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Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil

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Abstract

Beef and dairy productivity depends directly on the reproductive efficiency and genetic gain of the herd, which can be related to the appropriate use of biotechnologies, such as timed artificial insemination (TAI). When considering variations in synchronizations protocols, longer or shorter periods of progesterone (P4) device treatment could provide benefits to fertility. However, our studies evidenced that protocols with six (J-synch), seven, eight and nine days of P4 device treatment had similar pregnancy per AI (P/AI). In cyclic cows, the early prostaglandin (PGF) administration, moving from the day of P4 device removal to two days earlier, which results in four handlings of cows, or the administration of one extra dose of PGF at the onset of the protocol and a single PGF on the day of P4 device removal (three handlings) are both efficient to induce early luteolysis, reducing serum P4 concentrations and, therefore, stimulating LH pulsatility, which improves growth of the dominant follicle and results greater P/AI when compared with protocols with the administration of PGF only on the day of P4 device removal. Resynchronization is another valuable tool to reduce the interval between AI. Traditional Resynch is initiated at pregnancy diagnosis (28 to 32 days after TAI) and the interval between AI is around 40 days; Resynch 22 and Resynch 14 respectively initiates 22 and 14 days after the previous AI in all cows (unknown status of pregnancy) and reduces the interval between AI to 32 and 24 days. The novelty about Resynch 14 is the need to use of Doppler ultrasonography for pregnancy diagnosis [evaluation of corpus luteum (CL) vascularization]. Similar P/AI after Resynch 22 and 14 were found in Nelore cows. In dairy cattle, reproductive management is carried out throughout the year, thus, it is important to adapt the reproductive management to few established days of the week. Therefore, traditional Resynch and Resynch 25 were set to start 32 and 25 days after previous TAI, respectively. The hastening of reproductive age of Nelore heifers aims to reduce age at first calving and increase productivity. Factors such as age, weight, body condition score (BCS), uterine score (USC), average daily weight gain (ADG), withers height/depth of rib relationship (dRIB) and subcutaneous fat thickness (SCFT) were associated with an increase in the success of gestational establishment at TAI and can be used to select the heifers that are more suitable for reproduction. These technologies can contribute to improve the national production of kilograms of meat and liters of milk per hectare, and consequently improve livestock profitability.

Keywords: hastening of reproduction age, reproductive management, resynchronization programs, reproductive efficiency.

Introduction

Brazil has a distinguished position in the global beef industry. In addition to being one of the leaders in the beef export market, it is prominent in the scientific development and commercial application of applied reproductive biotechnologies. The correct use of biotechnologies in farms plays a critical role on productivity. Among the most used reproductive biotechnologies, timed artificial insemination (TAI) - which eliminates the need for estrous detection - deserves to be highlighted for facilitating management and by improving reproductive efficiency and genetic gain of the herds. Data comprised in our laboratory in 2016 showed that in 2015, TAI moved approximately R$567 million (~U$175 million) in Brazil, with an estimate of 3,500 veterinarians directly involved with this activity. Timed AI is currently implemented on 8.2 million beef cows, therefore generating an increase of 8% on calves' production, which represents approximately 656 thousand more calves per year or an additional income of R$820 million/year (~U$253 million) compared with natural service breeding. Time AI also hastens parturition and adds genetic gain to the herds, generating an average gain of 20 kg on the weaning weight of calves, which represents 3.3 million weaned calves with extra 20 kg or, extra R$400 million (~U$123 million). Also, from weaning to slaughtering, TAI calves gain an additional 15 kg of carcass, generating extra R$482.2 million (~U$149 million). Thus, TAI aggregates to the bovine beef chain around R$1.7 billion (more than half billion US $) per year (Baruselli, 2016).

As for dairy herds, TAI also has impact and adds around extra R$900 million (~U$278 million) per year by reducing the calving interval and using genetically superior bulls. It is estimated that TAI reduces one month of calving interval, increasing by 10% the annual milk production, what in Brazil represents extra 690 million liters of milk or R$759 million (~U$234 million) of additional income per year. Additionally, the use of bulls with superior genetics

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adds around 300 liters of milk per lactation of the daughter, resulting in an extra income of R$113.9 million (~U$35 million; Baruselli, 2016). Thus, it is estimated that the impact of TAI on dairy and beef chain together achieves around R$2.6 billion (~U$800 million) per year of extra income.

In 2016, TAI reached the mark of 11,034,119 procedures, which represents a growth of 5.1% in relation to the previous year (Fig. 1; non-published data from VRA-USP-Brazil; 2017), according to a carried out survey based on the number of protocols sold for TAI (information provided by companies in the sector) and the number of commercialized semen straws (Associação Brasileira de Inseminação Artificial - ASBIA, 2017). Currently, TAI procedures correspond to 85% of the insemination performed in Brazil (Fig. 1). Thus, it is evident that TAI holds a relevant place in the AI market. The strong progress made in recent years is indicative that the technology has been consolidated in the market, as it has positive results for livestock and qualified professionals for its execution. In spite of the proved benefits of TAI for reproductive efficiency, only 10 to 12% of females in reproductive age are in fact inseminated in Brazil (Baruselli, 2016). Thus, new strategies for expanding the AI application and optimizing the results should be incorporated to maximize its use and improve even more its economic impact.

