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History, evolution and perspectives of timed artificial insemination programs in Brazil

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Abstract

Currently, timed artificial insemination (TAI) can be applied routinely in the reproductive programs on farms. TAI protocols are designed to promote control of both luteal and follicular function, permitting the TAI with satisfactory pregnancy per AI (P/AI). A variety of protocols for TAI have been designed according to specific characteristics and requirements of different breeds, animal categories and types of management. The most common of these therapies use GnRH or estradiol plus progesterone/progestin (P4)-releasing devices and prostaglandin F2α. Moreover, TAI programs should be considered as an important tool of reproductive management to enhance the reproductive performance of cattle. Thus, the correct incorporation of these programs within the farm routine enhances reproductive efficiency of livestock to increase overall productivity of the farm unit.

Keywords: artificial insemination, cattle, reproduction, synchronization of ovulation.

Introduction

High reproductive performance is an essential requirement to ensure maximum livestock production and satisfactory economic return. In this context, the incorporation of reproductive programs in the routine of the farm seems like an organized approach to optimize the reproductive outcomes and profitability of dairy and beef cattle operations.

Artificial insemination (AI) promotes genetic and economic gains through the use of superior genetic bulls. Despite the technological advances of AI programs, the implementation of AI programs based on estrus detection is hampered mainly by postpartum anestrous and estrus detection (ED) failure (Bó *et al.*, 2007). These difficulties are aggravated when working with *Bos indicus* breeds because they exhibit estrus of shorter duration than *Bos taurus* (Figueiredo *et al.*, 1997; Bó *et al.*, 2003) or with high producing dairy cows because milk production is inversely proportional to estrus duration and reproductive performance of dairy cows (Lopez *et al.*, 2004; Wiltbank *et al.*, 2006).

To avoid the problems associated with AI programs utilizing ED, several research groups have developed different strategies to inseminate bovine females at an appointed time, eliminating the need for ED. The first positive results of this effort emerged in mid 1990s with the development of the Ovsynch protocol (GnRH-7 days-PGF-48h-GnRH-16h- timed AI; Pursley *et al.*, 1995). A number of protocols were designed to control both luteal and follicular function, which permit timed AI (TAI) with satisfactory pregnancy per AI (P/AI). Currently, TAI programs are applied routinely in dairy and beef herds providing a systematic approach to the use of AI (Macmillan *et al.*, 2003; Stevenson *et al.*, 2003; Baruselli *et al.*, 2004; Chebel *et al.*, 2004; Lucy *et al.*, 2004; Santos *et al.*, 2004; Thatcher *et al.*, 2006; Bó *et al.*, 2007; Galvão and Santos, 2008; Cerri *et al.*, 2009; Meneghetti *et al.*, 2009; Sá Filho *et al.*, 2009a; Santos *et al.*, 2010; Wiltbank *et al.*, 2011; Bisinotto and Santos, 2012).

A variety of protocols have been developed to design specific treatments for different animal categories and to minimize time and labor, yielding satisfactory pregnancy outcomes. Therefore, the objective of this review is to describe the history, evolution and the main perspectives of TAI programs in cattle.

History and evolution of synchronization of ovulation protocols for TAI

GnRH based TAI protocols

The GnRH administration induces the emergence of a new follicular wave after induction of ovulation (Macmillan and Thatcher, 1991; Twagiramungu *et al.*, 1992a, b, 1995; Wolfenson *et al.*, 1994; Schmitt *et al.*, 1996). When prostaglandin F2α (PGF) was given 7 days after GnRH treatment, fertility at the induced estrus was not reduced (Thatcher *et al.*, 1989; Twagiramungu *et al.*, 1992a). This became the basis for subsequent development of programs to control timed ovulation. The first synchronization of ovulation protocol, designated Ovsynch protocol, was assigned by Pursley *et al.* (1995) and consisted in a first injection of GnRH followed 7 days later with an injection of PGF, followed in 48 h by a second injection of GnRH; TAI could be performed 0 to 24 h (optimally 16 to 18 h) later. Following this first report, numerous protocols have been proposed and routinely applied in high production dairy cows (Wiltbank *et al.*, 2011).

The stage of the estrous cycle (Martinez *et al.*, 1999; Vasconcelos *et al.*, 1999; Moreira *et al.*, 2000) and cyclic status (Bisinotto *et al.*, 2010) at the time that GnRH is administered has been shown to affect Ovsynch results (Wiltbank *et al.*, 2011). Previous experiments found that the ideal phase to initiate the

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Ovsynch protocol is from days 5 to 12 of the estrous cycle (Vasconcelos *et al*., 1999; Moreira *et al*., 2000). Further researchers developed pre-synchronization systems that attempt to increase the proportion of cows in the ideal stage of the estrous cycle on the day of the first GnRH of Ovsynch (Moreira *et al*., 2000; Galvão *et al*., 2007; Souza *et al*., 2008; Kasimanickam *et al*., 2009; Chebel and Santos, 2010).

GnRH-based TAI protocols in beef cattle have been associated with inconsistent results, mainly by failures in the induction of the emergence of a new follicular wave following the first GnRH treatment of the Ovsynch protocol (Geary *et al*., 1998; Martinez *et al*., 1999; Baruselli *et al*., 2000; Bó *et al*., 2003; Colazo *et al*., 2009). GnRH-based protocols have also been evaluated to synchronize the ovulation in suckled *Bos indicus* cows (Barros *et al*., 2000; Fernandes *et al*., 2001; Baruselli *et al*., 2002; Williams *et al*., 2002; Sá Filho *et al*., 2009b). The overall P/AI was satisfactory only in cyclic cows (Fernandes *et al*., 2001) but, is still lower than the results achieved after progesterone (P4) plus estradiol (E2) based TAI protocol (Baruselli *et al*., 2002). Thus, collectively, these data indicate that Ovsynch protocol presents low efficiency when applied in lactating zebu cows under tropical pasture condition that are frequently associated with high incidence of postpartum anestrus (Barros *et al*., 2000; Fernandes *et al*., 2001; Baruselli *et al*., 2002).

