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Review

Strategies to overcome seasonal anestrus in water buffalo

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A B S T R A C T

Reproductive seasonality in buffalo (*Bubalus bubalis*) is characterized by behavioral, endocrine, and reproductive changes that occur over distinct periods of the year. During the nonbreeding season (spring and summer), the greater light-dark ratio (long days) suppresses estrus behavior and the occurrence of ovulation. Anestrous buffaloes have insufficient pulsatile of LH to support the final stages of follicular development, and subsequently, estrus behavior and ovulation do not occur, limiting reproductive efficiency, especially in artificial insemination (AI) programs. A number of therapeutic strategies designed to synchronize follicular wave emergence and ovulation have allowed for the use of AI throughout the year, overcoming seasonal anestrus in buffalo. These therapies also improve reproductive performance by increasing the service rate and pregnancy per AI in buffalo herds, regardless of reproductive seasonality.

1. Introduction

Buffalo (*Bubalus bubalis*) is a species that is characterized by seasonal reproductive activity. They become sexually active in response to a decreasing day length (short days) in late-summer to early autumn [1]. During the nonbreeding season, buffalo often exhibit anestrus, which extends the calving to conception interval and consequently, reduces reproductive performance [2]. This physiological characteristic adversely affects the buffalo dairy industry and without intervention, will result in seasonal calving and a dramatic variation on milk supply throughout the year [3].

To avoid this problem, it is necessary to implement management schemes to overcome reproductive seasonality, improving the milk production throughout the year [4]. Thus, hormonal therapies to induce estrus and ovulation in anestrous buffalo cows are important strategies to overcome seasonality. These hormonal treatments have been designed to control follicular and luteal function, synchronize estrus and ovulation and, more importantly, eliminate estrus detection by artificially inseminating cows on a preplanned schedule (timed artificial insemination [TAI]).

Among the hormonal therapies developed for cattle, GnRH plus PGF2α-based TAI protocols resulted in a reduced ovulatory response when applied in anestrous buffalo [5]. Also, during the nonbreeding season, when a high incidence of anestrus is expected, lower pregnancy rates are encountered in those cows synchronized with the Ovsynch protocol for TAI [6–11]. On the contrary, recent studies in buffalo have demonstrated similar pregnancy per TAI in both breeding and nonbreeding seasons after the use progesterone (P4), estradiol (E2), and equine chorionic gonadotropin (eCG)-based protocols [12,13]. Despite these results, i.e., similar pregnancy per TAI throughout the year, it is still not clear as to whether seasonality affects oocyte competence.

Thus, the present review aims to discuss a number of key points related to the potential impact of seasonality on the reproductive performance of buffaloes. The discussion

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will focus on (1) buffalo reproductive physiology during the nonbreeding season; (2) impact of seasonality on oocyte competence; and (3) hormonal control of follicular growth and ovulation to overcome seasonal anestrus in buffalo herds.

2. Buffalo reproductive physiology during the nonbreeding season

Domestic small ruminants and buffaloes are known as animals with marked seasonality of breeding activity. The annual cycle of daily photoperiod has been identified as the determinant factor of this phenomenon, whereas environmental temperature, nutritional status, social interactions, calving date, and lactation are considered to modulate reproductive seasonality [14]. Buffalo are classified as short-day breeders, becoming sexually active in response to decreasing day length in the late summer and early autumn. This physiological change is apparently orchestrated by melatonin release profiles [1]. Melatonin is produced and secreted during the night (dark). As days become shorter, exposure to melatonin increases and through a complex action on the hypothalamus-pituitary-gonadal axis simulates GnRH secretion by the hypothalamus. Thus, differences in day length are recognized and translated into signals that turn sexual activity on or off (reviewed by Di Francesco [15]).

