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Estrus Synchronization in Swamp Buffaloes

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Swamp buffaloes are distinctly of Chinese origin. There are 18 known breeds/strains in China while Indonesia has identified seven breeds/strains. Among the breeds of Indonesia, the spotted swamp buffalo is more unique and is largely raised for socio-religious purposes. The Swamp buffalo population in Indonesia is about 2,191,640 heads or 1.21% of the world's buffalo population [1]. Their productivity tends to decrease from year to year. Data published by the Department of Animal Husbandry and Health (DAHH), Riau Province (2011) showed that although there was an increase in the number of buffaloes, it was not significant. It is noted that in 2007 the population of buffaloes in the Riau Province was 56,309 heads and decreased to 51,697 heads in 2010. Conversely, cattle population in Kampar regency of Riau Province increased slightly from 22,548 in 2007 to 24,785 in 2010. The total buffalo population in Kampar regency is 43.5 % of the total population of buffaloes in the Riau Province. Based on data issued by Riau Livestock Services, within the last three years, the buffalo population in Riau decreased at a rate of 0.53%. The decrease in buffalo population was due to an increase of up to 0.17 % in the number of buffaloes slaughtered.

The main problem affecting the productivity of buffaloes is the long calving interval due to delayed postpartum estrus and delayed puberty [2]. This is due to the difficulty in detecting estrus in this species on account of poor signs of estrus (silent heat, quiet ovulation or subestrus). This is often mistaken for infertility in swamp buffaloes [3,4]. As a result, farmers do not know if their buffaloes are in estrus and therefore cannot be mated at the right time [5]. These limitations are exacerbated during the hot season, when fertility decreases dramatically [6,7]. Only 20% of Philippine Carabaos were diagnosed pregnant by rectal palpation, and farmers deliberately postpone breeding for working females thus further reducing breeding and pregnancies [8]. On the other hand, the number of bulls tended to decrease from year to year because many buffalo bulls were slaughtered as sacrifice animals when Muslims celebrate their pilgrimage [9]. Moreover, swamp buffalo bulls are known to have extremely low sperm concentration [10]. In addition, the fertility in swamp buffalo herds is low (average 30%-40%) [11]. Thus increasing the swamp buffalo population through natural mating appears difficult. One way to increase the buffalo population is to promote artificial insemination (AI) [12]. Implementation of AI requires proper detection of estrus, which can be difficult in swamp buffaloes due to poor estrus expression [11]. During the last couple of years, estrus synchronization by the hormone administration has emerged as a valid alternative to reduce labor costs, plan breedings and time the insemination in cattle [13]. The same approaches have been widely used in river [6,14,15] and swamp buffaloes [16-18]. The use of estrus synchronization techniques may overcome some of the difficulties of estrus detection and increase the efficiency of AI in swamp buffaloes.

Progestagens, gonadotropin releasing hormone (GnRH), prostaglandin F2alpha (PGF2α) and/or their different synthetic analogues have been used for estrus control in buffaloes with variable results [15,19]. Early studies on synchronization of time of estrus in buffaloes were based on protocols developed for cattle, aimed at either inducing premature luteolysis using prostaglandins or prolonging the luteal phase using progestagens [20]. Pursley et al., [21] were the first to use a protocol for estrus synchronization and ovulation in cows with fixed-time insemination. Thereafter, the model was adapted and tested in river buffaloes during different seasons [22-25] and also adopted to a limited extent in swamp buffaloes [26,26].

The applications of the Ovsynch protocol, a sequence of GnRH, PGF2α, and GnRH treatments for synchronized ovulation in lactating dairy cows, had been reported successful, resulting in fertility to timed AI (TAI) that was similar to that of cows inseminated after detection of estrus [27]. The Ovsynch programs have been employed with excellent results in bovine and buffalo cows [22,28]. Work on estrus synchronization in buffaloes had been conducted in three different regions using the Ovsynch protocol, namely: half-bred (Murrah-Mediterranean) buffaloes, Mediterranean buffaloes [29] and Egyptian buffaloes [30]. Data on estrus synchronization in swamp buffaloes are few and unorganized. In this chapter the available data on estrus synchronization in swamp buffaloes is mentioned.