Estradiol plus progesterone based protocol

Exogenous P4 suppresses LH release, alters ovarian function, suppresses estrus and prevents ovulation in cattle (Adams *et al*., 1992; Savio *et al*., 1993). Both P4 and progestins have been incorporated to the estrus synchronization protocols in cattle by oral sources such as melangestrol acetate (Patterson *et al*., 1989; Madureira *et al*., 1997; Hiers *et al*., 2003) or by insertion of intravaginal P4 device or progestin ear implants (Martinez *et al*., 2000a; Bó *et al*., 2002; Cavalieri *et al*., 2006). Progestins given for intervals that exceed the normal lifespan of the corpus luteum are associated with highly synchronous estrus upon withdrawal, but low fertility at the ensuing estrus (Revah and Butler, 1996). Therefore, P4 based TAI protocols have incorporated an inducer of ovarian follicular wave emergence at the beginning of the protocol with exogenous P4/progestin source which is normally removed after 7, 8 or 9 days (Bó *et al*., 2002; Baruselli *et al*., 2004; Meneghetti *et al*., 2009; Vasconcelos *et al*., 2009). Due to the emergence of a new follicular wave during the protocol and the short treatment period, the incidence of persistent follicles is reduced and fertility after TAI is close to that achieved following AI upon estrus detection (Bó *et al*., 2002; Santos *et al*., 2009; Teixeira, 2010).

Similar to the effect of GnRH treatment, described previously as an inducer of new follicular wave emergence, novel studies introduced the use of E2 plus P4 to control follicular wave dynamics in *Bos taurus* (Bó *et al*., 1991; Martinez *et al*., 2000b; Colazo *et al*., 2003) and *Bos indicus* cattle (Baruselli *et al*., 2006; Carvalho *et al*., 2008; Sá Filho *et al*., 2011a). The E2 and progestin/P4 combination followed by TAI has been a successful hormone therapy (Bó *et al*., 2002), allowing satisfactory P/AI following TAI in either *Bos taurus* or *Bos indicus* cattle (Martinez *et al*., 2000a; Bó *et al*., 2002; Macmillan *et al*., 2003; Baruselli *et al*., 2004; Cavalieri *et al*., 2006; Meneghetti *et al*., 2009; Souza *et al*., 2009; Teixeira, 2010). Nevertheless, the use of estrogens have been commercially limited in USA, New Zealand and in countries of the European Union, even though estrogen doses used to synchronize follicular wave emergence and ovulation only reach endogenous concentrations similar to those observed at estrus or during gestation.

Several studies (reviewed by Bó *et al*., 2002) found that E2 plus P4 treatment suppress the growing phase of the dominant follicle. The mechanism responsible for E2-induced suppression of follicle growth appears to involve suppression of FSH (Bó *et al*., 1991, 1993, 1996, 2002; O'Rourke *et al*., 2000) and LH (Burke *et al*., 1996). The administration of 5 or 2.5 mg of 17β-E2 (Bó *et al*., 2002) or 2.5 mg of estradiol benzoate (EB; Caccia and Bó, 2008) in P4/progestin-implanted cattle at random stages of the cycle was followed by synchronization of emergence of a new follicular wave approximately 4 days later. Furthermore the effect of EB on induction of new follicular wave occurs regardless of species (*Bos indicus*, *Bos taurus* or *Bos taurus indicus*) or breed (Beef = Angus, Nelore or Angus x Nelore or Dairy = Holstein, Gir or Holstein x Gir) of cattle (Carvalho *et al*., 2008).

The interval from E2 treatment to follicular wave emergence seemed to depend on FSH resurgence, which has been reported to occur after E2 concentrations decreased below a threshold level (O'Rourke *et al*., 2000). Estradiol valerate (EV) has a long circulating half-life which promotes a prolonged suppressing effect on FSH and ovarian follicular growth than 17β-E2 or EB (Bó *et al*., 1993; Martínez *et al*., 2005). This could be the reason for the variability and length of interval from EV treatment to follicular wave emergence. In *Bos indicus* cattle, the administration of EV (2.5 or 5.0 mg) with a norgestomet implant delayed the day of follicular wave emergence in comparison to treatment with 2.0 mg of EB (Sá Filho *et al*., 2011a). Furthermore, longer interval with higher dispersion from treatment to the emergence of a new follicular wave after EV treatment has been observed in *Bos indicus* heifers when compared to *Bos indicus* cows. Consequently, EV is not recommend to be used in zebu heifers (Sá Filho *et al*., 2011a).

Despite pharmacological differences, the estradiol esters (i.e., EV or EB) have been applied successfully in TAI synchronization protocols for synchronization of follicular wave emergence of suckled *Bos taurus* (Odde, 1990; Geary *et al*., 1998) and *Bos indicus* beef cows (Meneghetti *et al*., 2009; Sá Filho *et al*., 2009b, 2010, 2011a; Sales *et al*., 2012).

After luteolysis, TAI synchronization protocols use inducers of ovulation to achieve a synchronized ovulation. In E2 plus P4 protocols, a lower dose of E2 is normally given from 0 to 24 h after progestin removal to induce a synchronous LH surge (approximately 16 to 24 h after EB treatment) and ovulation approximately 24 to 32 h after the LH peak (Hanlon *et al*., 1997; Lammoglia *et al*., 1998; Martínez *et al*., 2005; Sales *et al*., 2012). The EB has been successfully used for inducing ovulations (Hanlon *et al*., 1997; Sales *et al*., 2012). Estradiol cypionate (EC) is another ester of E2 with a low water solubility that delays its release from the site of injection. Despite pharmacodynamics differences, both esters of estradiol (EB and EC) administered either at P4 device removal (EC) or 24 h later (EB) were effective in inducing an LH surge that resulted in synchronized ovulations and similar P/AI in suckled *Bos indicus* beef cows submitted to TAI (EB = 57.5%; 277/482 *vs*. EC = 61.8%; 291/471; Sales *et al*., 2012). In addition, the use of EC as the ovulatory stimulus given at the time of P4 device removal in the TAI protocol reduces cow handling, without reducing fertility.

As described previously, some countries have regulatory limitations to the use of estradiol on synchronization of ovulation protocols for TAI. Because of these restrictions, several studies were designed to evaluate the effect of using GnRH or different E2 esters to control the follicular growth and ovulation of beef and dairy cattle. These protocols have presented different pregnancy responses according to the animal category (dairy or beef cattle) and cyclic status. Based on the studies listed in Table 1, there was no difference between the use of GnRH or E2 ester as the ovulatory stimulus in either dairy or beef cattle. However, dairy and beef cows subjected to E2 + P4-based TAI protocol had greater P/AI than cows treated with GnRH-based TAI protocols. The greater response following E2 + P4 based TAI protocol could be associated with the better control of follicular wave emergence at the beginning of the protocol. However, it is important to mention that the majority of those studies was conducted in cattle under tropical conditions of South America. Therefore, influence of the incidence of anovular cows within herd, nutrition, management or season on pregnancy responses should be considered and requires further investigation.

Use of exogenous gonadotropin to enhance the ovarian responses during TAI programs

Anestrous cows have insufficient pulsatile release of LH to support the final stages of ovarian follicular development and ovulation. This condition limits the effectiveness of traditional TAI protocols (Baruselli *et al*., 2004). The treatment with equine chorionic gonadotropin (eCG) has been demonstrate as an alternative to increase final follicular development (follicular growth from the luteolysis induction and ovulation) and P/TAI mostly in anestrous or undernourished suckled beef cows (Bó *et al*., 2007; Sá Filho *et al*., 2009a; Sales *et al*., 2011) and in dairy cows in anestrous or with low body condition score at the beginning of the protocol (Souza *et al*., 2009; Garcia-Ispierto *et al*., 2011). Therefore, in beef and dairy cows with insufficient pulsatile release of LH to support the final stages of ovarian follicular development, treatment with eCG can improve the ovulatory response to the synchronization protocol and pregnancy outcome.

It is an important concern that, because eCG is a complex glycoprotein with a high molecular weight that is produced by the pregnant mare (Murphy and Martinuk, 1991), a potential immunological reaction may occur after repeated use in cattle (Drion *et al*., 2001). A PhD thesis (University of Sao Paulo) evaluated the potential adverse effects of the repeated use of eCG in cattle (Mantovani, 2010). A first experiment was designed to determine anti-eCG antibody production in response to 400 or 2000 IU of eCG, given once, twice or three times at 30-day intervals in *Bos taurus* and *Bos indicus* heifers. Animals were then submitted to weekly blood sampling for 63 days, and then at 30 to 60 day intervals for a total of 300 days. Antibody production was not affected by the number of eCG treatments; however, antibody production was higher in *Bos taurus* than in *Bos indicus* heifers. Higher antibodies levels were also observed in heifers receiving 2000 than 400 IU eCG. A second experiment conducted one year later focused on the evaluation of the cellular and humoral immunological memory of the *Bos taurus* heifers treated previously with 400 or 2000 IU of eCG. Humoral immunological memory response was not observed in animals treated previously with 400 or 2000 IU of eCG, regardless of the number of previous treatments. However, cellular immunological memory response was observed to be higher in animals subjected to increased numbers of previous treatments; but no evidence of adverse biological effects were observed. Results suggest that eCG, as used in synchronization protocols, is unlikely to have adverse effects following subsequent treatments.

Another alternative to improve the endogenous gonadotropin secretion and the ovarian responses during TAI programs is the use of calf removal. The temporary weaning increases LH pulse frequency and stimulates follicular growth and ovulation in cows >30 days postpartum (Mackey *et al*., 2000; Yavas and Walton, 2000). Several studies have demonstrated that the addition of temporary weaning on either GnRH-based (Geary *et al*., 2001; Williams *et al*., 2002; Sá Filho *et al*., 2009b) or E2 plus P4-based (Barreiros *et al*., 2003; Penteado *et al*., 2004) TAI protocols improved P/AI in suckled beef cows. Similar improvement on P/AI has been observed between calf removal and eCG treatment in suckling beef cows, and no additive effect has been found (Penteado *et al*., 2004).

Table 1. Effect of type of TAI program (GnRH or E2) on the pregnancy per artificial insemination (P/AI) of dairy and beef cattle.

	Type of TAI program				P-Value[3]	Reference (additional information)
	Ovulatory stimulus[1]		Base of protocol[2]			
	GnRH	E2	GnRH	E2		
	---------------------------- P/AI (n)	---------------------------				
Dairy	27.7 (314)	34.7 (366)	-	-	NS	Stevenson and Phatak, 2005[A]
	23.9 (309)	27.9 (412)	-	-	NS	Kasimanickam *et al.*, 2005[A]
	25.2 (127)	25.8 (132)	-	-	NS	Bartolome *et al.*, 2005
	58.7 (63)	48.3 (60)	-	-	0.05	Ambrose *et al.*, 2005[B,C]
	37.4 (179)	35.4 (192)	-	-	NS	Pancarci *et al.*, 2002[A,D]
	28.0 (157)	29.3 (164)	-	-	NS	Pancarci *et al.*, 2002[A,D]
	43.4 (488)	45.3 (483)	-	-	NS	Hillegass *et al.*, 2008[E]
	45.5 (44)	36.7 (30)	-	-	NS	Iwakuma *et al.*, 2008[B,C]
	28.9 (194)	30.9 (194)	-	-	NS	Souza *et al.*, 2009[B]
	33.8 (198)	29.1 (196)	-	-	NS	Souza *et al.*, 2009[B,F]
	36.4 (228)	32.9 (252)	-	-	NS	Shabankareh *et al.*, 2010
	45.7 (300)	39.9 (281)	-	-	0.07	Lima *et al.*, 2010[B]
	-	-	58.7 (63)	66.1 (56)	NS	Ambrose *et al.*, 2005[B,C]
	-	-	30.6 (98)	44.9 (98)	< 0.05	Veneranda *et al.*, 2006[B,D]
	-	-	37.8 (98)	30.0 (100)	NS	Veneranda *et al.*, 2006[B,D,F]
	-	-	41.0 (100)	52.0 (100)	NS	Veneranda *et al.*, 2006[B,D]
	-	-	24.2 (66)	30.6 (62)	0.009	Capitaine Funes *et al.*, 2009[G]
	-	-	27.4 (208)	40.8 (211)	0.03	Lima *et al.*, 2010[A]
	-	-	30.9 (97)	43.4 (99)	NS	Ranieri *et al.*, 2011[E,G]
	-	-	15.5 (200)	20.4 (201)	NS	Rodrigues *et al*; unpublished data
	-	-	10.4 (67)	25.0 (52)	0.04	Ayres and Ferreira; unpublished data[E,G]
Overall	35.2 (2,601)	35.0 (2,752)	28.7 (997)	37.3 (979)		
	35.0 (100)	30.8 (104)	-	-	NS	Fernandes *et al.*, 2001
	38.9 (190)	56.2 (178)	-	-	0.002	Sá Filho *et al.*, 2011b[B]
	50.9 (212)	51.8 (228)	-	-	NS	Sá Filho *et al.*, 2011a[B]
	48.7 (195)	44.8 (424)	-	-	NS	Sá Filho *et al.*, 2011a[B,C]
Beef	-	-	42.4 (92)	45.1 (91)	NS	Williams *et al.*, 2002[F]
	-	-	39.1 (92)	40.4 (99)	NS	Williams *et al.*, 2002[C]
	-	-	15.0 (100)	47.3 203)	0.01	Baruselli *et al.*, 2002
	-	-	65.0 (103)	61.5 (52)	NS	Martinez *et al.*, 2002[B]
	-	-	45.2 (166)	52.9 (174)	NS	Mialot *et al.*, 2003
Overall	44.8 (697)	47.1 (934)	42.0 (553)	48.6 (619)		
Total	37.2 (3,298)	38.1 (3,686)	33.4 (1,500)	41.7 (1,598)		

[1]Induction of ovulation: GnRH or an estradiol ester was used as ovulatory stimulus regardless the treatment used to induce the follicular wave emergence or the progesterone supplementation during the synchronization protocol; [2]Base of protocol: GnRH-based or E2 plus P4-based TAI protocols; [3]Effect of treatment (GnRH *vs.* E2); [A]Presynchronization was used; [B]Progesterone supplementation was used during the synchronization protocol; [C]Cyclic heifers; [D]Experiments I and II; [E]The E2 group also received GnRH at the TAI; [F]Females had their calves removed during 48 h before TAI or were treated with eCG; [G]Double Ovsynch protocol. NS = non-significant.

Impact of TAI on reproductive performance

Many studies compared the efficacy of the TAI protocol as tool of reproductive management for dairy cows (Cordoba and Fricke, 2002; Cavestany *et al.*, 2007; Gutiérrez *et al.*, 2009; Lima *et al.*, 2009; Teixeira, 2010; Herlihy *et al.*, 2011; Ribeiro *et al.*, 2011; Bisinotto and Santos, 2012). Similar P/AI has been found of cows bred upon estrus detection or following TAI (Santos *et al.*, 2009; Teixeira, 2010; Wiltbank *et al.*, 2011). Furthermore, timed synchronization protocols decreased the interval from parturition to first service and increased the proportion of cows becoming pregnant sooner after the voluntary waiting period (VWP; Cavestany *et al.*, 2007; Gutiérrez *et al.*, 2009; Teixeira, 2010; Herlihy *et al.*, 2011). In Brazil, we performed an experiment to evaluate the impact of TAI on the first day after the VWP on reproductive performance of high-producing dairy cows compared to the use of only AI upon ED (Teixeira, 2010). No difference ($P = 0.55$) was found in P/AI between dairy cows receiving the first service after ED (26.8%; 125/467) or TAI synchronization protocol (25.5%; 126/495). However, shorter interval from calving to first AI (78.3 ± 0.9 *vs.* 60.6 ± 0.1 days; $P < 0.01$ and from calving to conception (94.6 ± 1.8 *vs.* 87.4 ± 1.8 days; $P < 0.01$) were observed in cows receiving a TAI after the VWP (Teixeira, 2010).

In tropical countries, it is common to use a breeding season (BS) for beef herds during spring and summer months because there is higher availability of forage. For this pasture-based system, high pregnancy rates in the beginning of the breeding season are critical for herd profitability. Cows that become pregnant earlier in the breeding season will calf earlier in the next calving season, and, consequently, will have additional time to recover before the next breeding season. This improves their chances to conceive again and reduces the risk of involuntary culling (Rhodes *et al.*, 2003). Furthermore, calves born early in the calving season would be heavier at weaning, yielding additional income to the producer (Cutaia *et al.*, 2003; Bó *et al.*, 2005).

We designed two experiments to compare the performance of different reproductive programs that used natural service (NS), AI upon ED and TAI within a 90-day breeding season (Penteado *et al.,* 2005, 2008). In experiment 1, 594 suckled beef Nelore cows between 55 to 70 days postpartum were randomly allocated to one of four groups according the strategy of breeding. Cows in the TAI + NS group (n = 150) were synchronized with an E2 plus progestin-based TAI protocol. Bulls were placed with cows 10 days after the TAI and remained together until end of the BS. Cows in the TAI + ED + NS (n = 148) received TAI, then AI based on estrus detection for the next 45 days, and then NS for the last 45 days of the BS. Cows in the ED + NS (n = 147) were artificially inseminated based on twice daily estrus detections during the first 45 days of the BS and then exposed to NS for the last 45 days of the BS. Cows in the NS (n = 149) were bred by NS for the entire 90 days BS. Cows in the ED + NS or NS groups had decreased ($P < 0.001$) hazard of pregnancy compared to cows in either groups that received TAI at the onset of BS. Furthermore, cows receiving TAI had higher ($P < 0.01$) pregnancy rates at the end of the BS compared to cows that did not receive TAI (Table 2; Fig. 1). In experiment 2, 507 suckled beef cows (Nelore; *Bos indicus*; n = 303) and crossbred (Crossbred; *Bos taurus x Bos indicus*; n = 204) between 30 and 60 days postpartum were blocked by parity and breed, and assigned randomly to one of two groups at the onset of the BS. The NS group (n = 255) received only NS during the entire BS and TAI + NS group (n = 252) received TAI at the onset of the BS followed by NS until the end of a 90-day BS. Cows in the TAI + NS group had 63% higher hazard of pregnancy ($P < 0.001$) compared to cows in the NS group. This change in rate of pregnancy reduced the median days to pregnancy by 44 days (11 *vs.* 55 days). However, there was no difference ($P = 0.31$) in the proportion of pregnant cows at the end of the BS (TAI + NS = 77.0% *vs.* NS = 71.0%; Table 3, Fig. 2). In addition, pluriparous cows had greater ($P < 0.01$) P/AI, and had greater ($P < 0.01$) proportion of pregnant cows at 45 days and at the end of the BS than primiparous cows (Fig. 3). Cows with BCS ≥ 3.0 had greater ($P < 0.01$) P/AI and also greater ($P < 0.01$) proportion of pregnant cows at 45 days of BS than cows with BCS <3 (Fig. 4). Crossbred cows had greater ($P < 0.01$) P/AI, and also had greater proportion of pregnant cows at 45 days and at the end of the BS than Nelore cows (Fig. 5). Thus establishing pregnancy sooner after the VWP yields a marked increase in overall production efficiency of the herd both at the cow and calf level, yielding additional income to the producer.

Perspectives of TAI

Timed AI programs achieved a satisfactory stage of technological development; advances in reproductive management of insemination are widely available and routinely in use on commercial farms worldwide. Currently, dairy and beef cattle operations are incorporating TAI programs to increase the productive and reproductive performance. Supporting this evolution, the total of TAI performed in 2011 was greater than 50% of the total of AI performed in Brazil (Fig. 6; Baruselli and Sá Filho, 2012). In addition, the greater incorporation of TAI programs in the reproductive programs has also been associated with the general increase in the use of AI in Brazil (based on the total number of semen commercialized and the total number of females eligible to reproduction). It is interesting to note that the total Brazilian herd inseminated increased from 5 to 6% (2002) to almost 10% of the herd in 2011 (Fig. 7). The perspective is that the proportion of the Brazilian herd that is inseminated will increase continuously in the following years.

Regarding the commercial aspects, the TAI allows the producers to reach different market opportunities, such as enhance the use of AI on large scale, synchronize the parturition to the better seasons of the year for milk production and calf trade, improve the number of calves from bulls with high genetic merit and an increased calving of crossbred products, that have higher market value. In addition, the calf production may become more consistent and predictable with the use of large scale TAI programs in commercial herds.

Presently, it is accepted that the development of TAI techniques are state of the art, and that subsequent adaptations or adjustments exclusively in synchronization protocols will probably determine only slight modifications on pregnancy outcome. Further improvements on reproductive programs should be coupling to others technologies such as health and nutrition managements. Also, future researches should focus on strategies to enhance embryo survival, reducing the embryonic and fetal losses, mostly in anovulatory, heat stressed or high milk production cows. Furthermore, the success of assisted reproductive technologies is dependent on proper technology transfer to producers and its correct incorporation on routine reproductive management of each herd.

Table 2. Reproductive variables measured in suckled beef cows submitted to different breeding programs during a 90 days breeding season (BS).

Breeding strategy[1]	First 45 days of the BS			Pregnancy during the BS	
	Pregnancy per TAI % (n)	Service rate % (n)[2]	Pregnancy per AI % (n)[3]	45 days % (n)	end % (n)
TAI + NS	50.7 (76/150)	---	---	75.3 (113/150)[a]	92.7 (139/150)[a]
TAI + ED + NS	54.3 (81/148)	25.4 (17/67)	76.5 (13/17)	63.5 (94/148)[b]	91.9 (136/148)[a]
ED + NS	---	44.0 (66/150)	53.0 (35/66)	23.3 (35/150)[d]	85.0 (125/147)[b]
NS	---	---	---	44.3 (66/149)[c]	83.2 (124/149)[b]

[a,b,c,d]Different letters in the same column differ ($P < 0.05$). [1]Cows were subjected to different strategies during 90 days BS. TAI + NS: Cows received a timed AI (TAI) on Day 11 of the BS following natural service (NS) until the end of the BS; TAI + ED + NS: Cows received TAI at day 11, then were observed for estrus twice a day and AI 12 h after estrus detection (ED) during the first 45 days of the BS, followed by NS until the end of BS; ED + NS: Cows were artificially inseminated after estrus detection during the first 45 days of the following NS until the end of BS; NS: Cows received NS during the entire BS. [2]Number of cows artificially inseminated following ED in the TAI + ED + NS and ED + NS groups. [3]Number of pregnancies cows following artificial insemination after ED.

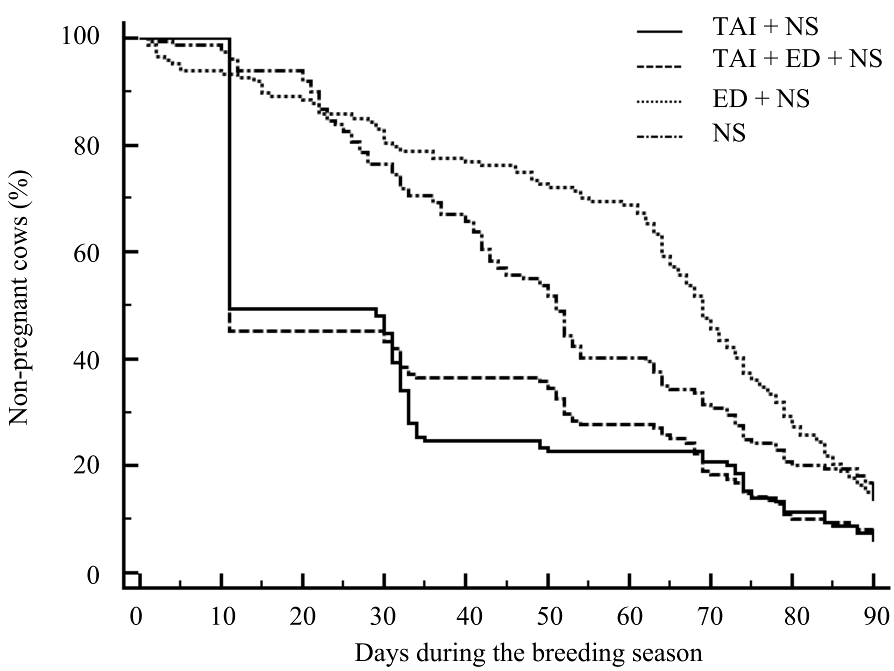

Figure 1. Survival curves for the proportion of non-pregnant cows by days of the breeding season for different breeding strategies during a 90-day breeding season (BS). TAI + NS: Cows received a timed artificial insemination on Day 11 of the BS followed by natural service (NS) until the end of the BS; TAI + ED + NS: Cows in the TAI + ED + NS received TAI at Day 11, then were observed for estrus twice a day and AI 12 h after ED during the first 45 days of the BS, followed by NS until the end of BS; ED + NS: Cows were artificially inseminated after estrus detection during the first 45 days of the BS followed by NS until the end of BS; NS: Cows received NS during the entire BS.

Table 3. Reproductive performance of suckled beef cows during the breeding season (BS) after different reproductive managements, according the parity (Primiparous or Pluriparous), cow's breed (Nelore or Crossbred) and BCS.

Item		Number	P/AI, % (n)	45 days, % (n)	End, % (n)
Breeding strategy	NS	255	----	46.3 (118)	71.0 (181)
	TAI + NS	252	52.4 (132)	63.5 (160)	77.0 (194)
		P value	----	0.001	0.31
Parity	Primiparous	250	41.3 (121)	36.8 (92)	58.0 (145)
	Pluriparous	257	61.8 (131)	72.4 (186)	87.6 (225)
		P value	0.002	<0.001	<0.001
Breed	Nelore	302	45.0 (151)	46.4 (140)	62.5 (197)
	Crossbred	205	62.4 (101)	67.3 (138)	84.4 (173)
		P value	0.007	0.03	0.05
BCS	Low (<3)	244	40.5 (121)	38.9 (95)	58.6 (143)
	Medium (≥3)	263	62.6 (131)	69.6 (183)	86.3 (227)
		P value	<0.001	0.005	0.005

P/AI: Pregnancy per IA; NS: Natural service.

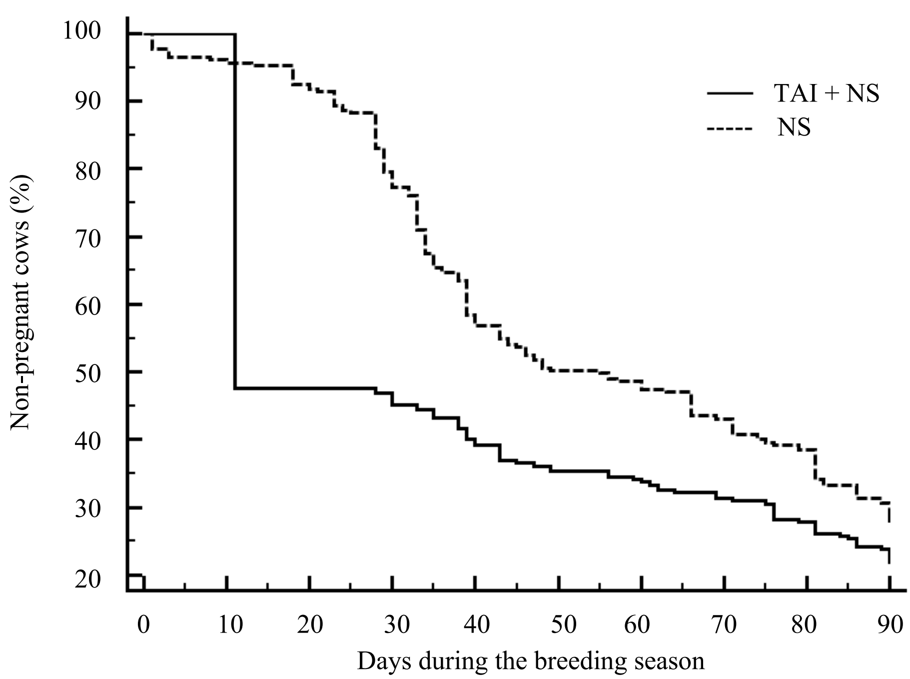

Figure 2. Survival curves for proportion of non-pregnant cows by days of a 90-day breeding season (BS) for suckled beef cows bred by natural service (NS; n = 255) or association between TAI at beginning of the BS following NS (TAI + SN; n = 252) during a 90-day BS. Median interval to pregnancy for NS and TAI groups was 55 days and 11 days (adjusted hazard ratio = 1.63; 95% confidence interval [CI] = 1.33 to 2.01), respectively.

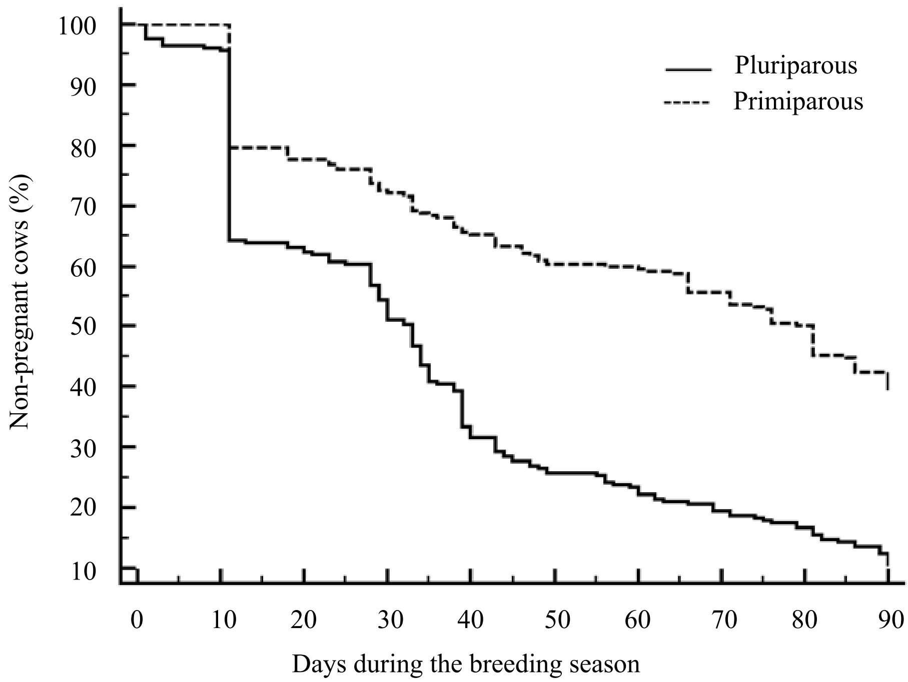

Figure 3. Survival curves for the proportion of non-pregnant cows by days of the breeding season (BS) for pluriparous (n = 257) or primiparous (n = 250) suckled beef cows bred by natural service or submitted to an association between TAI at beginning of the BS followed by NS during a 90-day BS. Median interval to pregnancy for primiparous and pluriparous cows were 79 days and 33 days (adjusted hazard ratio = 1.96; 95% confidence interval [CI] = 1.55 to 2.49), respectively.

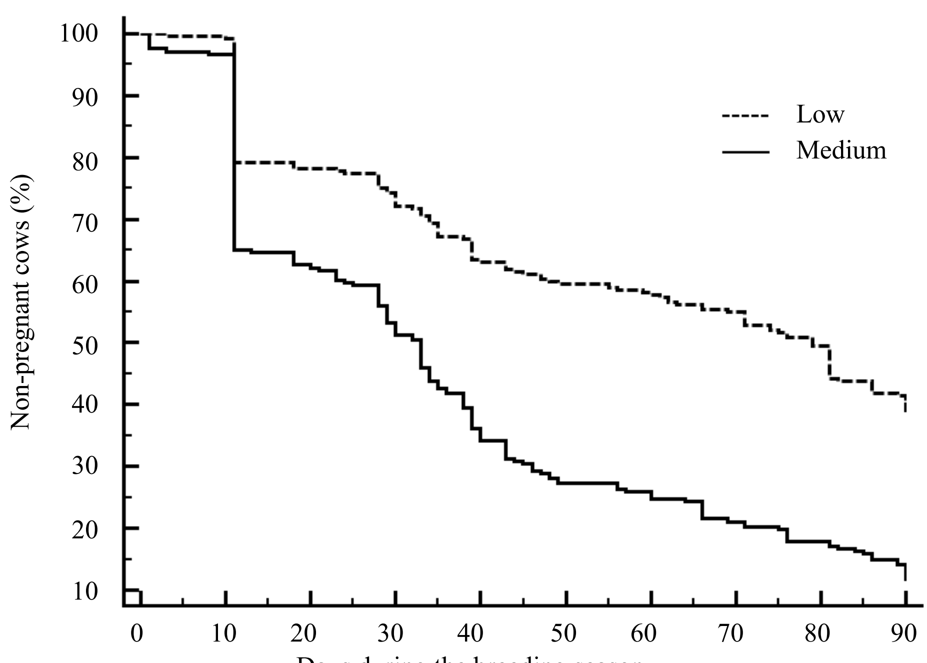

Figure 4. Survival curves for the proportion of non-pregnant cows by days of breeding season (BS) classified according to body condition score (BCS) as Low (BCS < 3.0; n = 263) or Medium (BCS ≥ 3.0; n = 244). Animals were suckled beef cows bred by natural service or submitted to an association between TAI at beginning of the BS followed by NS during a 90-day BS. Median interval to pregnancy for Low BCS and Medium BCS cows were 79 days and 33 days (adjusted hazard ratio = 1.79; 95% confidence interval [CI] = 1.35 to 2.21), respectively.

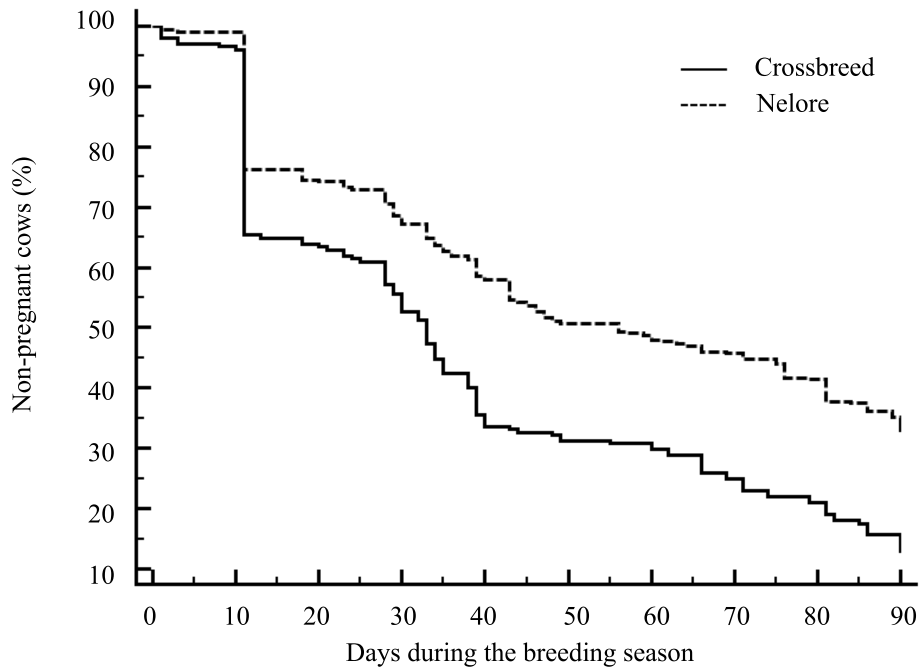

Figure 5. Survival curves for the proportion of non-pregnant cows by days of breeding season (BS) for Nelore (*Bos indicus*; n = 302) or Crossbreed (*Bos taurus* x *Bos indicus*; n = 205) suckled beef cows bred by natural service or submitted to an association between TAI at beginning of the BS followed by NS during a 90-day BS. Median interval to pregnancy for Nelore and Crossbreed cows were 56 days and 33 days (adjusted hazard ratio = 1.42; 95% confidence interval [CI] = 1.15 to 1.76), respectively.

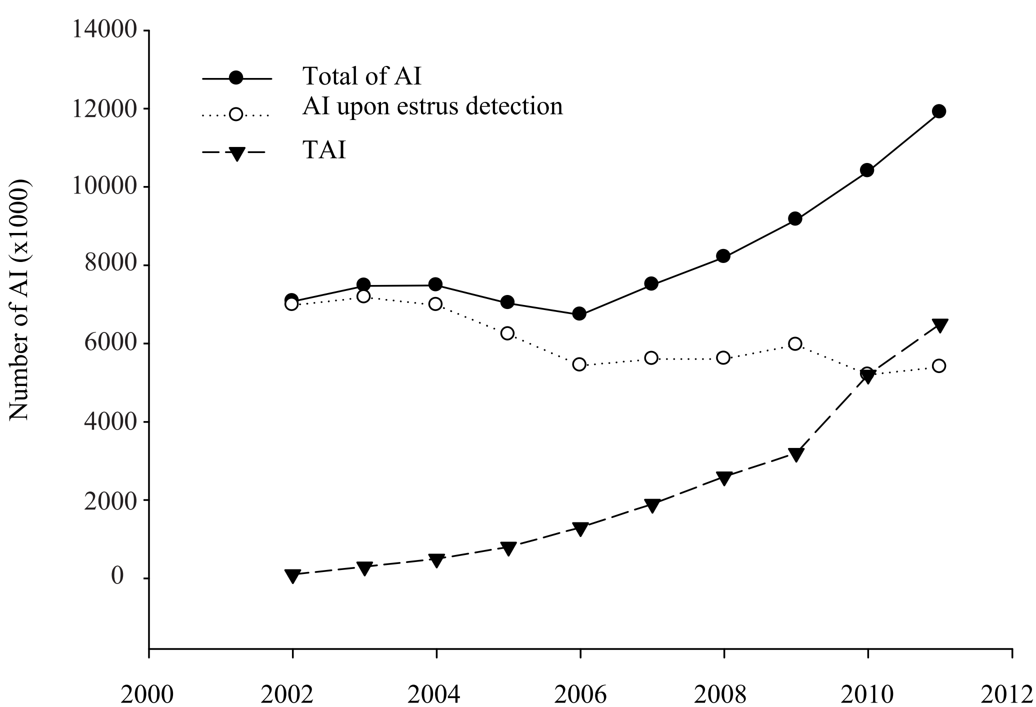

Figure 6. Evolution of TAI in Brazil based on the number of TAI protocols and doses of semen commercialized.

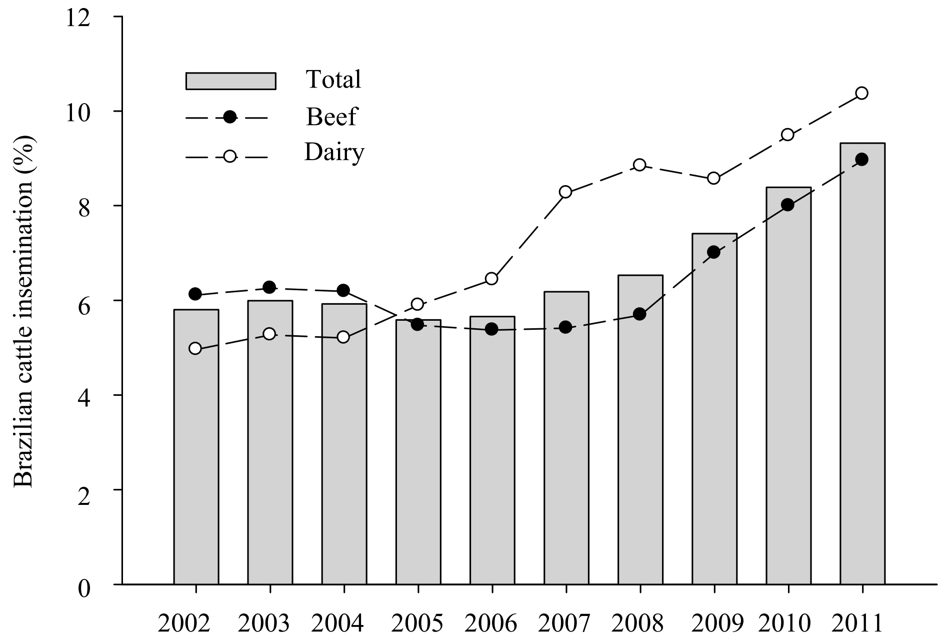

Figure 7. Percentage of cattle artificially inseminated in Brazil per year, based on the number of semen commercialized (ASBIA, 2011) and the number of females suitable to reproduction (beef and dairy) in the country (Anualpec, 2011). An average of 1.4 or 2.4 AI per female were considered for beef or dairy, respectively.

Conclusions

The protocols currently developed were designed to control both luteal and follicular function, allowing TAI with satisfactory pregnancy performance. There are specific differences among protocols designed to accommodate different animal categories and to minimize time and labor, yielding satisfactory pregnancy outcomes. The correct incorporation of these synchronization programs on reproductive management routine enhances reproductive efficiency of livestock.

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