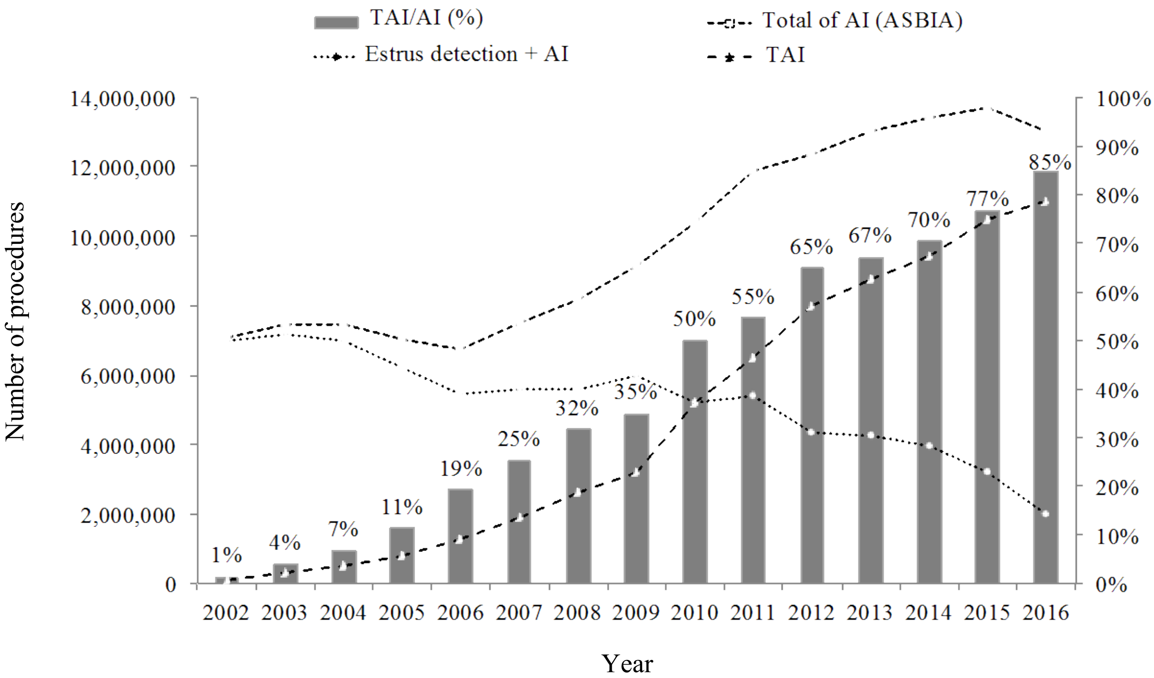

Figure 1. Time evolution of artificial insemination (AI) in Brazil. The numbers of AI done after estrus detection and following treatments for timed AI (TAI) are estimated based on the number of protocols sold for TAI (information provided by companies in the sector) and the number of commercialized semen straws (ASBIA, 2017). Data was organized by Departamento de Reprodução Animal-FMVZ-USP, São Paulo, Brazil, 2017.

Recent conquests for improving the TAI efficiency in the field

In this topic, the evolution and main perspectives of TAI programs used in beef and dairy cattle in Brazil will be discussed. Variations of current synchronization protocols, such as duration of the progesterone (P4) device and early prostaglandin F2α (PGF) treatment, were investigated to optimize animal handling and the efficiency of TAI protocols. Additionally, the evolution of programs to resynchronize ovulation, allowing subsequent management of TAI during the breeding season may reduce the calving interval and accelerate the genetic gain. Finally, the reduction of the heifers' age as they enter TAI programs is another great tool to maximize the calve production of genetic superior females earlier, hastening the age at parturition and accelerating the genetic advance.

The evolution and flexibility of TAI protocols

Over the past years, TAI protocols for beef and dairy cattle have gone through several modifications aiming to improve pregnancy outcomes. The length of treatment with P4 or progestin devices (summarized in Table 1) and early administration of PGF (from the day of device removal to two days before device removal; summarized in Table 2) were the main alterations evaluated. Briefly, in most experiments, the basic protocol used as control was the insertion of a P4 or progestin-releasing device plus the administration IM of estradiol benzoate (EB) at random days of the estrous cycle defined as day 0, device removal and intramuscular administration of PGF, estradiol cypionate (EC) and equine chorionic gonadotropin (eCG) on day 8 and TAI 48 h later.

The modifications of the duration of P4 or progestin device treatment in cows are based in the following evidences. The increased duration of device treatment, from 8 to 9 days, could provide more growth time to the dominant follicle, guaranteeing the ovulation of follicles with larger diameter with greater ovulation rates (Mantovani *et al*., 2005, 2010; Sá Filho *et al*., 2010) and, consequently generating larger corpus luteum (CL) with greater capacity of P4 synthesis (Mantovani *et al*., 2005). On the other hand, excessively long periods of follicular dominance may disrupt oocyte quality, resulting in reduced fertility (Cerri et al., 2009; Mihm *et al*., 1999; Roche *et al*., 1999; Lonergan, 2011). Another hypothesis is that the reduction of the period of P4 exposition (to six or seven days) may avoid adverse effects of follicular growth at the end of the protocol, extending proestrus and increasing estradiol concentration during this period (Bó *et al*., 2016). Briefly, none of these modifications tested and summarized in Table 1 improved pregnancy per artificial insemination (P/AI) following TAI. Protocols with six (J-synch), 7, 8, or 9 days of P4 device treatment were similarly efficient (Table 1). Similarly, previous studies have shown that the age of the ovulatory follicle did not influence P/AI in heifers and suckled beef cows (Abreu *et al*., 2014a, b). The absence of differences between protocols using P4/E2/eCG with various periods of P4 device treatment (6 to 9 days) may indicate that the new dominant follicle has a window of size and proestrus duration to ovulate an oocyte that is competent and suitable for fertilization. This would allow a flexible management for TAI protocols.

In countries that use GnRH and PGF based protocols, the beneficial effect of changing the duration of traditional Ovsynch protocol has been associated with greater circulating estradiol concentrations by prolonging the proestrus prior to ovulation and greater progesterone concentrations in the ensuing luteal phase, especially in the cows that do not ovulate after the first GnRH (Bridges *et al*., 2014). Furthermore, high estradiol concentrations in the proestrus period have been associated to a more appropriate uterine environment and smaller incidence of embryo loss (Jinks *et al*., 2013).

On the other hand, in the P4/E2/eCG-based protocol routinely used in Brazil, the lack of differences when using different periods for P4 device treatment may be due to diverse reasons: 1) the high efficiency in the synchronization of a new follicular wave at the beginning of the protocol; 2) the treatment with eCG to stimulate dominant follicle growth and to increase circulating P4 concentrations in the subsequent luteal phase; and 3) the treatment with estrogen that increase circulating estradiol concentrations prior to a synchronized ovulation.

The other proposal of protocol modification was related to earliness administration of PGF, from the day of device removal to two days earlier, aiming to induce early luteolysis and reduce serum P4 in cyclic cows. This would stimulate luteinizing hormone (LH) pulsatility and, consequently, improve the growth of the dominant follicle (Mantovani *et al*., 2005, 2010). In this context, greater P/AI was achieved when cycling Nelore cows (detection of CL at the onset of the protocol) received an early administration of PGF [(two days previous to device removal; day 7; 50.3% (86/171)]) compared with those receiving PGF on the day of device removal [day 9; 36.1% (56/155); $P < 0.05$]. Such difference was not observed in cows without a CL at the beginning of the treatment for TAI (Meneghetti *et al*., 2009). Increased P/AI was also observed with early PGF administration (from day 9 to day 7) for non-lactating Nelore cows (Peres *et al*., 2009). In dairy cows, the early administration of PGF from day 8 to day 7 also improved fertility to TAI and embryo transfer (Pereira *et al*., 2013).

Despite improving P/AI, the early administration of PGF demands an extra day of animal handling, which is undesirable, especially in beef farms. An alternative strategy to reduce P4 serum concentration during TAI protocol of cows with CL avoiding the extra handling is the inclusion of an extra dose of PGF at the onset of the protocol, keeping the second dose on the day of device removal (Carvalho *et al*., 2008). In this context, several studies showed similar P/AI when cows were treated with a single PGF administration on day 7 (four animal handlings) or PGF on day 0 and again on the day of device removal (three animal handlings) for Nelore cows (Carvalhaes *et al*., 2016; Mingoti *et al*., 2016), Girolando heifers (Mendanha *et al*., 2012) and crossbred Nelore-Aberdeen Angus heifers (Colli *et al*., 2016), as summarized in Table 2. Thus, the inclusion of an extra dose of PGF at the onset of the protocol allows improving fertility by reducing P4 serum concentration during TAI protocols, without the need of an extra day of handling.

