Oestrus Synchronization and Artificial Insemination at Fixed Times Protocols in Dairy Buffaloes

Adrian Bota*, Horatiu Bujdei, Remus Chiorean

Research and Development Station for Buffalo, Sercaia, 507195, street Campului, no 2, Brasov county, Romania

Abstract

The development of reproductive biotechnologies, such as oestrus synchronization (OS) associated with artificial insemination (AI) in buffaloes, could have a significant impact on milk yields obtained at farm level. The aim of this paper was to study the effects of OS and fixed-time AI in buffaloes. Thirty buffalo cows with anoestrus were divided into three groups L1, L2 and L3 (10/group) at the Research and Development Station for Buffaloes Sercaia. The following protocols were applied: L1 - GnRH on day 1, prostaglandins on day 7, GnRH on day 9, followed by AI at 10 days (resulted in 50% pregnancy rate); L2 - GnRH was administered on day 1, prostaglandin was administered on day 7, GnRH was administered on day 9, AI was performed at 48 hours after the prostaglandin administration (resulted in 80% pregnancy rate); L3 - prostaglandin was administered on day 1, GnRH was administered on day 12, prostaglandin was administered on day 19, GnRH was administered on day 21, within 17-24 hours after last administration of GnRH AI was performed (resulted in 50% pregnancy rate). The use of OS techniques in buffaloes can overcome some of the difficulties of detecting oestrus and increase the efficiency of AI.

Keywords: anoestrous, artificial insemination, dairy buffaloes, synthetic gonadotropin, prostaglandins.

1. Introduction

The reproductive seasonality of buffaloes is a physiological factor that has a major impact on the economic activity of their development. Over the past 20 years, artificial insemination (AI) become important for genetic improvement and a solution to control the period of breeding in buffalo. When compared with cattle, AI is more problematic in buffalo due to variable estrous cycles, reduced estrous behavior, and reproductive seasonality [1,2]. The highest percentage of calvings in this species is observed from March to September and determines the concentration of milk production until December, with a significant reduction or even the absence of milk yields in the winter-spring months. Given that is a short-day breeder, the buffalo has increased reproductive activity with decreasing day length [3,4]. An additional problem that affects buffalo productivity is the long interval between calving intervals due to delayed postpartum estrus. This is broadly associated with the difficulty in detecting estrus in this species because of the weak signs (quiet ovulation). Moreover, behavioral signs of estrus including bellowing, restlessness, tail raising and homosexual mounting conduct are present only in a slight proportion of buffalo and are characteristically exhibited during the night [5,6]. Thus, the development and implementation of reproductive biotechnologies, such as estrus synchronization associated with artificial insemination in buffaloes, could have a significant relevance on the productions such as milk yield. In the last years, estrous synchronization by hormone administration has emerged as a viable alternative to reduce labor costs, plan reproduction and insemination time in cattle. Studies on buffer estrus synchronization were based on protocols developed for cattle, which aimed to either induce

* Corresponding author: Bota Adrian, Phone: +40.268.245.890, Email: adrianbota68@yahoo.com

premature luteolysis using prostaglandins or prolong the luteal phase using progestogens. To overcome the seasonality of reproduction, a synchronization protocol must initiate follicular development by activating the hypothalamic-pituitary-ovarian axis. AI after estrus synchronization is the most practical useful method. Up-to-date, synchronized heats are more regular and a lower incidence of double ovulations is recorded compared to natural estrus [4]. Reproduction technique protocols have been established in buffalo to manipulate the estrous cycle and moreover, in certain cases, to control the timing of ovulation [7-9]. The synchronization success depends mostly by environmental and management factors, reproductive disorders or diseases, interval between calving and therapeutic protocol and body condition.

The aim of the present study is to illustrate the effects of oestrus synchronization and fixed-time artificial insemination in dairy buffaloes.

2. Materials and methods

Animal management

The study was conducted at the Research and Development Station for Buffalo, Șercaia (GPS: 45°50′N 25°8′E, altitude of site 445 m) during 2020, on 30 buffaloes with good body condition and in different physiological conditions, raised under identical conditions. Following the gynecological investigation based on the transrectal and ultrasound examination in each animal, buffaloes with reproductive disorders were identified. The animals were divided into 3 experimental groups (Table 1) aged between 11 and 13 years. The following disorders of the reproductive cycle were highlighted: active anestrus (caused by the presence of the corpus luteum), buffaloes with more than 2 months after calving, buffaloes which do not have sowing or mounting having a corpus luteum in the ovary, or have more than 3 months after the last sowing and are non-pregnant, passive anestrus (with ovary without yellow body), buffaloes that did not show heat signs and buffaloes programmed for gestational diagnosis.

During winter season buffalo cows received a daily ration consisting out of 25 kg corn silage and 8 kg of hill pasture hay, with no supplementation of concentrates. Animals were kept under tied stanchion barn conditions, on deep straw litter beds, with access to mineral and salt blocks and also ad libitum water.