Buffaloes during the nonbreeding season fail to exhibit estrus, as a result of an alteration in endocrine profiles leading to anovulatory or noncycling status [4]. Anestrous buffaloes have insufficient pulsatile LH to support the final stages of follicular development and the subsequent estrus behavior and ovulation [16]. In this context, cows calving during the nonbreeding season have an extended postpartum anestrous period with a significant proportion of females not resuming cyclic ovulations until the onset of the subsequent breeding season [2,3]. On the other hand, well-nourished buffalo raised in equatorial zones may display estrous cycles throughout the year and show little postpartum anestral activity [1,17–20].

3. Impact of seasonality on oocyte competence

Although high environmental temperatures have been shown to have a detrimental effect on the quantity, quality, and developmental competence of oocytes obtained from abattoir-derived buffalo ovaries [21], it is still not fully understood whether the seasonal photoperiod affects oocyte development competence. It has been suggested that cumulus oocyte complexes derived from follicles of buffalo females subjected to heat stress, nutritional stress, disease or other factors, such as reproductive seasonality might have oocytes with compromised competence [22]. In this regard, buffalo cows produced higher embryo yields in autumn compared to mid-winter and spring–summer [23], suggesting a possible seasonal effect on oocyte development competence. However, the morphologic features [23] and mitochondrial DNA analyses [24] of the oocytes did not vary substantially over these seasons. In addition, there was no effect of season on the percentage of oocytes that were suitable for *in vitro* embryo production, nor was there any effect on blastocyst production rate [25].

Studies conducted in both seasons in Brazil revealed no effect of season on the numbers of viable oocytes subjected to *in vitro* embryo production with an overall blastocyst rate of 44.9% (262/584) and pregnancy rates of 43.5% (50/115) and 37.1% (26/70) after fresh and vitrified embryo transfer, respectively, [26–28]. In addition, in a recent Brazilian study (unpublished data), conception rates did not differ in buffalos subjected to timed embryo transfer during the breeding and nonbreeding seasons (26.5%; 52/196 and 32.7%; 16/49, respectively). Similarly, no differences in pregnancy rates were observed when TAI was performed with the same batch of semen during the breeding and nonbreeding seasons [29]. Therefore, in tropical and subtropical climates, the long-day period would not appear to adversely affect oocyte quality and fertility. However, more studies need to be carried out on the oocyte competence during different seasons to confirm these findings.

4. Hormonal control of ovarian follicular growth and ovulation to overcome seasonal anestrus in buffalo herds

Studies designed to overcome the effects of the nonbreeding season involve the use of hormonal treatments that control both luteal and follicular function in buffalo, enabling the use of TAI during both the breeding and nonbreeding seasons [7,30–33]. Several studies have been conducted by our research group to evaluate the efficiency of the Ovsynch protocol (Day 0: GnRH; Day 7: PGF2α; and Day 9: GnRH; TAI 16 hours after the second GnRH injection; [34]) for TAI in buffalo [6,35–37]. In these experiments, cycling buffalo responded to hormonal treatments with approximately 50% pregnancy per TAI during the breeding season. Nevertheless, pregnancy rate was influenced by body condition score (high pregnancy per artificial insemination [AI] was achieved when body condition score $\geq$ 3.5; 1–5 scale), parity (primiparous animals had lower pregnancy rates than multiparous cows), and time of the year (higher pregnancy rate was observed during the breeding season than that in the nonbreeding season). Moreover, 40% to 60% pregnancy rates have been reported in other studies with the Ovsynch protocol in cycling buffalo synchronized during the breeding season [8,33,38].

However, when the Ovsynch protocol was used in anestrous buffalo (without CL), results were inferior to those obtained with cyclic buffalo. Souza et al. [5] verified that buffalo without a CL at the beginning of the protocol responded poorly to the first (42.0% vs. 89.8%) and second (52.0% vs. 87.8%) GnRH treatments, and this resulted in a lower pregnancy rate after TAI (20.0 vs. 65.3%, respectively) compared to the animals with a CL. Results of several other studies revealed a high incidence of anestrus during the nonbreeding season (spring and summer), and lower pregnancy rates after TAI were reported (7.0%–30.0%; [6–11]). Therefore, studies have been conducted in our laboratory to develop treatment protocols that would increase pregnancy rates in buffalo submitted to TAI during seasonal anestrus.