Table 1. Efficiency of TAI protocols with different periods of progesterone (P4) or progestin source in different categories of cattle.

Reference	P4/progestin source	N	Category	Device, days	Pregnancy per AI	P value
Barbuio *et al*. (2016)	New FertilCare 1200®	313	Lactating Nelore Cow	8 *vs*. 9	55.8% (87/156) *vs*. 56.1% (88/157)	0.96
Barbuio *et al*. (2016)	New Crestar®	297	Lactating Nelore Cow	8 *vs*. 9	59.6% (87/146) *vs*. 62.9% (95/151)	0.59
Barbuio *et al*. (2016)	1x Used Crestar®	214	Lactating Nelore Cow	8 *vs*. 9	46.7% (50/107) *vs*. 43.0% (46/107)	>0.05
Mingoti *et al*. (2016)	New Sincrogest®	288	Lactating Holstein Cow	*8 vs. 9*	28.3% (41/145) *vs*. 23.8% (34/143)	0.28
Santos (2016)	New CIDR®	655	Lactating Nelore Cow	7 *vs*. 9	56.2% (195/347) *vs*. 54.2% (167/308)	0.49
Motta *et al*. (2016)	2x Used FertilCare 1200®	211	Nelore Heifer	6 *vs*. 8	47.1 (48/102) *vs*. 48.6% (53/109)	>0.05
Motta *et al*. (2016)	2x Used FertilCare 1200®	574	Nelore *vs*.Angus Heifer	6 *vs*. 8	55.0% (159/289) *vs*. 55.4% (158/285)	>0.05
Elliff *et al*.(2017)*	Primer®	505	Lactating Holstein Cow	7 *vs*. 8	27% (68/255) *vs*. 25% (64/250)	0.72

*sent for publication - SBTE 2017: similar (P = 0.554) pregnancy per AI (day 40) was observed between groups treated with intravaginal device containing 1g P4 kept for 7 days (28%; 35/129) or 8 days (24%; 31/130) or containing 0.5g P4 kept for 7 days (26%; 33/126) or 8 days (27%; 33/120).

Table 2. Efficiency of TAI protocols with three (administration of PGF on day 0 and on the days of device removal) or four animal handlings (administration of PGF two days prior to device removal) in different cattle categories.

Reference	Duration and source of P4 device	N	Category	Handling number	Pregnancy per AI	P Value
Mendanha *et al*. (2012)	CIDR® (9 days)	451	Girolando heifer	3 *vs*. 4	40.3% (92/228) *vs*. 42.1% (94/223)	>0.05
Colli *et al*. (2016)	2x Used CIDR® (8 or 9 days)	367	Angus *vs*. Nelore heifer	3 *vs*. 4	57.3% (110/192) *vs*. 57.1% (100/175)	0.93
Mingoti *et al*. (2016)	Sincrogest®† & CIDR®†(8* or 9 days)	1.941	Lactating Nelore Cow	3 *vs*. 4	53.4% (518/971) *vs*. 53.9% (523/970)	0.71
Carvalhaes *et al*. (2016)	New CIDR® † (9 days)	289	Lactating Nelore Cow	3 *vs*. 4	57.1% (84/147) *vs*. 64.8% (92/142)	0.18

*3 cattle handlings with PGF treatment only on the day of P4 device removal. †New, 1x used and 2 used P4 device.

Resynchronization of ovulation (Resynch programs)

In order to achieve maximum reproductive efficiency, aggressive strategies to concentrate pregnancies early in the breeding season should be taken. For that, three steps should be considered: AI of all cows at the beginning of the breeding season, early identification of nonpregnant cows, and reinsemination of nonpregnant cows as early as possible. The most commonly management adopted to get nonpregnant cows pregnant soon after the first AI is the introduction of clean-up bulls for the remainder of the breeding season. However, modern alternatives known as Resynchronization Programs are a potential tool to reduce time for subsequent inseminations.

The term “resynchronization” or “Resynch” refers to the treatment for synchronization of follicular wave emergence and ovulation of a female that was previously subjected to TAI. The aim of the technique is to allow the subsequent AI of cows that have already gone through one or more AI without establishing pregnancy, eliminating the need for estrus detection, maximizing the use of selected bulls and improving reproductive efficiency associated with genetic gain. Consequently, it allows the reduction of the interval between AI and hastens postpartum conception, reducing the calving interval and accelerating herds’ genetic evolution.

At first, the Resynch program (so called traditional Resynch) was initiated at pregnancy diagnosis around 28 to 32 days after TAI (Stevenson *et al*., 2003; Marques *et al*., 2012, 2015). Cows diagnosed as nonpregnant immediately initiated a new protocol for TAI. This Resynch management is flexible (starts at date chosen for pregnancy diagnosis) and treatments are only performed in nonpregnant cows. However, the interval between AI is around 40 days and is still considered too long by some technicians compared with bull exposure, when mating occurs around 21 days after TAI. Although bull mating allows the reduction of the interval between two consecutive services, the service rate depends on the estrus return (around 50%; Sá Filho *et al*., 2013). The evolution of Resynch programs allowed the reduction of the interval between AI to compact the period of breeding season and the interval between two subsequent parturitions, with the benefit of guaranteeing 100% service rate. To reach this goal, the treatments should start earlier than pregnancy diagnosis and therefore should be done in all cows. The Resynch 22 was developed to initiate 22 days after the previous AI, eight days before pregnancy diagnosis (Sá Filho *et al*., 2014). At that time, cows diagnosed as pregnant are excluded from the following treatments and nonpregnant cows continue the synchronization treatment. The advantage of Resynch 22 is to reduce the interval between AI to 32 days, however, the first treatment (P4 device and estradiol) should be done in all cows and pregnancy diagnosis must be performed in a fixed schedule, differently from the traditional Resynch. Although most of the farms that use the traditional Resynch have a prescheduled date for pregnancy diagnosis in order to start the resynch protocol as soon as possible, this date is not mandatory (anytime the pregnancy diagnosis is done, it is possible to start a new protocol in open cows). On the other hand, for Resynch 22 or 14, the date of pregnancy diagnosis is mandatory because the protocol was already started eight days before.

