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Estrus induction and fertility in response to exogenous hormonal administration protocols in postpartum anestrus murrah buffaloes

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Abstract
The objectives were to determine the estrus and conception responses to the controlled internal drug release (CIDR), Crestar and Polyurethane sponge (vaginal implant containing 1.5 gm of Natural Micronized Progesterone) along with prostaglandin F2α and PMSG protocol and to compare the efficacy of this protocol. In Group I twelve buffaloes received CIDR inserts for 7 d and were treated with PGF2α on day 6th and inj PMSG (600 IU) immediately after CIDR withdrawal. In Group II, CIDR was replaced with Crestar, While In Group III and IV, exogenous progesterone source were Polyurethane sponge (vaginal implant containing 1.5 gm of Natural Micronized Progesterone). In Group 1and III, 100% of buffaloes showed estrus responses, while Group II and IV buffaloes showed 91.67% estrus response. The overall conception rate were 50.00, 63.64, 66.67 and 54.55% respectively. Intra-vaginal sponge impregnated with progesterone with conception rate of (66.67%) provided excellent fertility. The intra-vaginal sponge impregnated with progesterone can replace CIDR and Crestar in buffaloes and costs 1/10th the price, thus reducing the financial implications to livestock owner.

Keywords: CIDR, Crestar, polyurethane sponge estrus induction, ovulation, conception, progesterone and buffalo

1. Introduction
Post-partum anestrus is the period after calving during which dairy animals do not exhibit estrus. It is the prevalent, frustrating and challenging problem encountered in dairy animals. Field survey on reproductive disorder have revealed that anestrus is most common single cause of infertility in buffaloes among which inactive or non-functional ovaries are single cause of anestrus (EL-Wishy, 2007) [12]. Incidence of anestrus is more in buffaloes than cattle and problem is more severe in summers (Singh *et al*., 2010) [41]. In comparison to cows, buffaloes have lesser number of pre antral and antral follicles and greater tendency of follicular atresia (Danell, 1987) [8]. Different methods are employed to reduce the postpartum anestrus and subsequent inter-calving period in order to increase the fertility during the low breeding season. Exogenous administration of progestins are considered appropriate for non-cyclic or anestrus post-partum cows (Yaniz *et al.*, 2004) [48] and buffaloes (Lakra *et al.*, 2003) [20], for resumption of estrus which normal cycle length (Rhodes *et al.*, 2003) [35]. Progesterone both exogenous and endogenous suppress the estrus and ovulation by inhibiting the release of Leuinizing Hormone (LH) (Peter and Lamming, 1984) [34] and its withdrawal results in a gradual rise in plasma LH concentration culminating in a preovulatory LH surge which occurs approximately after 48 hours (Garcia-Winder *et al.*, 1986) [13]. Crestar is a recently used therapy for estrus induction in postpartum anestrus buffaloes (Nayak *et al*., 2009; Malik *et al.;* 2011; Nakrani *et al.*, 2014) [29, 21, 25]. The CIDR-B is the most common intravaginal progesterone available and contains 1.38 g of progesterone and is extensively used in various protocols for estrus synchronization, induction, fixed-time AI, fixed-time embryo transfer, and super stimulation programs. Polyurethane sponges impregnated with varying amounts of progesterone and of various lengths, diameters and densities have been used in cattle. In India, at present no locally made commercial progesterone-releasing intravaginal device is available for treatment of post-partum anestrus in buffaloes. Based on these views, the present study was proposed to evaluate the progesterone implants in post-partum anestrus buffaloes in relation to reproductive parameters.

2. Materials and Methods

The present study was carried out on 48 postpartum anestrus buffaloes from different villages of R. S. Pura area of Jammu district. History of each animal was recorded before including in the investigation. Buffaloes were selected on the basis of history, which had not exhibited any signs of estrus for 90 days or more postpartum. The anestrus period varied from 3 months to 6 months duration. Buffaloes were examined per rectally twice at 10 days interval to ascertain inactive, smooth ovaries, flaccid uterine horns and pale vestibule. Pathological causes of anestrus were ruled out. On the basis of selection criteria, 48 post-partum anestrus buffaloes were included in the trial and randomly divided into 4 groups consisting of 12 animals in each group. In Group I buffaloes progesterone were supplemented with CIDR (Controlled Intravaginal Drug Release, Pfizer Ltd.), In Group II progesterone was supplemented by Crestar ear implant, In Group III exogenous source of progesterone was Polyurethane sponge, a vaginal implant (containing 1.5 gm of Natural Micronized Progesterone) and In Group IV source of progesterone was Polyurethane sponge, a vaginal implant (containing 1.5 gm of Natural Micronized Progesterone along with 1% carboxymethylcellulose). All post-partum buffaloes in each group were treated with an injection of 600 I.U. PMSG (Folligon) administered intramuscularly on the day of removal of removal of source of exogenous progesterone. The buffaloes exhibiting estrus (behavioural symptoms) within 10 days after treatment were considered to have responded positively to treatment and were also examined per-rectally (Nautiyal, 2000) [28]. Artificial Insemination (AI) was done with good quality frozen thawed semen 12 h after the onset of estrus. The animals not conceiving at first estrus were again inseminated on subsequent estrus. Statistical Analysis was done by using analysis of variance (ANOVA) and General Linear Models of SPSS 16.0 computer programme**.** The significance was assayed at 5% (*P*< 0.05).

