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Therapy of ovarian inactivity in postpartum Bulgarian Murrah buffaloes by PRID and Ovsynch estrus synchronization protocols

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ABSTRACT

Objective: The aim of the present study was to assess therapeutical effect of modified Ovsynch and PRID estrus synchronization protocols in Bulgarian Murrah buffalo with inactive ovaries during the low-breeding season. **Methods:** The study was carried out in 46 Bulgarian Murrah buffaloes with small inactive ovaries established by two consecutive transrectal ultrasonographies on Day 40 and 50 postpartum. At the start of the therapy the buffaloes were randomly divided into three groups. Group I (n=18) was treated by PRID-based protocol; Group II (n=18) was treated by Ovsynch based protocol and Group III (control; n=10) was injected intramuscular with saline at the same days as in the first two groups and fertile bull was introduced after that. The animals in the different groups were submitted to ultrasound examination at day of artificial insemination or bull introduction. Ovulation was determined 7 days post insemination by ultrasound. The pregnancy diagnosis was done 30 days after insemination. Mean diameter of the largest follicles at the start of therapy and the day of AI was registered. In the hormonal treated buffaloes estrus clinical sings, ovulation rate and pregnancy rate after AI were determined. In the control group pregnancy rate after spontaneous estrus was established. The mean diameter of the largest follicles determined on Days 40 and 50 after calving was not over 9 mm for all buffaloes. **Results:** At day of AI the average diameters of the preo-vulatory follicles in PRID and Ovsynch treated buffaloes were significantly (P<0.01) greater than these on Day 0. The cases of a clear uterine mucus discharge during the induced estrus were significant more (P<0.05) for Group I (94.4%) than Group II (66.7%). The pregnancy rate after AI (56.6% and 38.8%) in PRID and Ovsynch program was significant higher (P<0.05) than pregnancy rate after spontaneous estrus (10%) in the control group. **Conclusions:** the treatment of buffalo ovarian inactivity could start on Day 50 postpartum. The PRID and Ovsynch estrus synchronization protocols by substitution of the second GnRH with hCG, could be successfully used for therapy of Bulgarian Murrah buffaloes with inactive ovaries during the low-breeding season.

1. Introduction

The post-partum anoestrus is a major factor for long calving interval and low reproductive efficiency[1-3]. This period is significant longer in buffaloes compared to cattle although the uterine involution completes around 35th day after parturition[4-6]. According to El-Wishy, only 34%-49% of buffaloes showed estrus during the first 90 days after calving and 31%-42% remain anestrous for more than 150 days[7].

Inactive ovaries are one of the most important reasons limiting the estrus behavior after partus[8]. Inadequate follicular development and absence of ovulatory follicle characterize this condition[9]. Nutrition, presence of calves, loss of body weight, season and poor management are predisposposing factors for the disease[7,10,11].

The heredity also determines suboptimal levels of

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gonadotropins and variable progesterone concentration in a systemic circulation[12]. However, the basic cause is a low secretion of follicle-stimulating (FSH) and luteinizing hormone (LH) and lack of pre-ovulatory LH surge[13].

In general, buffaloes are described with great differences of oestrous phase length (4–64 h), low expression of estrus sings and high variability (6–72 h) in the time of ovulation[14,15]. The reproductive inactivity is related with lack or reduced sexual activity-low or non-breeding season[16].

Different estrus synchronization protocols with or without timed artificial insemination (TAI) have been utilized for reduction of postpartum anoestrus, but the obtained results are still conflicting[17–20]. The differences of synchronization programs[16,21,22], breed and age of buffaloes[23,24], seasonal effects[25] or level of nutrition[26] are accepted like a reason for these discrepancies.

Progesterone-releasing intravaginal device (PRID) and controlled internal drug release (CIDR) or Ovsynch programs have been used for treatment of buffaloes with inactive ovaries[27,28,29,30].

The insertion of progesterone intravaginal coil or implant has been shown to exert negative feedback on hypothalamus-pituitary system, decrease LH pulse frequency and sensibilize hypothalamus to estrogens[12,31]. After the coil withdrawal blood progesterone concentration sharply drops and gonadotropin – releasing hormone (GnRH), LH and FSH increase leads to resumption of ovarian activity[32].

Many studies have carried out through PRID or CIDR introducing on Day 0, PGF2α injection on Day 6 or 7, pregnant mare serum gonadotropin (PMSG) administration at day of progesterone device withdrawal (Day 7), followed by GnRH 48 h latter and TAI 16–24 h after the second GnRH[33–37].

