate) on day 9 and fixed time artificial insemination was performed (FTAI) at 16-18 hours or/and at observed estrus after the second dose of GnRH.

**Group IV (COVS): CIDR –Ovsynch (CIDR- GnRH- $PGF_2\alpha$- GnRH)**

Postpartum lactating true anestrous buffaloes were subjected to CIDR-Ovsynch protocol which consisted of intramuscular injections of GnRH analogue (Buserelin acetate) @ 10µg on day 0 and CIDR was inserted in to vagina followed by administration of an intramuscular injection of Cloprostenol sodium @ 500µg on day 7 with removal of CIDR and a second dose of GnRH @ 10µg was administered on day 9 and fixed time artificial insemination was performed (FTAI) at 16-18 hours or at observed estrus after the second dose of GnRH.

**Group V: control**

Postpartum lactating anestrous lactating buffaloes that exhibited natural estrus after screening and before initiation of hormonal protocols were designated as control group. These buffaloes were inseminated with frozen thawed semen at standing estrus by adopting AM-PM rule during breeding and low breeding season.

**Estrus response rate (%)**

The number of animals in which estrus was induced after completion of the hormonal protocols was recorded and expressed in percentage.

**Intensity of estrus (%)**

The intensity of estrus at AI was classified as intense, intermediate and weak as per the method described by Rao and Rao (1981) [3] with slight modification (Table 2) and expressed in percentage.

**Conception and pregnancy rate (%)**

Conception rate at induced estrus was calculated as the percentage of buffaloes that became pregnant at first and subsequent insemination as confirmed by trans-rectal ultrasonography on day 28 and pregnancy rate was calculated when per-rectal palpation in between days 45 and 60 post insemination.

**Statistical analysis**

All the recorded data was tabulated and analyzed statistically as per the standard procedure described by Snedecor and Cochran (1989) [4].

**Results and discussion**

The present study recorded estrus response rate, intensity of estrus and conception rate in Group I to IV and control groups under farm and field during breeding and low breeding season (Table 3, 3a, 4, 4a, 5 and 5a).

No significant difference (P>0.05) was observed between treatment groups, whereas significant difference (P>0.05) was observed between treatment and control groups in both farm and field condition (Table 3). Significant difference (P>0.05) was observed among all the treatment groups in farm and field during breeding and low breeding season except in DPG group in farm (Table 3a).

The estrus response rate in Group I was collaborated with the earlier studies of Esposito *et al*. (2019) [5] recorded higher estrus response in buffaloes maintained in farm treated with double-PG protocol in buffaloes as 91.90 and reported that higher estrus response is due to PGF2 alpha induced the luteolysis of the CL in the treatment group and moreover decline in progesterone concentration at the time of estrus indicated that CL responded to the luteolytic dose of PGF2 alpha. Similarly in the present study at the time estrus low progesterone level was recorded in farm during breeding season. In contrary with the findings of Pant and Singh (1991) and Srivastava (2005) [6, 7], they recorded that 31.00 and 60.00 per cent estrus response rate in buffaloes maintained in farm and they recorded low estrus response. Higher estrus response in the present study might be due to lowering of progesterone concentration after injection of $PGF_2$ alpha, followed with development of follicle (in turn production of estrogen) as opined by Colazo and Mapletoft (2014) [8].

The estrus response rate in Group II in farm high breeding was contrary with the findings of Hirole *et al*. (2017), Ghuman *et al*. (2012) and Navanukraw *et al*. (2004) [9, 10, 11], they found lower estrus response rate as 83.33, 85.2 and 81.10 per cent, respectively. Interestingly, Ravikumar *et al*. (2014) [12] observed 100 per cent estrus response during breeding and low breeding season in field condition. Dejarnette and Marshall (2003) [13] observed lower estrus response rate (67%) in cow. Improved synchrony of estrus response in the present study might be due to pre-synchronization as opined by Dejarnette and Marshall (2003) [13].