1. Basis of Estrus Synchronization

There are two basic methods of estrus cycle synchronization in farm animals. These methods depend on either inhibiting LH secretion and ovulation or shortening the life span of the corpus luteum (CL) with the subsequent onset of estrus and ovulation [31]. More precise mechanisms of synchronization use agents such as GnRH to alter the dominant follicle and the CL in order to initiate a new follicular wave and estrus [31]. The reproductive physiology of the swamp buffalo females and males has been summarized in a previous chapter [32]. Swamp buffalo females have a smaller number of primordial follicles on their ovaries compared to river buffaloes and overall a poor reproductive function [32]. It is thus expected that the results of estrus synchronization in swamp buffaloes would be poor compared to river buffaloes.

Table 1. Estrus synchronization using prostaglandins, progestagens and combinations in swamp buffaloes in different studies.

Country	Drug Used	Estrus Rates	Conception Rates	Pregnancy/Calving Rates	Ref.
China	PG a single dose	79.10% 64.18% 43.33% 58.33% 50.0%	33.21% 41.68% - - -	- - - 54.17% -	[26] [47] [48] [49] [50]
China	PG two doses 11 days apart	-	-	50%	[58]
China	PG+CIDR CIDR	86.13% 78.02%	46.03% 43.66%	- -	[47]
China	PG+PMSG	84.21% 73.01% 84.5%	43.48% 45.6%	50% -	[49] [47] [51]
China	PRID+PMSG	-	-	47.16%	[59]
Thailand	PG two doses 11-12 days apart	- -	21.7%-81.2% 27.5%	21.7% - 63.6%	[52-54] [55]
Thailand	PRID PRID +PMSG 500 IU	- -	47.0% 6.3%-37.2%	- -	[55] [46]
Thailand	Norgestomet Norgestomet + PMSG	36% 45.5%	- -	30.77% 39.13%	[43]
Vietnam	CIDR CIDR+Fertagyl CIDR+PG	57.5% 75% 75%	- - -	56.52% 55.17% 40%	[42]
Philippines	PG two doses 11 days apart	-	-	31.41%-52.68%	[56]
Indonesia	PG two doses 11 days apart	100%	-	83.87%	[57]
Indonesia	PG two doses + 500 IU hCG	81.3%-100%	-	50%-86.6%	[60]

2. Extending the Luteal Phase

The first method involves long-term administration of low doses of progestogen so that the CL regresses naturally during the period when progestogen is being withdrawn. Continued progestins induce a new follicular wave and promote the atresia of follicles already present [33]. On progestagen withdrawal, follicular growth, estrus and ovulation occur within 2 to 8 days. The interval from withdrawal of progestogen to the onset of estrus varies among species and between methods of progestagen treatment which lasts for 14 to 21 days, depending on the species. Several methods for progestogen administration are commercially available. These include orally active progestagens, pessaries, ear implants, and intravaginal devices. The combination of progesterone/progestin and estradiol at the beginning of the protocol is effective in inducing the emergence of a new follicular wave due to suppression of both FSH and LH which promote the atresia of all follicles present on the ovaries [33].

Many previous reports on river buffaloes [34-38] depicted the efficacy of progesterone treatments on estrus synchronization and timed inseminations [39] with conception rates ranging from 14% to 60%. The addition of eCG at the time of progesterone withdrawal have been suggested to improve the overall conception rates due to a better growth of the ovulatory follicle [37,38] particularly during the low breeding season [40,41].

Improvement in estrus synchrony and pregnancy rates was observed in Vietnamese swamp buffaloes treated with CIDR + Fertagyl [42] and Thai swamp buffaloes treated with norgestomet + PMSG [43-45] or PRID +PMSG [46] (Table 1). Administration of low levels of a progestin (such as melengesterol acetate) in the absence of a corpus luteum can result in the formation of persistent follicles and poor subsequent fertility in cows [31], however, feeding oral progestins is not reported for river or swamp buffaloes.

