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## Seasonal fluctuations in the response of Italian Mediterranean buffaloes to synchronization of ovulation and timed artificial insemination

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#### ABSTRACT

A comprehensive study of the efficiency of synchronization of ovulation and timed artificial insemination (TAI) was undertaken in a large group of Italian Mediterranean buffaloes at a commercial dairy. A total of 2791 synchronization protocols were carried out on 857 animals over 3 years. Of these protocols, 823 (29.5%) did not proceed beyond Day 7 (due to the absence of a vascularized CL) and 620 (22.2%) were discontinued on Day 10 (due to the absence of follicles >1.0 cm and tonic uteri); hence, 1443 (51.7%) protocols did not progress to TAI. Data were analyzed for four periods: P1, transition to spring (from breeding season to low breeding season); P2, low breeding season; P3, transition to fall (low breeding season to breeding season); and P4, breeding season. No differences were found among the four periods in terms of the proportion of protocols that did not result in TAI. Of the 857 buffaloes, 660 (77%) conceived and delivered a calf. The average number of TAI per pregnancy was 2.1 and ranged from 1.9 to 2.3 across years. Logistic regression analysis showed that buffaloes that calved during P3 had a higher odds ratio for pregnancy (1.380; P < 0.05) than buffaloes that calved in other periods. Pregnancy was also influenced by the calving to service period (odds ratio = 0.977; P < 0.01) and the pregnancy per AI (P/AI) at successive TAI (odds ratio = 1.480; P < 0.01). The pregnancy per AI at the first TAI (424/857, 49.5%) was greater (P < 0.01) than in subsequent TAI. The occurrence of late embryonic mortality (between Days 27 and 45 after TAI) was similar among the four periods. These findings indicated that there are distinct seasonal differences in the response of Italian Mediterranean buffaloes to synchronization and TAI.

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#### 1. Introduction

The Italian Mediterranean buffalo has undergone selection for efficiency of production in intensive dairy systems that aim to achieve milk production throughout the year [1,2]. Continuous production of buffalo milk is required to meet the market demand for mozzarella cheese and other dairy food products derived from buffalo milk [3].

In the Mediterranean region, female buffaloes show annual fluctuations in reproduction with distinct breeding and nonbreeding seasons [4]. As buffaloes are short-day breeders, the annual peak in fertility coincides with decreasing day length from autumn to winter [5]. From winter to spring, females transition to the nonbreeding season in response to increasing day lengths [6]. The

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decline in fertility during the transition from the breeding to nonbreeding seasons is associated with a greater incidence of anestrus [4], a decline in the function of the CL [7], and an increase in embryonic mortality [8,9]. Natural mating is often preferred to assisted reproduction (estrus synchronization and AI) during the transition phase, and the nonbreeding season as assisted reproduction is thought to be less effective during the seasonal decline in fertility [10–12].

The efficiency of AI has doubled (25%–50% pregnancy rate) over a period of approximately 20 years [13–18], and the most successful protocols involve the synchronization of ovulation and timed artificial insemination (TAI) [19–21]. However, even with this protocol, Italian buffaloes would appear to show a decline in pregnancies during the transition to the nonbreeding season that is likely due to a decrease in activity of the CL and increase in the embryonic mortality that were noted above [22].

The aim in the present study was to undertake a comprehensive evaluation of the response of Italian Mediterranean buffaloes to synchronization of ovulation and TAI at different times of the year.

#### 2. Materials and methods

#### 2.1. Animals and management

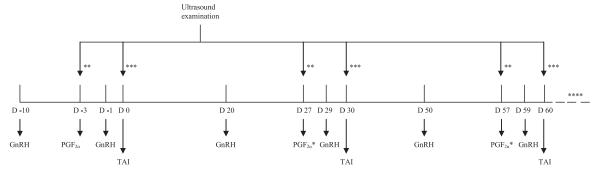
The study was conducted from 2010 to 2012 at a single commercial Italian buffalo dairy located in southern Italy (39.0°N and 41.5°N). The dairy was expanding, and the numbers of buffaloes utilized each year were as follows: 2010, 272; 2011, 289; and 2012, 296. The intercalving period was 416  $\pm$  2 days, and hence, individual buffaloes are represented more than once in the total of 857 buffaloes that underwent synchronization of ovulation and TAI from 2010 to 2012. The buffaloes were maintained in open yards that allowed 15 m<sup>2</sup> for each animal. They were fed once daily in a group pen with a total mixed ration consisting of 50% to 55% forage and 45% to 50% concentrate that contained 0.90 milk forage units per kilogram of dry matter and 15% crude protein/dry matter [23,24]. The buffaloes underwent clinical examination for any gross abnormalities, including atypical uterine secretion after calving [8].

#### 2.2. Synchronization of ovulation and fixed TAI

As a part of routine reproductive management, the buffaloes were treated with prostaglandin im  $(PGF_{2\alpha})$ analogue luprostiol, 15 mg; Prosolvin, Intervet) on Days 15 and 29 after parturition. Starting at Day 37 after calving, buffaloes with a minimum body condition score of 6.0 (the scale ranged from 1 to 9) [25] underwent synchronization of ovulation and TAI using a modified Ovsynch protocol that was adapted for Italian buffaloes [26]. Briefly, on Day 0, the buffaloes received an im injection of GnRH agonist (buserelin acetate, 12 µg; Receptal, Intervet, Milan, Italy). On Day 7, the buffaloes underwent transrectal ultrasonographic examination to confirm the presence of a CL and for color Doppler assessment of blood flow within the CL [22]. Buffaloes with a CL that had optimal blood flow and echogenicity [27] were given prostaglandin (Luprostiol, 15 mg) and a GnRH agonist im (buserelin acetate, 12 µg) on Day 9. These buffaloes were then inseminated once by TAI 20 hours later (Ovsynch-TAI). Ovaries were palpated per rectum on Day 10 (immediately before TAI) to assess estrous status (i.e., a follicle >1.0 cm and a tonic uterus) [26]. Buffaloes without the required selection criteria did not undergo TAI. Buffaloes excluded from TAI on the basis of these criteria used on Days 7 and 10 of the synchronization protocol were resynchronized (see below) and underwent TAI once the criteria for insemination were satisfied. The synchronization and TAI protocol is shown in Figure 1. All inseminations were conducted by the same operator by using frozen-thawed semen of 12 bulls. The bulls were of proven fertility and had yielded pregnancy rates greater than 30% when used previously in AI. The number of buffaloes, synchronization treatment, and bulls utilized throughout the study are shown in Table 1.

On Day 20 after TAI, at unknown pregnancy status, a GnRH agonist im (buserelin acetate, 12  $\mu$ g) was routinely administered to induce ovulation in order to (1) increase the progesterone in the circulation and reduce the likelihood of late embryonic mortality in the buffaloes that had conceived [9,28] and (2) resynchronize buffaloes that had not conceived.

On Day 27 after TAI, buffaloes underwent transrectal ultrasonography to determine the presence of an embryo



**Fig. 1.** Protocol for synchronization of ovulation and TAI in Italian buffaloes. \* PGF<sub>2α</sub> was administered only to nonpregnant buffaloes. \*\* Buffaloes without a vascularized CL were discarded. \*\*\* Buffaloes without a follicle greater than 1.0 cm and a tonic uterus with the presence or absence of mucous vaginal discharge were discarded. \*\*\*\* The same schedule of resynchronization was repeated seven times.

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