More recently, a new approach of early Resynch was proposed using the technology of color Doppler ultrasonography to perform an earlier non-pregnancy diagnosis by analyzing the vascular patterns of the CL (and not the presence of an embryonic vesicle in the uterus as usually done; Siqueira *et al*., 2013; Pugliesi *et al*., 2017). This management is called Resynch 14, because the treatment starts 14 days after the previous TAI, followed by pregnancy diagnosis eight days later (22 days after TAI) using Doppler ultrasonography (Vieira *et al*., 2014). Again, this Resynch protocol demands the initial treatment in all animals (unknown pregnancy status) and a mandatory fixed schedule for pregnancy diagnosis. Additionally, it demands specific ultrasound equipment and a well-trained technician to perform pregnancy diagnosis by the evaluation of CL vascularization grade. However, it allows an impacting reduction in the interval between AI to 24 days, which it close to what is achieved with an ideal 21 days service rate (Fig. 2).

Regarding the early diagnosis of nonpregnancy in cows using the color Doppler ultrasonography, high accuracy and close to 100% sensitivity were observed using only the CL vascularization patters as an indicative of luteolysis to identify nonpregnant dairy cows (Siqueira *et al*., 2013). As for beef cattle, 100% sensibility and 91% accuracy was reported when the association of CL vascularization and size were evaluated to diagnose early pregnancy (Pugliesi *et al*., 2014). Thus, color Doppler ultrasonography is considered an accurate tool to diagnose early pregnancy, because there is a low possibility of a wrong diagnostic of a pregnant cow as nonpregnant (close to 0% of false negatives). The occurrence of pregnancies diagnosed in a subsequent B mode ultrasonography exam as non-pregnancies may be related to long estrous cycles, pregnancy loss, or a lack of synchronization to the first TAI protocol, and not necessarily is due to a wrong pregnancy diagnosis. In these cases, the nonpregnant cows may start a subsequent synchronization of follicular wave emergence and TAI.

Some peculiarities are inherent to each Resynch protocol, specially related to the initial treatment. For the traditional Resynch and Resynch 22 the treatment starts 30 or 22 days after TAI, respectively, and it consists on the insertion of a P4 intravaginal device plus the administration of 2 mg estradiol benzoate (Pessoa *et al*., 2015). The dose of EB used in Resynch 22 was determined based on a study using 1,426 cows (768 *B. taurus* and 728 *B. indicus*; Pessoa *et al*., 2015). Pregnancy to the first TAI and pregnancy loss between 30 and 62 days following AI was similar between cows receiving 1 mg (44.0 and 3.8%) or 2 mg EB (44.0 and 5.5%) on D22. However, pregnancy to Resynch and cumulative pregnancy were greater ($P < 0.01$) in cows treated with 2 mg EB (47.3 and 68.2%) than with 1 mg EB (36.1 and 62.8%). This difference may be related to more effective follicle wave synchronization with 2 mg EB than 1 mg EB in cows (Caccia and Bó, 1998).

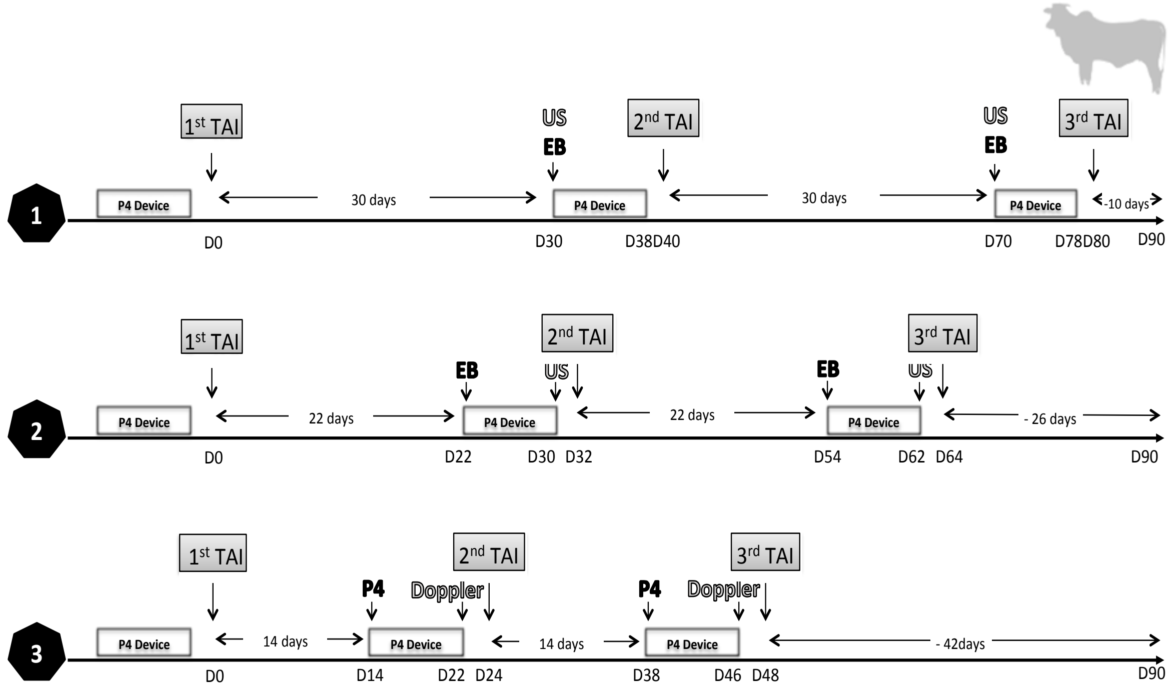

Figure 2. Scheme of three Resynch programs for timed artificial insemination (TAI) of beef females (based in a 90 day breeding season): 1) Traditional Resynch = treatment starts at pregnancy diagnosis (28-32 days after previous AI) in nonpregnant cows; 2) Resynch 22 = treatment starts in all cows (unknown pregnancy status) 22 days after the previous TAI and continue only in nonpregnant cows diagnosed eight days later by conventional ultrasonography (embryonic vesicle); 3) Resynch 14 = treatment stars in all cows (unknown pregnancy status) 14 days after the previous TAI and continue only in nonpregnant cows diagnosed eight days later by color Doppler ultrasonography (CL vascularization). TAI = timed artificial insemination; US = pregnancy diagnosis by conventional ultrasonography; P4 = progesterone; EB = estradiol benzoate.

However, for Resynch 14 the initial treatment starts 14 days after TAI and the administration of estradiol is replaced with the administration of 100 mg P4 IM. This is recommended based on previous studies showing that the use of EB 13 to 14 days after the previous AI induces luteolysis in some of the cows, reducing the previous AI conception rates (Cutaia *et al*., 2002; Vieira *et al*., 2014). This fact is not observed when estradiol is administered 22 days following AI for the Resynch 22 program, as reported previously (Sá Filho *et al*., 2014; Pessoa *et al*., 2015).

These results are in agreement with previous data showing a reduced ability of the CL to produce P4 when females are treated with EB during mid-diestrus (Munro and Moore, 1985; El-Zarkouny and Stevenson, 2004). This might be related to the induction of PGF release driven by the increase on estradiol concentration (Thatcher *et al*., 1986; Araújo *et al*., 2009). Alternatively to estradiol, P4 can be employed to promote the atresia of the dominant follicle and the emergence of a new wave. In this context, Rezende *et al*. (2016) demonstrated the growth of a new follicular wave 3.0 ± 0.7 after treatment with 100 mg P4 IM in Nelore cows. Thus, synchronization of follicular wave emergence in Resynch 14 programs must be performed replacing EB with P4 on the first day of treatment.