The estrus response rate in Group III was agreement with the findings of Negalia *et al*. (2003) [14], they recorded similar estrus response rate in buffaloes in farm (88.00). In some recent studies (Mujawar *et al.*, 2019) [15] recorded higher estrus response in ovsynch treated buffaloes (100 per cent) in both farm and field condition during breeding season. Ravikumar *et al*. (2014) [12] observed 100 per cent estrus response during breeding and low breeding season in field condition. Recently, Ravikumar *et al*. (2019) [16] observed 100 per cent estrus response during breeding and low breeding season in field condition. In contrary lower estrus response was recorded in earlier studies Wagon *et al* 2019 [17] in buffaloes in farm condition was 73 per cent.

The estrus response rate in Group IV was in par with the findings of Zhaobing *et al*., (2019) [18], recorded that estrus response as 94.1 per cent in farm during breeding season. In present study estrus response rate in lower breeding season was contrary with the reports of Kajaysri *et al*. (2015) [19], they recorded that 100 per cent estrus response rate in buffaloes, whereas Samir *et al*. (2019) [20] found that estrus response was 78 per cent in farm condition during breeding season. In some earlier study (Mujawar *et al*., 2019) [15] recorded higher estrus response in CIDR-Ovsynch treated buffaloes (100 per cent) in both farm and field condition during different season. Ravikumar *et al*. (2014) [12] observed 100 per cent estrus response during breeding and low breeding season in field condition. Higher estrus response in the present study might be due to the fact that the TRIU-B intra-vaginal device containing progesterone would have prevented ovulation and CL formation. The CIDR intra-vaginal device would have created artificial luteal phase in buffaloes. The estrus response after removal of implant could be due to the block on gonadotropins was due to negative feedback mechanism [21]. In conclusion to this, the PGF2α injection towards the end of the treatment would have induced luteolysis in the buffaloes that responded to the treatment. The non-responsiveness in some of the buffaloes could be attributed to low P4 concentrations and absence of a dominant follicle towards the end of the treatment period [22].

The estrus response rate in control group in farm high breeding was partial agreement with the findings of Hirole *et al*. (2018) [23] and estrus response as 66.66 per cent. Partial agreement with the reports of recent studies of Ravikumar *et al*. (2019) [16] observed 50 and 40 per cent estrus response during breeding and low breeding season in field condition. In contrary with the earlier reports of Srinivas Rao (2014) [24] and recorded lower estrus response as 36.36 per cent. In the present study contrary with the recent reports of Yendraliza *et al*. (2019) [25] and observed lower estrus response as 30.00 per cent in field during high breeding. Interestingly, Ravikumar *et al*. (2014) [12] observed 100 per cent estrus response during breeding and low breeding season in field condition.

In the present study the difference in estrus response might be due to breed, difference in protocols, age of animal, lactations, BCS, nutrition and environment as opined by Nanda *et al*. (2003) and Barile (2005) [26, 27].

In the present study observed intensity of estrus in all the treatment and control groups and buffaloes in farm and field were categorized into intense, intermediate and weak estrus based on score card (Table 2). Significantly ($p<0.05$) higher percentage of buffaloes showed intermediate estrus followed with intense and weak estrus within the treatment and control groups in farm and field (Table 4). Similarly, significant difference ($P>0.05$) was observed among all the treatment groups in farm and field during breeding and low breeding season (Table 4a).

The present study recorded higher percentage of intermediate estrus followed with intense and weak estrus in Group I was agreement with the reports of Phani (2017) [29], who observed intense, intermediate and weak estrus as 25, 34 and 22 per cent under field; 21.42, 75.00 and 3.57 per cent under farm during low breeding season, respectively. In contrary higher intense estrus followed with intermediate and weak estrus with reports of Patil *et al*. (2007) [30], who observed intense, intermediate and weak estrus as 50.00 and 33.33 and 16.67 per cent in farm during low breeding season.

The present study recorded higher percentage of intermediate estrus followed with intense and weak estrus in Group II was agreement with the reports of Dejarnette and Marshall (2003) [13].

The present study recorded higher percentage of intermediate estrus followed with intense and weak estrus in Group III was agreement with the reports of Ramalakshmi (2015), Ramakrishna (2016) and Phani (2017) [31, 32, 29], who observed intense, intermediate and weak estrus as 25.00, 62.50 and 12.50 per cent under field; 00.00, 50.00 and 30.00 under farm during high breeding and 7.14, 57.14 and 37.71 per cent under farm during low breeding season, respectively. In contrary higher intense estrus followed with intermediate and weak estrus with reports of Patil *et al*. (2007) [30], who observed intense, intermediate and weak estrus as 50.00, 25.00 and 25.00 per cent, respectively. Interestingly, equal intensity of estrus recorded by Jyothi (2011) [33], who observed the per cent of intense, intermediate and weak estrus as 33.33, 33.33 and 33.33, respectively.

The present study recorded higher percentage of intermediate estrus followed with intense and weak estrus in Group IV was agreement with the reports of Shah *et al*. (2017) and Salim (2018) [34, 35] who observed intense, intermediate and weak estrus as 54.28, 73.80 and 71.42 per cent; 50.00, 33.33 and 16.67 per cent, respectively. In contrary higher intense estrus followed with intermediate and weak estrus with reports of Ramalakshmi (2015), who observed intense, intermediate and weak estrus as 62.50, 37.50 and 00.00 per cent, respectively.

The present study recorded higher percentage of intermediate estrus followed with intense and weak estrus in control group was non-agreement with the reports of Parmar *et al*. (2016) [36] who observed intense, intermediate and weak estrus as 57.14, 42.86 and 0.00 per cent, respectively. Interestingly, equal intensity of estrus recorded by Ramakrishna (2016) [32], who observed the per cent of intense, intermediate and weak estrus as 10.00, 10.00 and 10.00, respectively.

In the present study the difference in intensity of estrus might be due to breed, difference in protocols, age of animal, lactations, BCS, nutrition and environment as opined by Nanda *et al*. (2003) and Barile (2005) [26, 27].

Conception rates (at induced and subsequent estrus) were recorded in treatment and control groups in postpartum Graded Murrah buffaloes in farm and field conditions during breeding and low breeding season (Table 5 and 5a).

In the present study the overall conception rate in Group I of buffaloes under farm conditions during the breeding season was on par with the observations of Honparkhe *et al*. (2008) [37] who reported the conception rate as 65.6 per cent, whereas the present findings are in contrary with findings of Yendraliza *et al*. (2019) [25] who recorded a higher conception rate of 70.00%. On the contrary, Group I buffaloes of the present reared under farm conditions during the low breeding season showed lowered conception rate when compared to the previous findings of Phani (2017) [29] who recorded a higher conception rate (86.66%). In the present study, 3 buffaloes had early embryonic mortality during the low breeding season under field conditions, which might be due to reduced secretion of $P_4$ by the corpus luteum or due to heat stress during the summer months as opined by Binelli *et al*. (2001) [38].

In the present study, the overall conception rate in Group II buffaloes of field condition during the breeding season was in tune with the findings of Konrad *et al.* (2013) [39] who found the conception rate as 55.80 per cent. On the converse, some earlier studies of Hoque *et al.* (2014) and Hirole *et al.* (2017) [40, 9] had recorded a lowered conception rates as 36.40 and 50.00%, respectively. Whereas, Ravikumar *et al.* (2014) [12] observed a higher conception rates during breeding (70.00%) and low breeding seasons (50.00%) under field conditions. In the present study higher conception rate was recorded in farm buffaloes during the low breeding when compared to the previous study of Tawab *et al.* (2019) [41] who recorded the conception rate as 35.98 and 36.64 per cent for insemination at standing heat and FTAI, respectively. On the contrary, Akoz *et al.* (2008) [42] found lowered conception (43.00%) rate in farm buffaloes during the low breeding season. While, Chebel *et al*. (2010) [43] recorded 36.20 and 33.00 per cent conception rate on day 40 and 65 post AI with a pregnancy loss of 8.80 per cent.

In the present study the overall conception rate in Group III buffaloes of farm conditions during the breeding season was in close agreement with the findings of Parmar *et al.* (2017) and Kalwar *et al*. (2019) [44, 45] who reported the conception rate as 57.14 and 60.00 per cent, respectively. On the contrary, a higher conception rate was recorded in earlier studies of Thorat *et al*. (2012) and Mujawar *et al.* (2019) [46, 15] who recorded the conception rate as 85.71 and 75.00%, respectively, whereas lowered conception rates were recorded by Warriach *et al*. (2008) [47] in buffaloes of farms during the breeding (30.40%) and low breeding seasons (29.40%).The present results were in partial agreement with the findings of Ramakrishna (2016) [32] who found the overall per cent conception rate and pregnancy rate as 60.00 and 60.00%, respectively in farm buffaloes during the breeding season. Recently, Ravikumar *et al.* (2019) [16] recorded a higher conception rate in buffaloes reared under field conditions during the breeding and low breeding season as70.00 and 50.00%, respectively. Lowered incidence of late embryonic mortality as recorded in the present study was similar with the reports of Veechio *et al*. (2012) [48] who recorded the late embryonic mortality as 5.30 per cent. The findings from the present study showed improved response to ovsynch during the peak breeding season compared to low breeding season which might be due to the presence of a large preovulatory follicle at the time of induced estrus as opined by Jabeen *et al*. (2012) [49].

In the present study the overall conception rate in Group IV of buffaloes reared under farm conditions during the breeding season was in close concurrence with the findings of Abhishek *et al.* (2018) [50] who recorded the conception rate (56.94%). On the contrary, Bhat and Dhaliwal (2018) [51] recorded a lowered conception rate when compared to present study (34.00), whereas a higher conception rate was recorded by Mujawar *et al.* (2019) [15] in buffaloes belonging to farms during the breeding season as 87.50%, respectively. The overall conception rate recorded in the present study for Group IV buffaloes pertaining to the field during the low breeding season was similar to the findings of Azawi *et al*. (2012) [52] who recorded conception rate as 31.80 per cent. On the converse, Ravikumar *et al.* (2014) [12] observed a higher conception rate during the breeding (70.00%) and low breeding seasons (50.00%) in buffaloes reared under field conditions.

In the present study the overall conception rate for control group buffaloes reared under farm conditions during the breeding season exhibited disagreement with the findings of Srinivas Rao (2014) [24] who recorded a higher conception rate (62.50%), whereas lowered conception rate (25.00%) was recorded by Hirole *et al.* (2017) [9] in buffaloes of farms during the breeding season. While Ramakrishna (2016) [32] recorded very low conception rate of 20.00% in buffaloes belonging to farms during the breeding season. The findings of the present study showed agreement with the reports of Ravikumar *et al.* (2019) [16] who observed 20.00 per cent conception rate during the breeding and low breeding seasons, respectively under field conditions.

## Conclusion

The present study concluded that estrus and fertility response in postpartum lactating anestrous buffaloes could be improved by DPG, POVS, OVS and COVS protocol. Among DPG, POVS, OVS and COVS protocol, POVS hormonal protocol has been effectively improve the fertility response in sub-estrus buffaloes, whereas OVS hormonal protocol has been effectively improve the fertility response in true anestrous buffaloes in breeding and low breeding seasons.

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**Table 1:** Treatment protocol for postpartum lactating sub-estrus and true anestrous buffaloes

| Treatment Groups | Farm and Field | No. of buffaloes |
|---|---|---|
| Double prostaglandins (DPG) | University Dairy farm | 46 |
| | Buffaloes in the villages | 286 |
| | **Overall** | **332** |
| Ovsynch | University Dairy farm | 12 |
| | Buffaloes in the villages | 42 |

| | | |
|---|---|---|
| (OVS) | **Overall** | **54** |
| Presynch-Ovsynch (POVS | University Dairy farm | 14 |
| | Buffaloes in the villages | 46 |
| | **Overall** | **60** |
| CIDR-Ovsynch (COVS) | University Dairy farm | 14 |
| | Buffaloes in the villages | 38 |
| | **Overall** | **52** |
| Control | University Dairy farm | 16 |
| | Buffaloes in the villages | 45 |
| | **Overall** | **61** |

**Table 2:** Score card for estrus response

| **Behavioural changes** | | **Points** | | |
|---|---|---|---|---|
| I | 1. Motor activity (Restlessness and alertness | | | 5 |
| | | 1 | | |
| | 2. Mounting on other animals | 1 | | |
| | 3. Standing to be mounted | 1 | | |
| | 4. Posture | 1 | | |
| | 5. Chin resting and rubbing | 1 | | |
| II | **Physiological changes** | | | 5 |
| | 1. Vulval edema | | 2 | |
| | a) Highly edematous | 2 | | |
| | b) edematous | 1 | | |
| | c) Not edematous | 0 | | |
| | 2. Urination | | 1 | |
| | 3. Genital discharge | | 2 | |
| | a) Large volume, stringy | 2 | | |
| | b) Moderate volume stringy to viscus | 1 | | |
| | c) Sparse volume viscus | 0 | | |
| III | **Gynaecological observation** | | | 5 |
| | 1. Fern pattern | | | |
| | a) Typical fern pattern | 2 | | |
| | b) Atypical fern pattern | 1 | | |
| | c) No fern pattern | 0 | | |
| | 2. Cervical relaxation | | 1 | |
| | 3. Uterine tonicity | | 2 | |
| | a) Highly tonic | 2 | | |
| | b) Tonic | 1 | | |
| | c) No tonic | 0 | | |
| TOTAL | | | | 15 |

Intense: 10-15 points Intermediate: 5-10 points Weak: <5 points

**Table 3:** Estrus response rate at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field conditions.

| **Parameters** | **Farm & Field** | **Treatment** | | | | **Control** |
|---|---|---|---|---|---|---|
| | | **Sub-estrus** | | **True anestrous** | | |
| | | **DPG** | **POVS** | **OVS** | **COVS** | |
| No. of animals treated | Farm (n=102) | 46 | 14 | 12 | 14 | 16 |
| | Field (n=457) | 286 | 46 | 42 | 38 | 45 |
| No. of animals in estrus | Farm (n=88) | 42 | 12 | 11 | 13 | 10 |
| | Field (n=374) | 245 | 37 | 34 | 33 | 25 |
| Estrus response rate (%) | Farm | 91.30 | 85.71 | 91.66 | 92.85 | 62.50 |
| | Field | 85.66 | 80.43 | 80.95 | 86.84 | 55.55 |

**Table 3a:** Estrus response rate at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field conditions during seasons.

| **Parameters** | **Farm and Field** | **HB and LB** | **Treatment** | | | | **Control** |
|---|---|---|---|---|---|---|---|
| | | | **Sub-estrus** | | **True anestrous** | | |
| | | | **DPG** | **POVS** | **OVS** | **COVS** | |
| No. of animals treated | Farm | HB | 21 | 7 | 6 | 7 | 8 |
| | | LB | 25 | 7 | 6 | 7 | 8 |
| | Field | HB | 147 | 22 | 22 | 18 | 22 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | LB | 139 | 24 | 20 | 20 | 23 |
| No. of animals in estrus | Farm | HB | 20/21 | 7/7 | 6/6 | 7/7 | 6/8 |
| | | LB | 22/25 | 5/7 | 5/6 | 6/7 | 4/8 |
| | Field | HB | 139/147 | 19/22 | 20/22 | 17/18 | 14/22 |
| | | LB | 106/139 | 18/24 | 14/20 | 16/20 | 11/23 |
| Estrus response rate (%) | Farm | HB | 95.23 | 100 | 100 | 100 | 75 |
| | | LB | 88.00 | 71.42 | 83.33 | 85.71 | 50 |
| | Field | HB | 94.55 | 86.36 | 90.90 | 94.44 | 63.33 |
| | | LB | 76.25 | 75 | 70 | 80.00 | 47.82 |

**Table 4:** Intensity of estrus at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field conditions.

| Treatment Group | Farm and Field | Intense estrus (%) | CR (%) | Intermediate estrus (%) | CR (%) | Weak estrus (%) |
|---|---|---|---|---|---|---|
| **DPG** | Farm | 11.90 | 40.00 | 69.04 | 65.51 | 19.05 |
| | Field | 15.10 | 15.00 | 62.85 | 72.00 | 22.04 |
| **POVS** | Farm | 41.67 | 80.00 | 58.33 | 71.42 | 0 |
| | Field | 56.76 | 71.42 | 27.03 | 70.00 | 16.21 |
| **OVS** | Farm | 27.27 | 33.33 | 54.55 | 83.33 | 18.18 |
| | Field | 17.65 | 33.33 | 52.94 | 77.77 | 29.41 |
| **COVS** | Farm | 46.15 | 50.00 | 53.85 | 57.14 | 00.00 |
| | Field | 45.45 | 26.66 | 39.39 | 69.23 | 15.15 |
| **Control** | Farm | 00.00 | 00.00 | 60.00 | 83.33 | 40.00 |
| | Field | 8.00 | 50.00 | 28.00 | 57.14 | 64.00 |

**Table 4a:** Intensity of estrus at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field conditions during seasons.

| Treatment Group | Farm and Field | HB LB | Intense estrus (%) | CR (%) | Intermediate estrus (%) | CR (%) | Weak estrus (%) |
|---|---|---|---|---|---|---|---|
| **DPG** | Farm | HB | 13.04 | 33.33 | 60.86 | 64.28 | 26.08 |
| | | LB | 10.52 | 50.00 | 57.89 | 72.72 | 31.57 |
| | Field | HB | 16.53 | 19.04 | 72.44 | 82.60 | 25.19 |
| | | LB | 13.55 | 12.5 | 52.54 | 51.61 | 18.64 |
| **POVS** | Farm | HB | 42.85 | 100.00 | 57.14 | 75.00 | 00.00 |
| | | LB | 40.00 | 50.00 | 60.00 | 6.66 | 00.00 |
| | Field | HB | 61.90 | 69.23 | 28.57 | 66.66 | 9.5 |
| | | LB | 50.00 | 75.00 | 25.00 | 75.00 | 25.00 |
| **OVS** | Farm | HB | 33.33 | 50.00 | 66.66 | 75.00 | 16.66 |
| | | LB | 20.00 | 00.00 | 40.00 | 100.0 | 20.00 |
| | Field | HB | 20.00 | 25.00 | 60.00 | 75.00 | 35.00 |
| | | LB | 14.28 | 50.00 | 42.85 | 83.33 | 21.42 |
| **COVS** | Farm | HB | 57.14 | 50.00 | 57.14 | 75.00 | 00.00 |
| | | LB | 33.33 | 50.00 | 50.00 | 33.33 | 00.00 |
| | Field | HB | 47.36 | 22.22 | 36.84 | 71.42 | 15.78 |
| | | LB | 42.85 | 33.33 | 42.85 | 66.66 | 00.00 |
| **Control** | Farm | HB | 00.00 | 00.00 | 66.66 | 75.00 | 33.33 |
| | | LB | 00.00 | 00.00 | 50.00 | 100.00 | 50.00 |
| | Field | HB | 16.66 | 50.00 | 33.33 | 50.00 | 75.00 |
| | | LB | 00.00 | 00.00 | 23.07 | 66.66 | 53.84 |