The exogenous gonadotropin-releasing hormone induces rapid release of luteinizing hormone (LH surge) during any stage of estrous cycle in cattle and buffaloes. This promotes the ovulation of dominant follicle or luteinization and/or atresia of the predominant follicle[38–40].

Ovsynch protocol includes GnRH administration on Day 0, PGF2α on Day 7, second GnRH on Day 9 and TAI 16–20 h after the second GnRH injection[16,30]. The presence of ovulatory follicle after the second GnRH injection is a prerequisite for effective synchronization and high pregnancy rate[31]. These regimes have been applied in cyclic and non-cyclic buffalo cows[19,41], but the conception and pregnancy rate were unsatisfactory. Several studies reported to increase ovulation and conception rate by replacement of the second GnRH with human chorion gonadotropin (hCG)[42,43].

Previous experiments indicate that synchronous ovulatory response depended on the size and stage of ovarian follicles at the beginning of the treatment and buffalo breed [36,44–46]. The success was lower when the treatment is performed during the non breeding season and various modified protocols have been used to eliminate the problem[47]. Recently, ultrasonography has been applied to clarify some questions connected to follicular development after estrus synchronization in anestrous buffaloes[48,49].

The above features of buffalo species suggest development and implementation of new estrus synchronization programs or elaboration of available one for therapy of buffaloes with inactive ovaries. Until present time clear standpoint about replacement of GnRH with hCG in estrus synchronization schedules absent. The information associated with application of different synchronization protocols before Day 60 postpartum is rather insufficient. Bulgarian Murrah buffalo is an indigenous buffalo breed and data about the effects of estrus synchronization protocols for true anoestrus reduction are not available.

The objective of the current study was to assess therapeutical effect of modified Ovsynch and PRID-based estrus synchronization protocols in Bulgarian Murrah buffalo with inactive ovaries during the low-breeding season.

2. Materials and methods

The study was carried out with 46 Bulgarian Murrah buffalo cows, body weight 480–520 kg, at the age of 4–6 years, average parity 2.8±0.2 and body condition scores 3.0–3.5 by the scale of Edmonson *et al*[50]. The average daily milk production was (5.4±0.6) kg with milking morning and evening.

The animals were with normal parturition, completed uterine involution and lack of endometritis during the experimental period. Calves were separated from dams immediately after the parturition and fed with milk replacer. Buffaloes were reared in a farm located at latitude of 42.183 N, longitude 25.567 E.

The daily mixed ration consisted of 40% concentrate with composition of dry matter (DM) basis-crude protein-13%; Ca-0.5%; P-0.35% and NEL 6.8 MJ/kg dry matter and 60% forages including hay, straw, 6 h controlled grazing and water *ad libitum*. The study was conducted during the low-breeding season (from August to October).

The experimental animals were with small inactive ovaries established by two consecutive transrectal ultrasonographies 10 days apart (Day 40 and Day 50 postpartum). Ultrasound examination was done by ultrasound scanner SonoScape A5 Vet (SonoScape Co. LTD, Shenzhen, China) and 7–12 MHz linear transducer. The criterion for ovarian inactivity was absence of corpus luteum (CL) and follicles > 10 mm in diameter. Completed uterine involution was confirmed by ultrasonography[6]. All examinations were accomplished by the same operator.

At the start of the therapy (Day 0 = Day 50 postpartum) the buffaloes were randomly separated into three groups – group I (PRID), group II (Ovsynch) and group III (Control).

In the first group (n=18) intravaginal device (PRID, Ceva Santé Animale, Libourne, France), containing 1.55 g progesterone in inert silicone elastomer coil was inserted on Day 0. Immediately after the coil withdrawal (Day 7), PGF2α 500 mg cloprostenol (PGF Veyx forte, Veyx-Pharma GmbH, Schwarzenborn, Germany) and 500 IU PMSG (Folligon, Intervet International, B.V, Boxmeer, Holland) were administrated. Forty eight hours later 1 500 IU hCG (Chorulon, Intervet International, B.V, Boxmeer, Holland) were injected intramuscularly.

The buffaloes in the second group (n=18) were treated with 100 μg GnRH (Depherelin, Veyx-Pharma GmbH, Schwarzenborn, Germany) on Day 0, 500 mg cloprostenol on Day 7, followed by 1 500 IU hCG on Day 9. The animals from the first and second group were artificially inseminated 16 and 28 h after hCG administration with frozen semen from one certified bull.