All research activities were performed in accordance with the European Union’s Directive for animal experimentation (Directive 2010/63/UE).

Table 1. Data on experimental batches and applied therapeutic protocols

Experimental group	Buffalo cows (n)	Total days of reproductive inactivity at the start of the therapeutic protocol (days)	Average age (years)	Therapeutic protocol
Experimental group 1	10	481.6	11.9	- day 1 - GnRH (synthetic gonadotropin); - day 7 - prostaglandin administration; - day 9 - GnRH (synthetic gonadotropin) - day 9-10 - AI (16-24 hours after the second administration of GnRH or when observing the heat signs if they appear earlier).
Experimental group 2	10	226.2	7.3	- day 1 - GnRH (synthetic gonadotropin); - day 7 - prostaglandin administration; - day 9 - GnRH (synthetic gonadotropin) at the same time the AI is realized (48 hours after the administration of prostaglandin).
Experimental group 3	10	188.1	13.2	- day 1 - prostaglandin administration; - day 12 - GnRH (synthetic gonadotropin); - day 19 - prostaglandin administration; - day 21 - GnRH (synthetic gonadotropin); -within 17-24 hours of GnRH administration - AI

[1]*GnRH – synthetic gonadotropin;* [2]*AI – artificial insemination*

Experimental design and therapeutic protocols

Experimental group 1 (n = 10) consisted of buffaloes that did not respond to the simple prostaglandin-based therapeutic protocol. For the estrus synchronization at a planned mounting date without the need for heat signs detection, the following therapeutic protocol was chosen:

- on the first day - administration of GnRH (synthetic gonadotropin);
- day 7 - prostaglandin administration;
- day 9 - administration of GnRH (synthetic gonadotropin);
- day 9-10 - artificial insemination (16-24 hours after the second administration of GnRH or when observing the heat if they appear earlier).

Doses administered:

- prostaglandin F2α (active substance alfaprostol - synthetic analogue of PGF2α) available on the market as the commercial veterinary medicinal product under the name of *Alfabedyl* - administered dose - 2.5 ml i.m./buffalo cow.
- GnRH - gonadotropin-releasing hormone (active substance gonadorelin - synthetic analogue of GnRH) available on the veterinary medicine market as a commercial product under the name of *Receptal* - administered dose - 2.5 ml i.m./buffalo cow.

Experimental group 2 (n=10) was set up after consulting the reproduction records. Following the evaluation of the data, it was found that 10 buffaloes showed anestrus after calving, anestrus after mounting (active and passive anestrus).

For the estrus synchronization at a planned mounting date without the need for heat detection, the following therapeutic protocol was chosen:

- on the first day - administration of GnRH (synthetic gonadotropin);
- day 7 - prostaglandin administration;
- day 9 - administration of GnRH (synthetic gonadotropin) at the same time the artificial insemination is done (48 hours after the administration of prostaglandin).

Doses administered:

- prostaglandin $F_{2\alpha}$ (active substance alfaprostol - synthetic analogue of $PGF_{2\alpha}$) available on the market under the name of *Alfabedyl* - administered dose - 2.5 ml i.m./ buffalo cow
- GnRH - gonadotropin-releasing hormone (active substance gonadorelin - synthetic analogue of GnRH) available on the veterinary medicine market as a commercial product under the name of *Receptal* - administered dose - 2.5 ml i.m./ buffalo cow.

Experimental group 3 (n=10) was set up after consulting the reproduction data. Following the evaluation of the data, it was found that these buffaloes (10 heads) showed anestrus after calving, anestrus after mounting (active and passive anestrus).

For the synchronization of estrus at a planned mounting date without the need for heat detection, the following therapeutic protocol was chosen:

- first day - administration of prostaglandin;
- day 12 - administration of GnRH (synthetic gonadotropin);
- day 19 - administration of prostaglandin;
- day 21 - administration of GnRH (synthetic gonadotropin);
- within 17-24 hours of GnRH administration - artificial insemination

Doses administered:

- prostaglandin $F_{2\alpha}$ (active substance alfaprostol - synthetic analogue of $PGF_{2\alpha}$) available on the market of the veterinary medicinal product as a commercial product under the name of *Alfabedyl* - administered dose - 2.5 ml i.m./buffalo cow.
- GnRH - gonadotropin-releasing hormone (active substance gonadorelin - GnRH synthesis analogue) available on the veterinary medicine market as a commercial product under the name of *Receptal* - administered dose - 2.5 ml i.m./buffalo cow.

3. Results and discussion

Of the 10 buffalo cows from the first experimental lot after the first treatment, 5 cows remained pregnant, the fecundity being 50.00%, of the 10 buffaloes with prolonged treatment from experimental lot 2, a number of 8 remained pregnant, the fecundity obtained being 70.00% and of the 10 buffaloes with treatment from experimental lot 3, 5 cows remained pregnant with the fecundity being 50.00%. In total, the fecundity obtained was 56.66% (Figure 1).

In accordance with our studies, with different applied protocol, respectively Ovsynch protocol were the results of De Rensis et al., 2005 [10]. Results concerning reproduction efficiency in Romanian Buffalo are in accordance with those previously reported by Georgescu et al. [11] and Vidu & Bota (2014) [12].

Following the diagnosis of gestation in buffaloes from experimental group 1, it resulted that the administration of GnRH determined at 5 ends the regression of the existing dominant follicle on the ovary, causing the growth of a new wave of follicles. Injection of $PGF_{2\alpha}$ 7 days later caused lysis (regression) of the corpus luteum, stimulating the growth of a new dominant follicle, which ovulated. Following the insemination, the 5 buffaloes became pregnant.

Following the diagnosis of gestation in buffaloes from experimental group 2, it turned out that the administration of GnRH determined at 8 ends the regression of the existing dominant follicle on the ovary, causing the growth of a new wave of follicles. Injection of $PGF_{2\alpha}$ 7 days later caused lysis of the corpus luteum, stimulating the growth of a new dominant follicle, which ovulated. The administration of the second dose of GnRH induced ovulation and after artificial insemination up to 64 hours after the injection of $PGF_{2\alpha}$, the 8 buffaloes remained pregnant.

Following the diagnosis of gestation in buffaloes from experimental group 3, it turned out that the administration of $PGF_{2\alpha}$ for luteolysis of the corpus luteum, 12 days before GnRH injection, caused regression at 5 ends of the existing dominant follicle on the ovary, causing a new wave of follicles. Administration of $PGF_{2\alpha}$ 19 days later caused lysis of the corpus luteum, stimulating the growth of a new dominant follicle, which ovulated. The administration of the second dose of GnRH (at 21 days) induced ovulation, and following artificial insemination up to 24 hours after GnRH injection, the 5 buffaloes became pregnant.

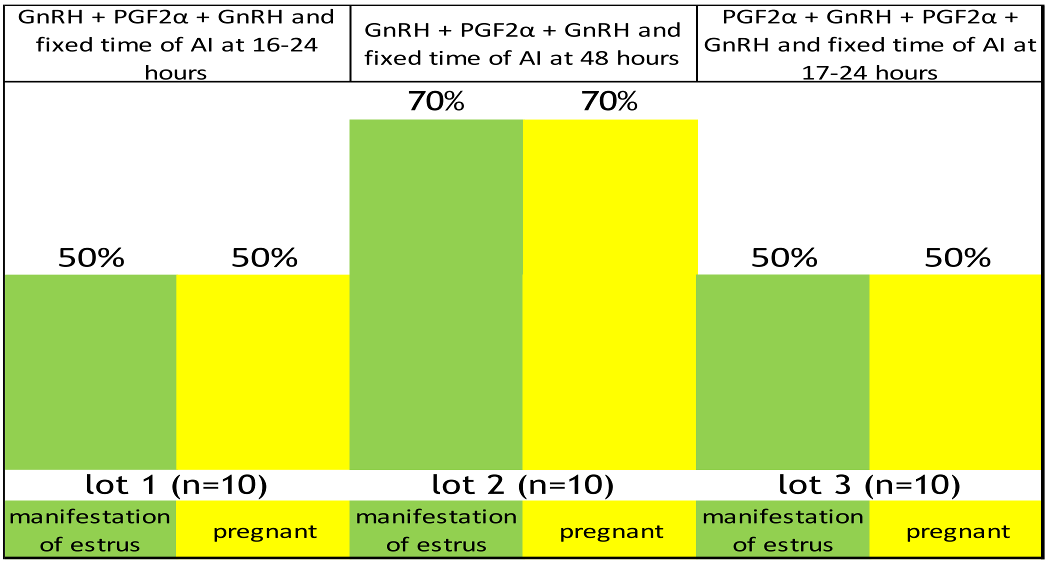

Figure 1. Graphical representation of reproduction indicators in the 5 batches of buffaloes after treatment with GnRH and PGF2α

Conception rates registered during this research trials are comparable with those previously reported by Gavojdian & Nicolae [13] for the Romanian buffalo breed, during normal reproduction season.

4. Conclusions

The hormonal protocols used in this study showed a satisfactory efficiency for the synchronization of estrus and the gestation rate in buffaloes. The application of the three protocols allows the concentration of calvings during the period of low or no milk production, which is an important improvement of business for farmers. The protocol using GnRH + $PGF_{2\alpha}$ + GnRH and fixed insemination time at 48 hours, results in a higher percentage of ovulation and higher gestation rates (70%). The use of products based on $PGF_{2\alpha}$ and GnRH helps to increase the percentage of pregnancies and, implicitly, the number of calves obtained, leading to an increase in milk production. The use of estrus synchronization techniques in buffaloes can overcome some of the difficulties of detecting estrus and increase the efficiency of artificial inseminations. When dealing with dairy species, reproduction is the main constraint in the onset and maintenance of lactation.

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