3. Results and Discussion

Response of post-partum anestrus buffaloes to different hormone protocols *viz* CIDR (Group I), Crestar (Group II), Intra-vaginal sponge (Group III) and Intra-vaginal sponge with CMC (Group IV) is summarized in table 1.

Table 1: Response to estrus induction treatment

Sr. No.	Particulars	Group I (N=12)	Group II (N=12)	Group III (N=12)	Group IV (N=12)
1	Estrus Induction Response	12 (100%)	11 (91.67%)	12 (100%)	11 (91.67%)
2	Onset of estrus (hrs)	37.07±1.58^{ab} (28-42)	33.32±2.23^{a} (23-40)	39.33±2.24^{b} (28-48)	40.33±2.41^{b} (35-50)
3	Duration of estrus (hrs)	22.31±0.79^{a} (18-27)	21.08±0.69^{a} (18-26)	27.08±0.69^{b} (24-31)	23.64±0.50^{c} (20-29)
4	Conception rate				
	First Service Conception Rate	3/12 (25.00%)	4/11 (36.36%)	5/12 (41.67%)	3/11 (27.27%)
	Second Service Conception Rate	3/9 (33.33%)	3/7 (42.86%)	3/7 (42.86%)	3/8 (37.50%)
	Overall Conception Rate	6/12 (50.00%)	7/11 (63.64%)	8/12 (66.67%)	6/11 (54.55%)

Means bearing different superscript within a row differ significantly (*P*<0.05).

In Group I, the estrus induction response of buffaloes treated with CIDR and PMSG was 100%. The present findings are in collaboration with observations of Caesar *et al.* (2011) [7] and Kajaysri *et al.* (2015) [18] in buffaloes. The average time required for onset of estrus in Group I was 37.07±1.58 hrs (28-42 hrs). Which are in the agreement with the findings of Kajaysri *et al.* (2015) [18] and Lakra *et al.* (2003) [20] who reported 36-72 hrs time required for inducting estrus in buffaloes after CIDR and PMSG treatment. In the present study, the average duration of estrus in Group I was 22.31±0.79 hrs (18-27 hrs). These finding are in collaboration with Kajaysri *et al.* (2015) [18] and Saini *et al.* (2017) [36]. In Group I, out of 12 buffaloes, 3 (25.00%) conceived at first estrus. 3 (33.33%) out of 9 conceived at second estrus. The overall pregnancy rate was 6/12 (50.00%). These findings are in agreement with Lakra *et al.* (2003) [20] and Naseer *et al.* (2013) [26] in buffaloes. High progesterone in the blood supply suppresses GnRH neurons in the tonic center of the hypothalamus with a flow-on to suppression of both LH and FSH from the pituitary. Upon CIDR removal the rapid drop in circulating concentrations of progesterone promotes the release of a surge of GnRH, followed by FSH and LH from the anterior pituitary causing the resumption of ovarian cyclicity (Zaabel *et al*., 2009; Azawi *et al*., 2012) [49, 4].

In Group II, the estrus induction response of buffaloes treated with Crestar and PMSG was 91.67%. Following removal of implant, the resumption of follicular development and maturation might be due to flux of the gonadotropins from the pituitary. The use of PMSG along with Norgestomet ear implant may be useful as PMSG is known to increase blood estrogen level and in turn leads to induction of behavioral estrus signs (Singh *et al*., 2004a) [40]. The present findings are in close collaboration with Nakrani *et al.* (2014) [25], Parmar *et al.* (2015) [31] and Jerome *et al.* (2016) [17]. The average time required for onset of estrus in Group II was 33.32±2.23 hrs (23-48 hrs)**.** The time interval of estrus expression in this study corroborated with findings of Thangapandian *et al.* (2015) [45] and Dhalkari *et al.* (2018) [10]. The time interval to exhibit estrus after hormonal treatment observed in the present study might be due to the administration of PMSG in combination with progesterone and estrogen (Nath *et al.*, 2004) [27]**.** The average duration of estrus in Group II was 21.08±0.69 hrs (18-26 hrs), which collaborate with finding of Parmar *et al.* (2015) [31] and Thangapandiyan *et al.* (2015) [45]. In Group II, out of 11 buffaloes, 4 (36.37%) conceived at first estrus. 3 (42.86%) out of 7 conceived at second estrus. The overall pregnancy rate was 7/11 (63. 64%).The findings in the present study are in collaboration with Vikas *et al.* (2014) [46], Parmar *et al.* (2015) [31] and Kumar *et al.* (2016) [19].

In Group III and IV, the estrus induction response of buffaloes treated with Intra-vaginal sponge and PMSG was 100 and 91.67% respectively. Progesterone sponge was found to be more effective in anestrus buffaloes by slow release of progesterone from the sponge and absorbed through blood vessel in vagina. It has a negative feedback effect on anterior pituitary gland to inhibit estrous cycle (Thammakarn, 2010) [44]. When the sponge is removed from vagina, the progesterone in blood stream was decreased immediately and the reproductive cycle of cattle becomes active again (Devipriya *et al.*, 2015) [9]. The present findings are in collaboration with Visha *et al.* (2014) [47] and Jayachandran and Muralidharan (2015) [16]. In the present study, the average time required for onset of estrus in Group III and IV were 39.33±2.24 hrs (28-48 hrs) and 40.33±2.41 (35-50 hrs) respectively. These findings are in collaboration with Saini *et al*. (2017) [36]. The average time required for onset of estrus in Group III and IV was significantly higher compared to Group II. This may be due to increased half-life and delayed metabolism of micronized progesterone (Malik and Krishnaprasad, 2016) [22]. The average duration of estrus in Group III and IV were 27.08±0.69 hrs (24-31 hrs) and 23.64±0.50 (20-29 hrs) (Table 4.1) respectively. On the contrary, Saini *et al.* (2017) [36] reported lower duration of estrus. Estrus duration in Group IV was significantly higher compared to Group III as CMC may have adhered to vaginal wall even after removal of sponge leading to not much abrupt fall in progesterone concentration. The mean duration of estrus was significantly higher ($P< 0.05$) in Group III and IV compared to Group I and II. The duration of estrus was higher in these group due to micronized natural progesterone having increased half-life (Malik and Krishnaprasad, 2016) [22] and due to suprabasal progesterone, which causes prolonged estrus (Bage *et al.*, 2002) [5]. In Group III, out of 12 buffaloes, 5 (41.67%) conceived at first estrus. 3 (42.87%) out of 7 conceived at second estrus. The overall pregnancy rate was 8/12 (66.67%) and in Group IV, out of 11 buffaloes, 3 (27.27%) conceived at first estrus. 3 (37.50%) out of 8 conceived at second estrus. The overall pregnancy rate was 6/11 (54.54%). These findings are in collaboration with Devipriya *et al.* (2015) [9] and Jaychandran and Muralidharan (2015) [16]. On the other hand lower pregnancy rates were reported by Singh *et al*. (2010b) [42]. The pregnancy rate was higher (66.67%) in intravaginal sponge without CMC compared to intravaginal sponge with CMC (54.54%). In group IV (intravaginal sponge with CMC) has led to local infection and which might have resulted in inadequate / excess absorption of micronized natural progesterone in some of animal which in turn led to insufficient follicular development (Hixon *et al.*, 1981) [14] or prolongation of follicular development attributed to low pregnancy rate (Stock and Stolla, 1995 [43]). Further, 30-40 per cent conception obtained at second cycle in anestrus buffaloes induced to cycle proved that all the estrus induction protocols used to induce estrus and 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 anestrus cows and buffaloes by many workers (Naikoo *et al*., 2010; Bhoraniya *et al*., 2012; Ammu *et al.*, 2012; Parmar 2013; Patel *et al.*, 2013; Savalia *et al*., 2013; Dhami *et al.*, 2014) [24, 6, 3, 30, 32, 38, 11].

Suboptimal functioning of the hypothalamus-pituitary-gonadal axis and low FSH and LH peaks have been related by several authors to anestrus in buffalo under tropical and subtropical conditions (Janakiraman *et al.*, 1980, Aboul-Elah *et al.*, 1983 and Saini *et al.*, 1988) [15, 1, 37]. The high progesterone primes the brain for the actions of estrogen and addition of eCG subsequent to progesterone treatment increased plasma concentration of LH and FSH and enhanced the LH peak in non-cyclic buffalo heifers (Saini *et al.*, 1988) [37] due to rapid decline in systemic progesterone concentration on its removal (Perry *et al*., 2004) [33] leading to induction of behavioral estrus signs (Singh *et al.*, 2004a) [40] due to synergistic effect of exogenous gonadotropin on folliculogenesis, oocytes maturation and development of corpus luteum (Agarwal *et al.*, 2005) [2]. Thus, administration of eCG probably helps complete the recovery of hypothalamus-pituitary-gonadal axis function already stimulated by the progesterone treatment (Murugavel *et al.*, 2009) [23].

4. Conclusion

Amongst the different hormonal protocol CIDR and Intra-vaginal sponge impregnated with progesterone treatment group resulted in 100% estrus induction response while Intra-vaginal sponge impregnated with progesterone with conception rate of (66.67%) provided excellent fertility. It can be concluded that intra-vaginal sponge impregnated with progesterone can replace CIDR and Crestar in buffaloes and costs $1/10^{th}$ the price, thus reducing the financial implications to livestock owner.

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