The control group (n=10) received 2 mL intramuscular injection of saline (0.9% NaCl) on Days 0, 7 and 9, respectively.

All animals were submitted to ultrasound examination at day of AI and size of the largest follicle was determined. The method described by Loeffler *et al.*[51] was used for estrus sings detection in treated buffaloes. At the same time fertile bull was introduced in the control group. Ovulation was given 7 days after AI by ultrasound checking for disappearance of the pre-ovulatory follicle and visualization of newly created corpus luteum.

Ultrasound pregnancy diagnosis was performed on Day 30 after AI and bull introduction in synchronized and control buffaloes, respectively.

After data processing a mean diameter (mm) of the largest follicles at the start of therapy and the day of AI was registered. In the hormonal treated buffaloes estrus clinical sings (clear mucus discharge and ease of cervical passage) (%), ovulation rate (%) and pregnancy rate after AI (%) were determined.

In the control group pregnancy rate after spontaneous estrus was established. Ultrasound appearance of the embryo was used as a criterion for pregnancy.

Statistical analysis was performed with the Stat-Soft 1984-2000 Inc. statistical software (Copyright©1990-1995 Microsoft. Corp.) by means of non-parametric analysis for comparison of two means and proportions, using Student's t-criterion. Differences were considered significant in P-values ≤ 0.05.

3. Results

The mean diameter of the largest follicles on Days 40 and 50 after calving in all buffaloes was not over 9 mm (Figure 1A). At the start of the therapy the values were (7.2±1.4), (7.8±1.2) and (7.3±1.4) mm for the first, second and third group, respectively, but significant differences were not registered (Table 1).

Table 1

Effects of PRID and Ovsynch protocols in Bulgarian Murrah buffalo with inactive ovaries.

Groups	Mean diameter of the largest follicle (mm)		Induced estrus sings (%)		Ovulation rate (%)	Pregnancy rate (%)
	At the start of therapy	At day of AI	Clear mucus discharge	Ease of cervical passage		
Prid (n=18)	7.2 ± 1.4^{a}	14.1 ± 1.5^{bc}	94.4^{a} (17/18)	88.9 (16/18)	72.2 (13/18)	55.6^{ab} (10/18)
Ovsynch (n=18)	7.8 ± 1.2^{a}	12.0 ± 2.3^{bc}	66.7^{b} (12/18)	66.7 (12/18)	55.6 (10/18)	38.8^{b} (7/18)
Control (n=10)	7.3 ± 1.4^{a}	7.6 ± 1.3^{a}	-	-		10.0^{c} (1/10)

Values within a column are significantly different at P<0.05.

The average diameter of a pre-ovulatory follicle in PRID [(14.1±1.5) mm] and Ovsynch [(12.0±2.3) mm] group at day of artificial insemination (Figure 1B) was significantly (P<0.01) greater than diameter on Day 0. In the control group similar difference was not registered (P>0.6). Besides, the values in synchronized animals differed in comparison with obtained one [(7.6±1.3) mm] in the control buffaloes (P<0.05).

The cases with presence of a clear mucus discharge were considerable more (P<0.05) in PRID (94.4%) than these (66.7%) in Ovsynch group. Although, the parameter ease of cervical passage was higher in group I (88.9%) than group II (66.7%), significant difference was not registered.

The ovulation rate in PRID and Ovsynch based protocols was 72.2% and 55.6%, respectively. On day 7 after insemination five non-ovulated animals from the first and seven buffaloes from the second group showed follicles in different stage of development. Ovarian cysts were observed in three Ovsynch treated buffaloes (Figure 1C).

The pregnancy rate after AI (56.6% and 38.8%) in PRID and Ovsynch program was significant higher (P<0.05) than pregnancy rate after spontaneous estrus (10%) in the control group. Overall pregnancy rate for synchronized buffaloes (17/36) was 42.2%. On Day 35 post AI an echoic embryo surrounded by non-echoic amniotic fluid was observed in the uterus (Figure 2).

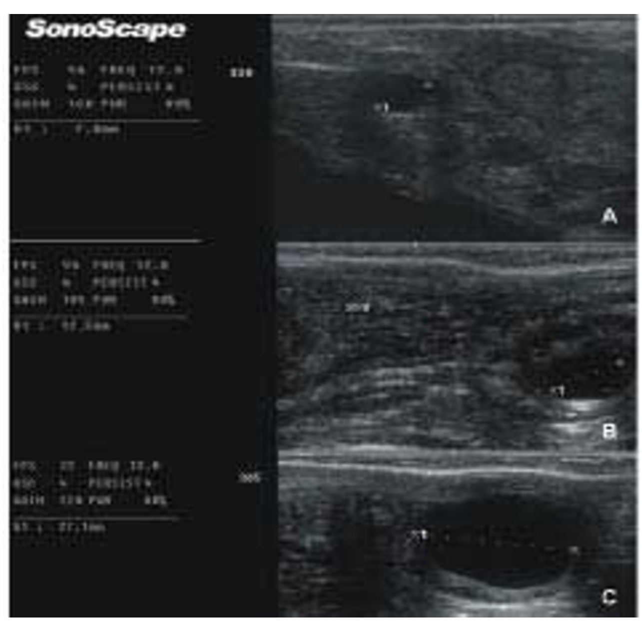

Figure 1. Follicular status at the start of the therapy (A), at the day of artificial insemination (B) and follicular cyst (C).

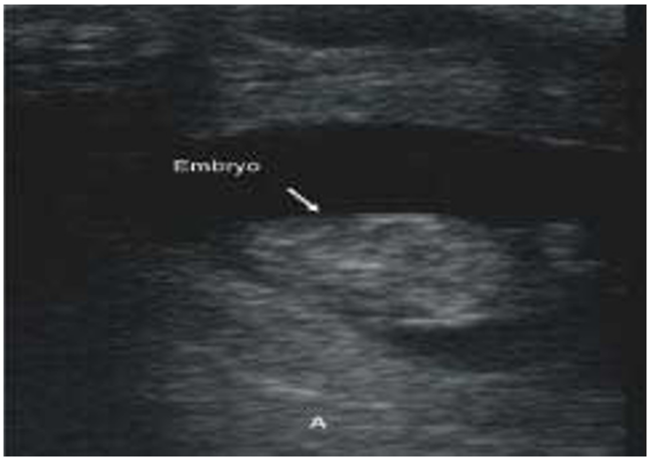

Figure 2. Cross-sectional ultrasonic images of a pregnancy on Day 35 after artificial insemination.

4. Discussion

Different methods for therapy of buffalo cows with postpartum anestrous are intensively discussed in the last few years[12,19,45,48]. Most of the estrus synchronization protocols have been used during the breeding season or after Day 60 postpartum [20,30,41].

The present study describes the reproductive response of buffaloes with inactive ovaries, when hormonal therapy starts on Day 50 postpartum. Moreover our results are received during the low-breeding season. One investigation in 5 742 Bulgarian Murrah buffalo cows reported to significant decrease of conception rate between August and September[52].

The absence of corpus luteum and follicles >10 mm at the start of therapy is an evidence for ovarian inactivity. Sah and Nakao[3] and Azawi *et al.*[20] also defined ovarian inactivity when palpated corpus luteum and follicles are lacking.

According to previous authors[53,54] follicles less than 8 mm fail to ovulate after administration of exogenous GnRH. This could explain lower reproductive effects obtained by Ovsynch protocol. Probably follicular atresia and lack of a sensitive luteal structure at the time of prostaglandin injection could be a reason for inadequate response in some buffaloes. Similar influence of follicular development stage on GnRH induced response was reported by Dharani *et al*[55].

In contrast, these follicles have adequate response of treatment with progesterone (P4) and PMSG. A study of Bartolomeu *et al.* also stated to positive effect after exogenous progesterone administration (CIDR) and PMSG when the follicular diameter was (7.5±1.0) mm at the beginning of the treatment[36].

The significant (P<0.01) greater mean diameters of pre-ovulatory follicles at day of AI in comparison to diameter on Day 0 in PRID and Ovsynch regimes indicates ovarian activity resumption. Barile *et al.* determined pre-ovulatory follicle diameter from (14.4±0.6) mm to (16.4±0.3) mm in Italian Mediterranean buffaloes using PRID-PMSG-PGF2α treatment[56]. By Ovsynch protocol Oropeza *et al.* estimated ovulatory follicle size (15.2±0.2) mm in Indian Murrah buffaloes[45], whereas in study of Derar *et al.* with Egyptian buffalo cows it was (13.6±0.2) mm[24].

The registered average follicular diameters at Day of AI in hormonal stimulated Bulgarian Murrah buffalo are closed to the reported one by the above researches. The significant difference (P<0.05) between the values in treated and control animals shows successful induction of ovarian activity, when the therapy starts on Day 50 post partum.

The present investigation states that buffaloes submitted to PRID protocols have better (P<0.05) estrus sings than Ovsynch treated animals, irrespective of similar follicle sizes. This data are in agreement with the study of Azawi *et al*[20]. According to Malik *et al.*[57] only 40% from anestral buffaloes responded with good mucus discharge after Ovsynch treatment. We speculate that the PRID induced follicles produce more estrogens (E_2) than these after Ovsych stimulation. Similar results in postpartum cows (greater peak of circulating concentrations of E_2 in CIDR than Ovsynch treatment) were registered by Herlihy *et al*[58].

The obtained ovulation rate for exogenous progesterone schedule is close to the reported one by Shah *et al.*[59] in Nili-Ravi breed and by Murugavel *et al.*[41] in non-cyclic Murrah buffalo cows. Previous investigators also used PRID-PMSG-PGF2α treatment for improvement of fertility rate during the low breeding season[30,60,61].

The ovulation rate after Ovsynch is significant lower than the registered 90% by Oropeza *et al.*[45], but it is close to the determined 62.5% by Warriach *et al.*[62]. Different factors (season, breed or animal nutritional status) are shown as a reason for this discrepancy[23,25,46]. We suggest that inadequate E_2/P_4 proportion after Ovsynch therapy leads to lower expression of LH receptors and suppress ovulation. The detection of cysts on Day 7 post AI confirms this attitude. Moreover the applied therapy includes human chorion gonadotropin (LH analog with extended half-life in the blood) which should stimulate the ovulatory follicle and improve its differentiation into a CL[63]. According to Kasimanickam *et al.*[64] if the dominant follicle presented at the time of GnRH injection fails to express LH receptors, it will never ovulate.

The pregnancy rate (55.6%) in PRID based protocol falls with showed 56.25% by Barile *et al.*[56] on day 28 after insemination and is close to the obtained 65.2% by Mansour *et al.*[65]. In disagreement with our data are registered values (44.5% and 70.5%) in studies of Neglia *et al.*[17] and Presicce *et al*[35].

The information about this parameter in Ovsynch programs is also controversial. Pregnancy rate reported by Oropeza *et al.*[45] and Neglia *et al.*[17] is close to the obtained 38,8% in this survey, but in other experiments[66,67] it varied from 6.9% to 42.2%. Using the same protocol Carvalho *et al*. received 58.8% conception rate in buffalo crossbred (Murrah x Meditteranian) cows[68].

A simple explanation for these contradictions could not be given, because these authors are used different type of coils (witht or without estradiol benzoate) and time for exposition, doses PMSG, GnRH and PGF2α analogues or buffalo breed reared under specific climate conditions.

In our opinion the start of the therapy (breeding season, low breeding season or seasonal anoestrus) is a very important factor.

The present study shows that hormonal treatment could starts on day 50 postpartum when uterine involution is completed and calves are separated immediately after parturition. In this aspect Palta and Madan announced that pituitary responsiveness to GnRH does not appear to be limiting factor for resumption of ovarian activity by Day 35 postpartum[69].

The used PRID and Ovsynch schedules lead to resumption of ovarian activity, but non-conceived buffaloes remain acyclic for a long period. This acyclicity could be explained with seasonal effects. The period between August and the middle of October is a low breeding season for this buffalo breed with the highest summer temperatures[62]. Terzano *et al*.[12] reported to lower basal LH level and lack of pre-ovulatoy surge in buffaloes with ovarian inactivity during the summer, such as the LH frequency and amplitude of pulses in follicular phase were significantly lower during summer than the winter season. The significant (P<0.05) lower pregnancy rate (10%) in the control group than others groups notwithstanding bull presence supports this affirmation.

The present study shows that inclusion of used PRID and Ovsynch protocols on Day 50 postpartum is an effective method for elimination of ovarian inactivity in Bulgarian Murrah buffaloes during the low breeding season. Moreover the overall pregnancy rate (42.2%) is acceptable and could reduce days open and calving interval.

The treatment of buffalo ovarian inactivity could start on Day 50 postpartum in completed uterine involution proven by ultrasonography and separation of calves from dams immediately after parturition. The performance of PRID and Ovsynch estrus synchronization protocols by substitution of the second GnRH with hCG, could be successfully used for therapy of Bulgarian Murrah buffaloes with inactive ovaries during the low breeding season. The combination of both hormonal treatments could reduce days open and calving interval.

Conflict of interest statement

No actual or potential conflict of interest in relation to this article exists.

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