**Table 5:** Conception and pregnancy rate at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field condition

| Parameters | Farm and Field | Sub-estrus | | True anestrous | | Control |
|---|---|---|---|---|---|---|
| | | DPG | POVS | OVS | COVS | |
| 1st service CR at induced estrus (%) | Farm | 60.86 | 66.66 | 71.42 | 57.14 | 57.14 |
| | Field | 59.39 | 60.00 | 66.66 | 64.28 | 53.84 |
| 2nd service CR at subsequent estrus (%) | Farm | 26.08 | 22.22 | 28.57 | 28.57 | 28.57 |
| | Field | 33.83 | 28.00 | 23.80 | 28.57 | 30.76 |
| 3rd service CR at subsequent estrus (%) | Farm | 13.04 | 11.11 | 00.00 | 14.28 | 14.28 |
| | Field | 6.76 | 12.00 | 9.52 | 7.14 | 15.38 |
| Over all CR (%) at 30 days (TRUS) | Farm | 50.00 | 64.28 | 58.33 | 50.00 | 43.75 |
| | Field | 46.50 | 54.34 | 50.00 | 36.84 | 28.88 |
| Pregnancy rate (%) at 45-60 days (PRE) | Farm | 50.00 | 64.28 | 50.00 | 42.85 | 37.50 |
| | Field | 45.45 | 54.34 | 47.61 | 34.21 | 26.66 |

**Table 5a:** Conception and pregnancy rate at induced estrus in treatment and at natural estrus in control groups in postpartum Graded Murrah buffaloes in farm and field condition during seasons

| Parameters | Farm & Field | HB & LB | Sub-estrus | | True anestrous | | Control |
|---|---|---|---|---|---|---|---|
| | | | DPG | POVS | OVS | COVS | |
| 1st service CR at induced estrus (%) | Farm | HB | 69.23 | 80.00 | 75.00 | 75.00 | 33.33 |
| | | LB | 50.00 | 50.00 | 66.66 | 33.33 | 75.00 |
| | Field | HB | 66.66 | 75.00 | 81.81 | 66.66 | 66.66 |
| | | LB | 50.81 | 46.15 | 50.00 | 60.00 | 42.85 |
| 2nd service CR at subsequent estrus (%) | Farm | HB | 28.57 | 33.33 | 25.00 | 50.00 | 40.00 |
| | | LB | 22.22 | 00.00 | 33.33 | 00.00 | 00.00 |
| | Field | HB | 38.66 | 35.71 | 28.57 | 3.33 | 33.33 |
| | | LB | 27.58 | 18.18 | 14.28 | 20.00 | 25.00 |
| 3rd service CR at subsequent estrus (%) | Farm | HB | 20.00 | 16.66 | 00.00 | 20.00 | 25.00 |
| | | LB | 00.00 | 00.00 | 00.00 | 00.00 | 00.00 |
| | Field | HB | 8.04 | 18.75 | 16.66 | 12.50 | 12.50 |
| | | LB | 4.34 | 00.00 | 00.00 | 00.00 | 20.00 |
| Over all CR (%) at 30 days (TRUS) | Farm | HB | 60.86 | 66.66 | 66.66 | 57.14 | 50.00 |
| | | LB | 39.13 | 60.00 | 50.00 | 42.85 | 37.50 |
| | Field | HB | 55.63 | 61.90 | 59.09 | 50.00 | 40.00 |
| | | LB | 38.56 | 48.00 | 40.00 | 29.16 | 20.00 |
| Pregnancy rate (%) at 45-60 days (PRE) | Farm | HB | 60.86 | 66.66 | 66.66 | 57.14 | 50.00 |
| | | LB | 39.13 | 60.00 | 33.33 | 28.57 | 25.00 |
| | Field | HB | 55.63 | 61.90 | 59.09 | 50.00 | 40.00 |
| | | LB | 36.60 | 48.00 | 35.00 | 25.00 | 16.00 |

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