3. Shortening the Luteal Phase

The second method involves the premature regression of the CL (luteolysis). The two primary luteolytic agents are estrogen and prostaglandin F2α, or its analogues (for example cloprostenol). Estrogen is luteolytic in ruminants at least during certain phases of CL development. However, due to the potential adverse effects on milk yield in buffaloes, estrogen is not suggested as a luteolytic agent in lactating buffaloes [61]. Estradiol has been used in combination with ovsynch protocol in buffalo [62]. Seventy percent of buffaloes treated with estradiol after an ovsynch treatment (GnRH + PG + GnRH) displayed estrus compared to 40% of the buffaloes which were not treated with estradiol. Similar to cows and river buffaloes [63], a single injection of PGF2α to swamp buffaloes administered after palpation of a CL on day 5 of natural estrus resulted in a decline in plasma progesterone and lysis of the CL within 24 h [64] and buffaloes showed estrus within 48-72h and estrus lasted for 4-5 days. Plasma progesterone levels reflected age-dependent changes in cyclic swamp buffaloes [65]. Estrus and ovulation can also be synchronized in cyclic animals through a combination of a progestogen and a luteolytic agent. This approach uses a luteolytic agent to regress the CL and the progestagen to mimic the action of progesterone and prevent estrus until the withdrawal of the progestagen source. Several previous reports reported on the use of prostaglandins alone [36,63,66-70] or in combination with other hormones for estrus synchronization in river buffaloes.

Single or double dose injections of prostaglandins alone (11-13 days apart) [26,48-50,52,53,56], or in combination with hCG [59] or PMSG [47,49] have been used for estrus synchronization in buffaloes from China, Thailand, the Philippines and Indonesia with estrus rates varying from 43.3% and pregnancy rates varying from 21.7% to 83.87% (Table 1).

4. Estrus Synchronization Using GnRH

GnRH and prostaglandins have been used in combination for estrus synchronization. Studies by Pursley et al., [21] confirmed that the administration of GnRH after PGF2α injection increases the rate of synchronized ovulations in bovines. The gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2α) method for estrus synchronization has proven to be very successful in synchronizing estrus in cattle and buffaloes [25,71-73].

Similar to the natural release of GnRH at onset of standing estrus, an injection of GnRH (100 μg) causes an LH surge that induces ovulation or luteinizes the largest follicle(s) present in the ovaries. Cows then start a new follicular wave one to two days later. When GnRH is followed by a PGF2α injection seven days later, most cows will have a mature dominant follicle of similar size at CL regression, resulting in a more synchronous heat response [74-77]. Additionally, the GnRH induced luteinization of dominant follicles will stimulate cyclicity in many anestrus cows [78]. Similar changes also probably occur in buffaloes [3,15].

Negila et al., [24] observed a pregnancy rate of 45% in Italian buffalo cows synchronized with PGF2α alone and 48.8% when PGF2α was combined with GnRH at the time of AI. In another study, the pregnancy rate in cyclic buffaloes synchronized with Ovsynch was 36% compared with 28.2% of buffaloes synchronized with PRID [23]. Prostaglandin induces a premature regression of the CL, as a consequence circulating progesterone concentration decreases allowing a sequence of hormonal and ovarian events that culminate in estrus and ovulation. The pregnancy rate achieved in relation to the use of different protocols with fixed-time artificial insemination in river buffaloes ranged between 30-50% [12]. The use of Ovsynch protocol (GnRH followed by prostaglandin 7 days later and a second dose of GnRH 2 days later) has been documented for estrus synchronization of river buffaloes in several studies [23,29,79-83]. The synchronization of time of ovulation was 70–90% and the conception rate was 33–60% [3,28,30].

Many variations to the Ovsynch protocol for estrus synchronization have been used in cattle [71,76] and river buffaloes [84,85] in an effort to better control the onset of estrus and timed inseminations [14,86]. However, conception rates were low during the low breeding season in river buffaloes [35,83,87,88] compared to those obtained during the peak breeding season.