P/AI after Resynch 22 and Resynch 14 were recently compared in 244 postpartum Nelore cows (Penteado *et al*., 2016). For that, cows subjected to the first TAI were allocated into one of the two Resynch programs, Resynch 22 (ECC = 3.0; n = 126) or Resynch 14 (ECC = 3.0; n = 118). Resynch 22 cows were treated with a P4 device and 2 mg EB IM 22 days after the previous AI (day 22). On day 30, the device was removed and pregnancy was diagnosed based on the presence or absence of an embryonic vesicle in the uterus (conventional ultrasonography). Nonpregnant cows had the P4 device removed and received 0.530 mg sodium cloprostenol (PGF), 1 mg estradiol cypionate and 300 IU of eCG IM, followed by TAI 48 h later on day 32. Resynch 14 cows were treated with a P4 device plus IM administration of 100 mg P4 (Afisterone®, HertapeCalier) 14 days after the previous AI (day 14). On day 22, pregnancy diagnosis was done by the assessment of CL vascularization using Collor Doppler ultrasonography. Cows with absence or low CL vascularization were considered open and proceeded the treatment (device removal, PGF, estradiol cypionate and eCG IM), and were TAI 48 h later on day 24. Cows with moderate or strong CL vascularity were considered pregnant and had the device removed without further treatments. Similar P/AI were observed for Resynch 22 and Resynch 14 cows following the first AI (48 *vs*. 53%; P = 0.57) and resynchronization (56 *vs*. 51%; P = 0.37), respectively. The cumulative pregnancy after 32 and 24 days of breeding season did not differ (P = 0.77) for Resynch 22 (77%; 97/126) and Resynch 14 cows (75%; 89/118), respectively. Besides keeping similar P/AI after subsequent TAI and reducing the interval between AI to 24 days, Resynch 14 considerably improved 21 days service rate from 66 to 87.5% in relation to Resynch 22 (Fig. 3).

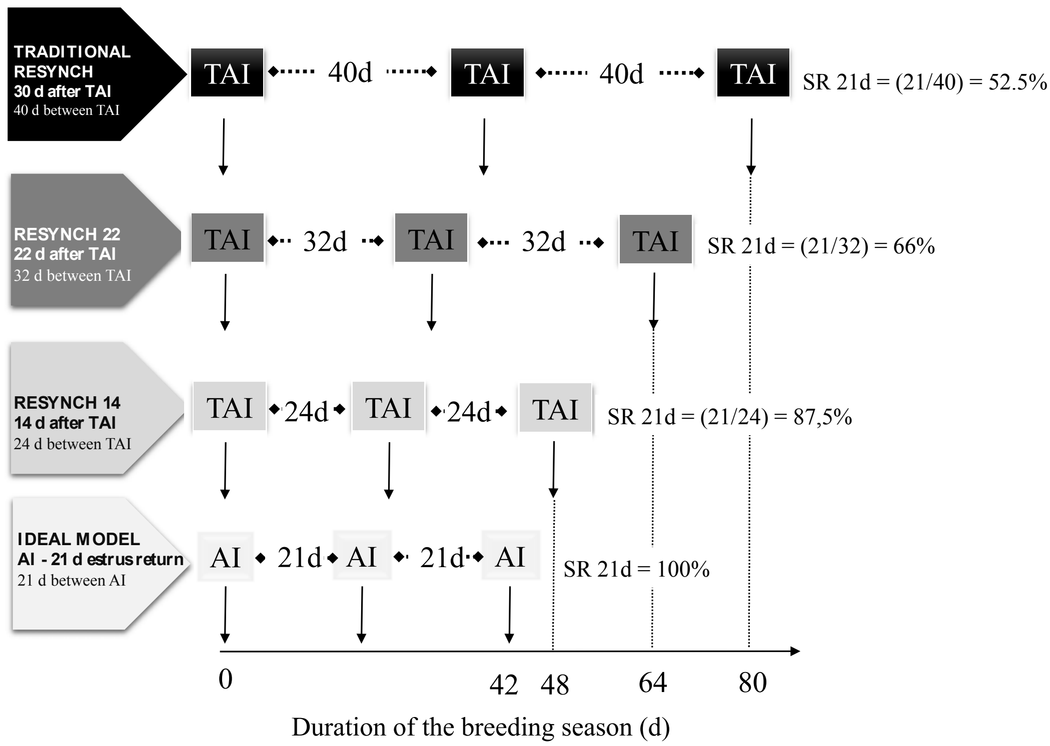

Figure 3. Service rate (SR) for different Resynch programs: Resynch 14 (starts 14 days after previous timed artificial insemination (TAI), with 24 days interval between AI and SR = 87.5%), Resynch 22 (starts 22 days after previous TAI, with 32 days interval between AI and SR = 66%), and traditional Resynch (starts after pregnancy diagnosis 30 days after previous TAI, with 40 days interval between AI and SR = 52.5%). Ideal model refers to a 21 day-interval between AI and SR = 100%.

The benefits of using Resynch programs are leading to the adoption of management exclusively with TAI, eliminating the need for clean-up bull in several farms. The use of three consecutive TAI using Resynch 22 (3 TAI) had similar pregnancy rates than those achieved with bull exposure after two TAI using Resynch 22 (2 TAI + bull) and greater pregnancy rate than one TAI followed by bull exposure (1 TAI + bull; Crepaldi *et al*., 2017). In this study, it was possible to achieve 87.4% of cumulative pregnancy rate at the end of a 64-day breeding season after three TAI using Resynch 22 (Fig. 4).

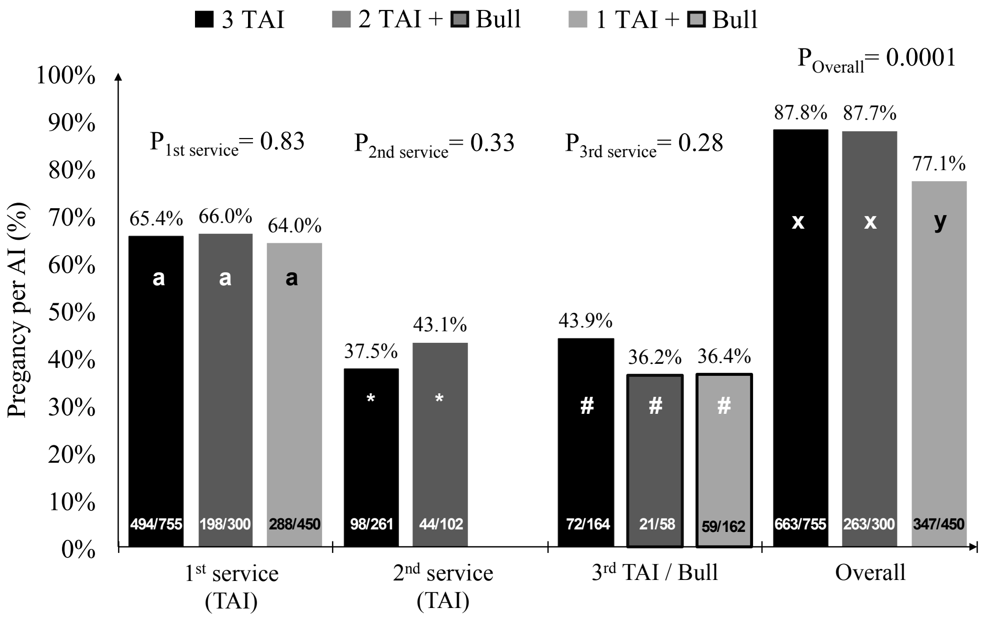

Figure 4. Pregnancy rate in *Bos indicus* beef cows after timed artificial insemination (TAI) with subsequent resynchronization for sequential TAI or bull exposure. Resynchronization was done using Resynch 22 program starting 22 days after the previous TAI. Groups were: 3 TAI (TAI + Resynch 22 and TAI + Resynch 22 and TAI; n = 450); 2 TAI (TAI + Resynch 22 and TAI + clean-up bulls; n = 300); 1 TAI (TAI+ clean-up bulls; n= 755). Pregnancy diagnosis of the 3rd TAI and bull mating were done at the end of the breeding season. Adapted from Crepaldi *et al*., 2017.

More recently, young Nelore heifers (12-15 months old) were subjected to three subsequent TAI using Resynch 14 program and achieved P/AI of 42.8% (270/631) at 1st AI, 34.1% (107/314) at 2nd TAI, 34.3% (59/172) at 3rd TAI and 72.1% (455/631) of cumulative pregnancy rate at the end of a 48-day breeding season (Colli *et al*., 2017; Fig. 5).

Pregnancy diagnosis was based on CL area and vascularization. Heifers with an area of CL ≥2 cm² and/or ≥25% CL blood flow were diagnosed as pregnant. False positive heifers were those diagnosed as pregnant at Doppler evaluation 22 days after AI and then as nonpregnant at pregnancy confirmation 30 days after AI. The false positive rate was 14.8% (47/317) and the P/AI of these heifers for the second time inseminated in D48 was 40.4% (19/47), increasing 2% in P/AI of day 48 (3rd TAI + false positive, 35.6%, 78/219).

These studies demonstrate that modern Resynch programs seems to be feasible and efficient on reducing the period of breeding season with similar cumulative pregnancy outcomes as obtained with bull exposure in traditional 90-day breeding season. It also brings the advantage of improving the number of pregnant animals by AI, accelerating the farms' genetic gain. Additionally, it concentrates calving births in the favorable calving season, consequently improving the weaning weight and accelerating the use of young females in the subsequent breeding season.

For dairy cattle, the Resynch protocols follow the same basis as for beef cattle. However, differently from beef, dairy herds reproductive management is accomplished all over the year, usually lacking a delimitated breeding season, and animals are managed several times a day. Thus, it becomes crucial to adapt the reproductive management so they do not interfere with the other daily management employed in a dairy farm. A good strategy is to concentrate the reproductive-related activities into established days of the week. In this basis, the traditional Resynch and Resynch 25 were set to start 32 and 25 days after previous AI, respectively (Fig. 6). This standardization of reproductive activities in specific weekdays enables the establishment of a well-planned routine in dairy farms, as shown in Figure 6.

In order to calculate reproductive efficiency of dairy cows in a Resynch 25 program, we considered that the first service occurs 58 days after parturition (average), 30% P/AI until third service, 20% P/AI between the fourth and sixth services (sixth service was established as the animals' last service) and 15% of pregnancy loss between 30 and 60 days of pregnancy. In this simulation, the herd achieves 82% of pregnant cows at 246 days in milk, 110.4 days interval between parturition and conception and 12.9 months of calving interval, which can be considered good reproductive efficiency. Therefore, studies were conducted in order to verify the applicability and viability of Resynch 25 in dairy properties in Brazil.

In one study, the efficiency of the association between gonadotropin-releasing hormone (GnRH) or EB and P4 at the onset of Resynch 25 to synchronize the new wave of follicular growth in Holstein cows without previous pregnancy diagnosis was evaluated (Vasconcellos *et al*., 2014). The authors verified that both associations (GnRH+P4 or EB+P4) were effective to synchronize the new wave of follicular growth and ovulation. Given this information, a second study was conducted to verify the possible influence of the administration of 2 mg EB at the onset of Resynch 25 on the establishment and maintenance of the previous pregnancy in Holstein cows (Vieira *et al*., 2015). Similar P/AI following first AI at 33 [EB: 33.0% (66/200), GnRH: 35.0% (69/197), EB+GnRH: 34.3% (70/204); P = 0.61] and 65 days [EB: 26.0% (52/200), GnRH: 28.9% (57/197), EB+GnRH: 26.5% (54/204); P = 0.26], as well as similar pregnancy loss between 33 and 65 days after first TAI [EB: 21.2% (14/66), GnRH: 17.4% (12/69), EB+GnRH: 22.9% (16/70); P = 0.47], were observed in cows resynchronized with EB, GnRH or a combination of both. The CL vascularization was also accessed by Doppler ultrasonography every 48 h, between days 25 and 33 in 42 lactating cows used in the previous experiment [EB: n = 15; GnRH: n = 12; EB+GnRH: n = 15]. It was found that the CL vascularization rate remained similar between the three groups during the experimental period (day 25: 76.1%, day 27: 79.1%, day 29: 77.5%, day 31: 76.3% and day 33: 77.1%). Thus, the resynch treatment using EB, GnRH or a combination of both after TAI results in similar P/AI after 1st TAI and after resynchronization. Also the administration of EB in pregnant lactating cows 25 days after AI does not induce pregnancy loss and does not compromise the CL vascularization.

Therefore, Resynch 25 can be an alternative for reproductive management of dairy properties. This program allows systematization and planning of the annual reproduction calendar of the farm, concentrating conceptions at the beginning of lactation. However, it is important to mention that other factors, such as nutrition and sanitary management, as well as organization and availability of qualified personal, may significantly influence the results of TAI programs with resynchronization in dairy herds.

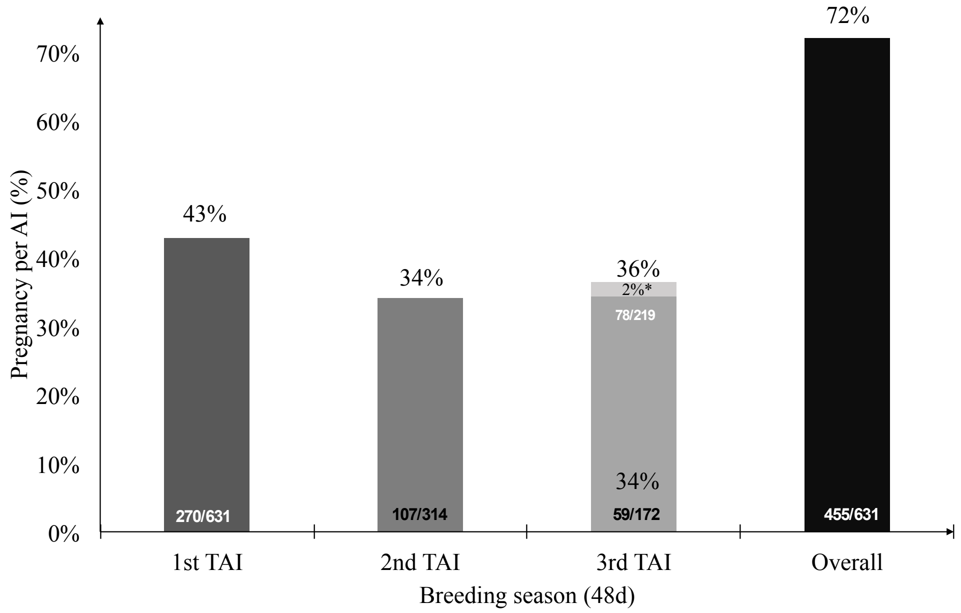

Figure 5. Pregnancy rates in young *Bos indicus* beef heifers (12-15 months old) after timed artificial insemination (TAI) with subsequent resynchronization using Resynch 14 (program starting 14 days after the previous TAI in all heifers, with unknown status of pregnancy). *Refers to heifers diagnosed as pregnant at first evaluation and them diagnosed as nonpregnant and reinsemination for the second time with the 3rd service heifers. Adapted from Colli *et al*., 2017.

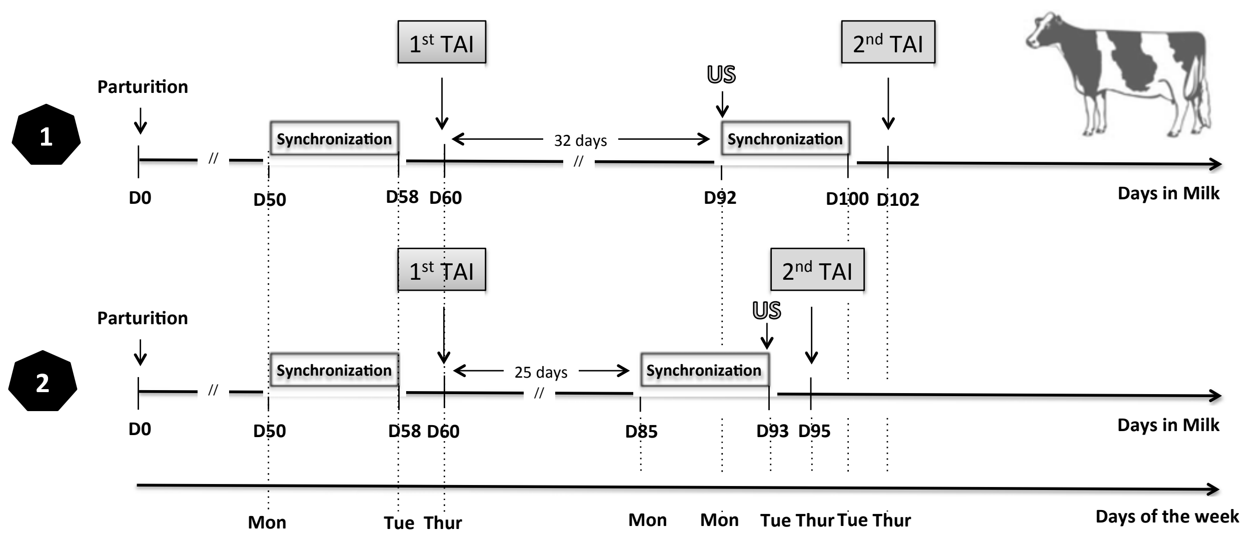

Figure 6. Scheme of two Resynch programs for timed artificial insemination (TAI) of dairy females: 1) Traditional Resynch = treatment starts at pregnancy diagnosis (32 days after previous AI) in nonpregnant cows, with 42 days interval between AI; 2) Resynch 25 = treatment starts in all cows (unknown pregnancy status) 25 days after the previous TAI and continue only in nonpregnant cows diagnosed eight days later by conventional ultrasonography (embryonic vesicle), with 35 days interval between AI. US = pregnancy diagnosis by conventional ultrasonography.

Hasten of reproductive age of Nelore heifers

Many factors may influence hasten or delay in the sexual maturity of heifers, such as age, weight after weaning and development of the reproductive tract. Knowing the real impact of these factors on the reproductive efficiency would be determinant for the development of future strategies that would accelerate even more the onset of reproduction of Nelore heifers in Brazil. Thus, the need to establish indicators of body development that determine improvements on the reproductive efficiency of yearling zebu heifers is evident. The main objective is to reduce age at first calving and increase productivity in Brazils' beef herd.

Recently, Freitas (2015) evaluated the body development and functioning of the reproductive tract in relation to gestational success in 650 14-month-old (13.9 ± 0.03 months) Nelore heifers that underwent TAI protocols. The heifers were kept on a pasture-based system. They were evaluated during 10 days before the onset of TAI protocol (day -10 to day 0). The evaluated characteristics were age (months), weight (kg, weight scale for squeeze chute), body condition score (BCS, 1 to 5 scale), withers height (hWIT, cm) and withers height/depth of rib relationship (dRIB, %), reproductive tract score (RTS, 1 to 5 scale), cyclicity (presence of a CL) and subcutaneous fat thickness (SCFT, mm). All heifers were synchronized for TAI (Norgestomet year implant + 2 mg EB – 8d – PGF + 300 IU eCG + 0,6 mg estradiol cypionate - AI 48 hs). The cyclicity (presence of CL at day -10 and/or day 0) and P/AI 30 days after TAI were determined by ultrasonography. Briefly, hWIT and RTS were not associated with an increase in the success of gestational establishment. However, heifers that were older, heavier, had greater BCS, dRIB and SCFT had greater P/AI (Table 3).

Table 3. Cut-off points of the evaluated parameters and calculated by the receiver operation characteristics (ROC) curve, based on the pregnancy rate.

Parameter	N	Area under curve	95% confidence interval	P value
Age			0.540 a 0.630	0.0003
> 13.8 months	351	58.5		
< 13.8 months	298			
Weight				
> 248.00 kg	429	56.8	0.529 a 0.607	0.003
< 248.00 kg	311			
BCS				
> 3.0	271	62.9	0.590 a 0.666	< 0.0001
< 3.0	469			
hWIT				
> 119.0 cm	544	50.2	0.462 a 0.541	0.94
< 119.0 cm	196			
dRIB				
> 44.0 %	393	61.0	0.571 a 0.648	< 0.0001
< 44.0 %	347			
SCFT				
> 2.5 mm	411	59.6	0.552 a 0.640	< 0.0001
< 2.5 mm	314			

BCS = body condition score; hWIT = withers height; dRIB = rib depth; SCFT =subcutaneous fat BCS = body condition score. Adapted from Freitas, 2015.

It was found that older females showed greater success in pregnancy (> 13.8 months = 43.0 *vs*. ≤ 13.8 months = 27.2%; P = 0.04). No association was observed between hWIT and P/AI (> 119.0 cm = 34.1 *vs*. ≤ 119.0 cm = 40.0%; P = 0.46). In contrast to hWIT, heifers with greater dRIB had greater P/AI compared with heifers with smaller dRIB (> 44.0% = 41.9 *vs*. ≤ 44.0% = 27.0%; P = 0.02). In addition to dRIB, another characteristic that influenced P/AI was SCFT (> 2.5mm = 44.4 *vs*. ≤ 2.5 mm = 23.4%; P = 0.0003), heifers with greater SCFT showed greater probability of P/AI (Fig. 7; Freitas, 2015).

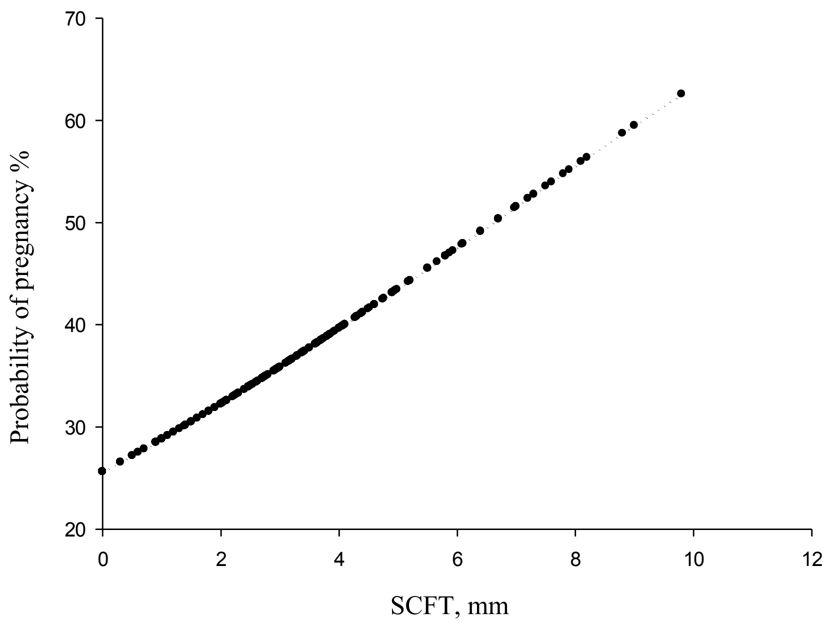

Figure 7. Probability of pregnancy to timed artificial insemination (TAI) as a function of the subcutaneous fat thickness (SCFT) of Nelore heifers (n = 650) with a mean of 13.9 months of age [Logit (SCFT) = 1.0662 + 0.1612 * SCFT; P = 0.0003]. Adapted from Freitas, 2015.

The zootechnics characteristics related to body development have different influence on P/AI. In the aforementioned study, hWIT and RTS isolated have no effect on pregnancy to TAI. However, age, weight, BCS, dRIB and SCFT are associated with an increase in the success of gestational establishment at TAI. When associated, the variables dRIB, age and SCFT are the characteristic that exert the most influence on P/AI (Freitas, 2015).

Garcia *et al*. (2002) showed that body weight accounted for most of the variation associated with the onset of puberty followed by the contribution of circulating leptin concentrations, which increased during a period of 16 weeks before first ovulation. Others studies found that the onset of puberty is related to growth rate and the amount of body fat (Nogueira, 2004). In a recent study, heifers were weaned at approximately 4 month of age and fed diets to promote relatively low (0.5 kg/day) or high (1.0 kg/day) rates of body weight gain until 8.5 month of age (Alves *et al*., 2015). Heifers that gained body weight at a greater rate exhibited greater circulating concentrations of leptin and reduced overall NPY expression in the arcuate nucleus. The authors suggest that such changes may mediate the nutritional programming of the reproductive neuroendocrine axis and facilitate an early onset of puberty in heifers (Alves *et al*., 2015).

More recently, two experiments were conducted with the objective of studying the factors that affect the pregnancy rate of 14 months old Nelore heifers submitted to TAI (n = 404) and natural breeding (n = 893; bull to heifer ratio was 1 to 30; Martins *et al*., 2017). On both studies, presence of CL, uterine score (USC; A = uterine horns diameter > 2 cm; B = uterine horns with diameter between 1.5 and 2 cm; and C = uterine horns with diameter < 1.5 cm), diameter of the largest follicle, daily average weight gain (DAWG), loin eye area (LEA), SCFT and hWIT were analyzed at the beginning of the breeding season. On TAI experiment, none of the heifers was cycling or had an A USC on the beginning of the breeding season. The P/AI was greater on heifers with greater USC [B = 41.1% (122/297) *vs*. C = 17.8% (19/107); P = 0.0005]. Pregnancy probability was greater for animals with greater SCFT (r^2 = 0.208; P = 0.005) and DAWG (r^2 = 0.168; P = 0.0007). The LEA (r^2 = 0.115; P = 0.13) and hWIT (r^2 = 0.309; P = 0.28) characteristics did not affect P/AI. This findings are in agreement with previous (Freitas, 2015). On natural breeding experiment, the cyclicity rate was 5.3% (47/893) at the beginning of the breeding season. Pregnancy diagnosed on day 50 of the breeding season was greater (P > 0.0001) for cycling (53.2%; 25/47) than anestrous (13.4%; 113/846) heifers and for heifers with greater uterine development [USC: A = 64.0% (16/25); B = 17.9% (116/647) and C = 3.2% (7/221); P < 0.0001]. The pregnancy probability was not influenced by SCFT (r^2 = 0.096; P = 0.42) and LEA (r^2 = 0.061; P = 0.61). However, DAWG positively influenced the probability of cyclicity (r^2 = 0.263; P < 0.0001) and pregnancy (r^2 = 0.093; P = 0.005); and hWIT negatively influenced pregnancy probability (r^2 = -0.082; P = 0.03). Pregnancy probability was also increased according to diameter of the largest follicle (r^2 = 0.117; *P* = 0.0004). Thus, it was possible to verify that heifers with greater SCFT and DAWG had greater pregnancy probability to TAI. