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Article

The new method for increasing the genetic potential in a nucleus of Romanian Buffaloes, by unicornual artificial insemination with sexed semen, after stimulation with OvSynch protocol

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Abstract: Although artificial insemination can mean a slow and progressive improvement of animal genetics, in buffalo-cow its practical application is difficult the results are incomparably lower than cattle. This article develops a new method, a well-known technique to make AI biotechnology more applicable to the buffalo, in order to improve the conception rate. The protocol we follow is to stimulate hormonal ovarian activity, inducing the dominant follicle, causing ovulation, and scheduling the moment of sexed artificial insemination deep in the uterine horn only ipsilateral to the ovary that will ovulate. The experiment was performed on 40 primiparous buffaloes-cows in two groups for AI separated by two bulls with 2 Millions female sperm straws. The groups were compiled after a thorough gynecological examination, and subsequently, the OvSynch therapeutic protocol was started. The results were 82,5% of buffaloes (33/40) had dominant follicle (DF) and inseminated, on hot/cold seasons the distribution was 75%vs90%. The conception rate was 63,6% (21/33), on hot/cold seasons 60%vs66,6%, and after calving 92.5% (20/21) female cattle were obtained. Thus, by implementing the UcFTAI protocol, we state that the goal of increasing the genetic potential of CIB becomes achievable and can be extended to a larger scale. Our Improved Protocol (UcFTAI) aims to reduce waste and maximize OvSynch hormone therapy.

Keywords: hormones, Carpathian Indigenous Buffalo, FTAI, sexed semen, OvSynch

1. Introduction

Genetic input and obtaining individuals with superior production quality [29], can be done within several generations by known breeding protocols, classic using for breeding by animals only animals with validated productivity. Because the buffalo population is small and the production value is low, it is necessary to use modern and current reproductive biotechnologies, such as artificial insemination (AI), as a measure of improvement and genetic progress in the buffalo herd. The methods and techniques are and must be in continuous development, and only those that are suitable should be used, they are applicable and specific for the farmer, species, race [10, 20, 26, 31].

The premier problem affecting the productivity function of buffaloes is the long calving interval due to delayed postpartum estrus and delayed puberty [22].

Poor endocrine status of buffaloes [32], and lower number of primordial an evolutins follicles on the ovary [13, 36], the high incidence of quiet estrus, weak palpable ovarian structures on transrectal examination [25], seasonality of reproduction and poor fertility [18], the high incidence of the follicular atresia in ovary structures[30] and a fewer number of antral follicles at all stages of the sexual cycle [23.], are some of the limiting factors which probably resulted in moderate responses of buffalo-cows to reproduction biotechnology [34].

Major methods for diagnosing estrus include sign-based methods, the manual method, the bull method, the chin marker, the dyeing of the tail, the analysis of reproductive records, the use of devices or laboratory diagnosis. [19, 22].

Moreover, the small number of bulls tested compared to cattle and the low conception rates obtained in AI programs discouraged buffalo farmers from adopting this technique. For these reasons, the genetic improvement of buffalo species is slow. The first step in breeding and selection activities for dairy animals is the daily recording of milk production and productivity of each animal. This step, together with the testing of offspring performed on young bulls, to select sires to be used in AI breeding programs, are considered important means to obtain the genetic improvement of a species. [1].

Although artificial insemination (AI) can mean a slow and progressive improvement of animal genetics, in buffalo-cow its practical application is difficult the results are incomparably lower then cattle [8, 11]. Although reproduction management protocols are similar to those of cows. It has limited application due to the low visibility of estrus and its poor detection in humans, the variable duration of estrus, and the difficulty in predicting the time of ovulation [9].

As the number of local buffaloes in Romania is decreasing in recent years, the application of current reproductive biotechnologies to these breeds is limited [8]. However, this AI is used occasionally in private small farms. In Italy, the country consecrated with tradition, in the production of milk buffaloes, AI has the most extensive use, but a large part of farms still using mount bull, and combined AI and natural breed [18].

Using hormonal protocols associated with FTAI (scheduled artificial insemination) makes buffalo breeding more helpful and practical, especially during the season with sexual inactivity or reduced sexual activity [2, 3].

Some veterinary drugs such as progestagens, gonadotropin-releasing hormone (GnRH), prostaglandin F2alpha (PGF2α) have been used to control estrus [12], in buffaloes with variable, inconsistent and inconclusive results [4, 7].

Early studies on synchronization of time of estrus in buffalo-cows were based on protocols developed for cattle, aimed at either inducing premature luteolysis using prostaglandins or prolonging the lutheal phase using progestagens [33]. Pursley et al., [35] he was among the first to use a protocol for estrus synchronization and ovulation in cows with fixed-time insemination. In cattle, it has been shown that synchronization of a follicular waves and subsequent estrus can be achieved by the association of too drugs GnRH and prostaglandin. The high LH release induced by GnRH injection causes ovulation or luteinization of the follicle or dominant follicles (DF) leading to the emergence of a new follicular wave. The subsequent injection of prostaglandin (PGF) produces the regression of the CL. The low progesterone environment stimulates the development of the newly formed dominant follicles DF and estrus and ovulation to occur 2-3 days, after prostaglandin injection [6, 17].

Subsequently, the model was adapted and tested on river buffaloes in different seasons [5, 15] and also implemented to a limited extent in swamp buffaloes [24].

Using Ovsynch protocols of stimulations during the breeding season with FTAI at 16-20h after the second GnRH injection, obtained an acceptable CR (35%-48%-56%) in buffaloes although lower conception rates (CR) have also been reported [1]. The difference appears to depend on the stage of the eastern cycle at the beginning of treatment, as the degree of synchronization after Ovsynch in cyclic animals could be improved by initiating treatment in the presence of a DF [15] and ultrasound monitoring of follicular progression.

However, there are few studies on deep unicornual insemination, ipsilateral to the ovary diagnosed by preovulatory follicle ultrasound [8]. Hormonal treatments like OvSynch combined with monitoring of follicular growth and dehiscence the calculation of unicornual AI time is described.

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2. Results

Thus, by implementing UcFTAI modified protocol was observed in both the cold and warm seasons, and encouraging ovarian appropriate reaction of the 40 buffaloes in our study. Before insemination, 33/40 buffaloes (82.5%) were diagnosed by ultrasound, which had on one of the ovaries a new dominant follicle of at least 0.9 cm in diameter. This FD is an anticipated reaction of the applied treatment and only they will be able to release a fertilizable oocytes. We note that 82.5% of females had a complete response and consider them as a positive reaction to the induction of estrus with / or without behavioral clinical signs.

These females were inseminated with semen from the two buffalo bulls, 85% of Oro's group and 80% of Aton's (Table no1). On the distribution of the seasons, the dominant follicle was detected in 75% (15/20) of the buffaloes in the warm season and 90% (18/20) in the (cold) breeding season.

The total conception rate was 63,6% (21/33). The distribution between the two seasons seems to be similar hot/cold seasons 60% (5/19) vs 66,6%.(12/18), the influence seems to have had the conditions of maintenance and comfort provided to counteract the effect of thermal stress and ovarian inhibition characteristic of buffalo.

By categories of bulls, the percentages were also similar 60% in Oro (9/15) and 66,6% in Aton (12/18). Only in the summer season, they observed a difference in fertility between bulls, 71.7% (5/7) in Aton's group and 50% (4/8) in Oro.

Regarding the sex ratio, after calving 92.5% (20/21) female cattle were obtained, only in Aton's group, only one male was born.

3. Discussion

Proper buffalo breeding and season require the use of advanced breeding technologies in this species [8], be able to get a calf regularly at 13-14 months. To maintain this interval between calvings in buffaloes, insemination must take place in the interval of 85 to 115 days after calving. Complete restoration of the uterus, resumption of ovarian activity, and the onset of heat usually take place around 20-50 days postpartum; therefore, there is a 35-95 day window for a buffalo to remain pregnant to maintain the desired interval between calvings.

The reproductive activity of buffaloes is strongly influenced by environmental cues, nutrition, photoperiod. The buffalo is considered a seasonal breeder, as most buffaloes are cyclical only in the colder months of the year. High temperatures and long days reduce cyclicity and lead to suppression of ovarian function. High prolactin (PRL) secretion has been identified as a contributing factor to reduced acyclicity and fertility by decreasing progesterone secretion in the summer months.

The seasonal effect on reproductive function is governed by the pineal gland that secretes melatonin, which in turn influences the circadian rhythm and alerts the biological function of hormones involved in regulating reproductive function. Other factors that influence estrus behavior are genetic predisposition, age, uterine inflammation, calving time and their relationships.

The seasonal buffalo anestrous in the countries north of the equator has been attributed either to thermal stress (India, Pakistan, Egypt), low environmental humidity (Venezuela), or photoperiodicity (Italy and European Countries). In Romania, the breeding season at the Romanian Indigenous Buffalo is considered during the cold season (autumn-winter) [8].

We know that artificial insemination (AI) is a current and promising biotechnical method in animal husbandry, a method that intensifies the reproduction and production of animals. Artificial insemination must be understood in the context of the continuous evolution of smart technologies of biological sciences. Genetic improvement is a dynamic

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process that must evolve over time, supporting and responding to the needs of breeders, the market, and the local context in which companies operate.

Recent research such as the development of protocols for synchronizing ovulation and programmed artificial insemination in buffaloes has been used to overcome these constraints and to be able to use large-scale artificial insemination on farms. These aspects were the basis for the development of this protocol of ours.

Ovulation induced in heifers following the GnRH - PGF2α protocol can be anticipated within approximately 28 hours after administration of the second dose of GnRH, while spontaneous ovulation can be anticipated within approximately 31 hours after estrus onset or approximately 19 hours after insemination [8, 11]. In this regard, Dolezel et al., [16] recommend the administration of GnRH at the end of the growth phase or at the beginning of the static phase of the dominant follicle, when GnRH will be administered within 72, 48, or 24 hours after PGF2α, in cows with small, medium or large follicles in the time of initial treatment.

The results got in this study are superior to other publications referring only to the OvSynch protocol reported a conception rate: of 56.5% during the mating season [14], of 56.7% in multiparous females [37], lor 48.8% during the mating season, and 6.9% during the non-mating season [3]. Other results vary between 36.0% and 42.55% if used in breeding season [28].

Extreme ambient temperatures (too hot or too cold) increase the duration of the sexual cycle and shorten the duration of estrus by decreasing feed intake. Decreased thyroid activity may indirectly influence reproductive efficiency. The susceptibility of buffaloes to thermal stress generated by high temperatures is due to the small number of sweat glands per unit area, which prevents thermoregulation [27]. In our study, we observe uniformity of CR results between the hot and cold seasons, and because the maintenance and comfort conditions were optimal, heat and frost were artificially avoided.

Our Improved Protocol (UcFTAI) aims to reduce waste and maximize OvSynch hormone therapy.

Table 1. Hormonal treatments to control estrus and ovulation in order to apply UcFTAI in Romanian Buffaloes (CIB). Use of the Ovsynch Protocol (GnRH + PGF2α + GnRH) and conception rate (CR) after use of sexed and genomically semen

Bufflo-Bull semen (S.&G.)	CIB OvSynch		Uc FTAI		Conception Rate (C R)		Calving
	Hot season	Cold season	Hot season	Cold season	Hot season	Cold season	female fetus
Oro (n-20)	10/20 (50%)	10/20 (50%)	8/10 (80%)	9/10 (90%)	4/8 (50%)	6/9 (66,6%)	10/10 (100%)
	20/20 (100%)		17/20 (85%)		10/17 (58,8%)		
Aton (n -20)	10/20 (50%)	10/20 (50%)	7/10 (70%)	9/10 (90%)	5/7 (71,1%)	6/9 (66,6%)	10/11 (90,9%)
	20/20 (100%)		16/20 (80%)		11/16 (68,7%)		
total	20/40 (50%)	20/40 (50%)	15/20 (75%)	18/20 (90%)	9/15 (60%)	12/18 (66,6%)	20/21 (95,2%)
	40/40 (100%)		**33/40 (82,5%)**		**21/33 (63,6%)**		

*** S.&G. - Sexed and Genomically tested Bufflo-Bull semen, CIB – Carpathian Indigenous Bufflo / Romanian Buffalo, OvSynch -Protocol for increase the follicles and ovulation, Uc FTAI- UniCornual Fixed Times for Artificial Insemination, CR – Conception Rate**

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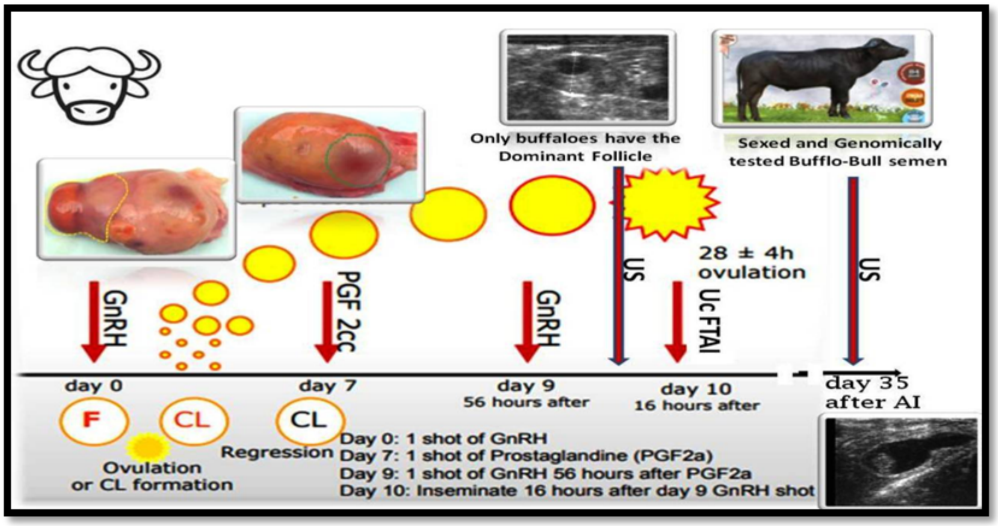

Figure no 1 Scheme of the UcFTAI method with semen sexed according to OvSynch protocol in buffalo

4. Materials and Methods

The research in this study was organized in one of the few buffalo farms in Romania from Eastern Europe, which has a sufficiently large number of animals for such a study and which creates constant optimal conditions regardless of the season. As the number of buffaloes in Romania is decreasing in last decades, and it was difficult to form homogeneous buffalo clays from the local variety.

4.1. Buffalo-cow biological material

The experiment was performed on a number of 40 primiparous buffalo-cows Carpathian Indigenous Buffalo (CIB) variety, similar to traditional Romanian Buffalo, belonging to the Mediterranean breed [8] and through this study we wanted to form a nucleus of females with high genetic potential.

Young calves buffaloes were selected by age (more than 22 months), weight (more than 250 kg), and gynecological. The average body score was 3, the females had a completely. From the point of view of sexual cyclicity, only cattle that had passed the onset of puberty and had active physiological formations on the ovaries were selected. The ovaries showed no signs of pathology, and their average size was 2 cm.

The group (n-40) was organized after a thorough gynecological and general examination (see figure no 1), in two experimental lots. To be homogeneous, the group was made up / divided of cattle suitable for the breeding sea (n-20) son and the off-season (n-20) (throughout the year). Locally the breeding season is in the cold months (cold season).

4.2. Buffalo-Bull semen

Two batches of semen were ordered from two validated buffalo bulls to be used for the insemination of cows. Two lots of 20 cows were created for each. This lot was intended for both climate seasons each, 10 buffalo-cows. In order to obtain a higher number of females with genetic value, parturition stems of semen sexed from two buffaloes genomically tested from Associatzione Nazionale Allevatori Specie Bufalina (Italy): Oro Mediterraneo and Aton del Parco. The semen was purchased, transported, and stored in optimal conditions by a local importer, with 2 Millions female sperm straws. The semen sees the following characteristics: **Oro Mediterraneo** IT059990127603, Fsexed-yes, genotyped-yes, Morphological value - 84p, PKM-98.22 and MY-426.42 and **Anton del Parco** IT071990106584, Fsexed-yes, genotyped-yes, Morphological value - 82p, PKM- 52,58 and MY-167.

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4.3. Ultrasound monitoring

The ovarian response of buffalo-cows to follicular stimulation treatment was monitored by transrectal ultrasonography (Honda HS-1600V®, Japan ultrasound scanner equipped with 5 -7,5 MHz Dopler transducer). Genital ultrasonography per rectum was used in three essential moments: before compiling the lots of cattle to exclude those with uterine/ovarian pathology and to retain the cyclical ones; A few hours before the time set by the AI to select only calves that have DF on the ovary, 35 days after AI, for the diagnosis of pregnancy and the calculation of the conception rate (figure 1).

4.4. Hormone treatment management

Ovulation synchronization can be produced using the hormonal cascade GnRH - prostaglandin after 7 days - GnRH after 48 hours. This system synchronizes follicular maturation with CL regression before GnRH-induced ovulation and fixed-date insemination.

The groups were compiled after a thorough gynecological and general examination, and subsequently, the Ovsynch therapeutic protocol (Gn-RH, PGF, Gn-RH) was started. According to the protocol, the females received on day 0 and 9, 0.01mg buserelin acetate (Receptal®, MDS-Intervet, Holland) and PGF received on the fifth day, cloprostenol 500 µg.IM (Estrumate®, MDS, Holland).

4.5. Artificial insemination and time management (Uc FTAI)

We use the OvSynch protocol, similar to that of cattle, and has the action of synchronizing estrus after injection with GNRH (day 0) and PGf2a (day 7), and synchronizing ovulation after the second injection with GnRH (day 9), according to Pursley et al. (1995), [35].

To prevent waste, they were AI only buffaloes that were at least interested in the bull, and that had a dominant follicle (DF) on the ovary (at least .9 mm). The AI method was tactile recto cervical, females were inseminated once at 18 hours after the second Gn-RH injection (FT-fixed times) with the deposition of sexed sperm only in the congenerous uterine horn of the ovary carrying the dominant follicle (deep Uc- nicornual).

The seeding technique used was the classical Anglo-Saxon method, by locating the transrectal cervix and its bimanual passage with the Cassou pistol. Pelvic, cervical, and uterine anatomical features of the buffalo were taken into account. A plastic protective shirt was also used to prevent bacterial contamination of the pipette.

4.6. Care management

All conditions of biosecurity, nutrition, well-being, maintenance, vaccination, and deworming were met. From the point of view of accommodation, the animals had thermal comfort preventing heatwaves in summer and frost in winter. So that the reproduction activity is continuous, the well-known seasonality should not be installed.

5. Conclusions

The results can prove it. Our Improved Protocol (Uc FTAI) aims to reduce waste and maximize OvSynch hormone therapy. The efficiency of the ovarian response would come from the careful gynecological selection of females, the preferential use of primiparous, and the bio-stimulation with bulls. Ultrasound identification of the ovary will prevent the waste of sexed and possibly genotyped seminal material (not cheap at all) with the dominant preovulatory follicle. Ultrasonography is done with a few hours of FTAI, and this is done only in buffaloes with FD and deep unicornual, ipsilateral ovary that will ovulate.

Thus, by implementing UcFTAI modified protocol, we state that the goal of increasing the genetic potential of CIB by becomes achievable and can be extended to a larger scale.

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