In Thai, Chinese and Indonesian swamp buffaloes the use of Ovsynch protocols resulted in estrus and conception rates of 83.3%-100% and pregnancy rates of 32.7% to 100% (Table 2). Few studies reported on the use of Select Synch protocol (GnRH on Day 0 + PG on Day 7 followed by AI at detected estrus) in Indonesian buffaloes [27,57,89] with high conception rates. Select Synch protocol in swamp buffaloes using different doses of GnRH induced estrus in all buffaloes at 27.8 h after administration of PG with 100% pregnancy rates at the doses of 300 mg of GnRH [90].

Ovulation rates in Thai swamp buffaloes were high (83.3%) when an Ovsynch protocol was preceded by a Pre-Synch protocol [91]. Timed inseminations 12-24 h subsequent to an Ovsynch [18] or 16h after a progestagen treatment [42] have been mentioned to a limited extent for swamp buffaloes.

A limited number of studies in swamp buffaloes report on the combination of different estrus synchronization protocols such as progestagens +PG [42], PG+gonadotrophins [47,49,51,59], progestagens +gonadotrophins [43,44,58] and progestagens+Ovsynch [92] with improvement in estrus and pregnancy rates compared to when each of the agents was used alone.

5. Factors Affecting Synchronization

Estrus synchronization programs improve reproduction efficiency by reducing the length of breeding and calving seasons and by increasing the weight of calves at weaning. Artificial insemination can also be used more efficiently. Environmental factors cause seasonal anestrus in buffaloes by affecting ovarian and hypophyseal hormonal secretion and lead to lower peaks of luteinizing hormone (LH) and variable progesterone levels [32]. In order to overcome the seasonality of breeding, a synchronization protocol should initiate follicular development by activating the hypothalamo-pituitary-ovarian axis.

Synchronization success depends on environmental and managerial factors, reproductive disorders or disease and the interval between calving and treatment [6,15,75]. Reproductive function is a major factor determining the economic importance of buffaloes [94]. The reproductive status is mainly influenced by the age of sexual maturity onset [95,96], stage of the estrous cycle [97,98], the prolonged interval between calvings and the weak ovarian activity during the hot months of the year [6,99,100]. Estrus synchronization yielded better results in adult swamp buffaloes compared to buffalo heifers [101,102] and lower synchrony was observed in swamp compared to river buffaloes [26]. The presence or absence of a CL before a timed insemination affects the outcome [103].

In addition to the type of protocol selected, the following factors must also be addressed when using any regimen in buffaloes: (a) selection of animals that are in good body condition score and free from disease; (b) minimize stress during treatment administration and AI, especially under tropical conditions, when animals may be herded together, tethered or moved to other locations; and (c) where seasonal differences exist, scheduling treatments for the more favorable periods or during the peak of the breeding season when the majority of animals are likely to have regular estrous cycles.

Table 2. Estrus synchronization with Ovsynch in swamp buffaloes in different studies

Country	Type of Protocol	Estrus Rates	Ovulation Rates	Conception Rates	Pregnancy Rates	Ref.
China	Ov-Synch	88.46% 83.90%	- 75.42%	46.38%	- -	[26] [49]
Thailand	Ovsynch plus Fixed Time AI 12 and 24 h after 2nd GnRH	-	-	38.1%	32.7%	[18]
Thailand	Ovsynch	-	-	-	34.6%	[93]
Thailand	Pre Synch+ Ovsynch	83.3%	-	-	-	[91]
Indonesia	Select Synch	100%	-	-	100%	[27]
Indonesia	Ovsynch Select Synch	100% 100%	- -	- -	64.71% 77.14%	[57]

6. Procedures and Products Used in Synchronization of Estrus

Hormones common to many protocols are prostaglandin F2α (PGF2α), gonadotropin releasing hormone (GnRH) and progestins. They are available as the following commercial products:

GnRH: Cystorelin®, Factrel®, Fertagyl®, OvaCyst® - administered IM
PGF2α: EstroPLAN®, Estrumate®, In-Synch®, Lutalyse®, ProstaMate® - administered by IM injection
Progestagens: CIDR®, PRID®, Cue-Mate® - by intravaginal application

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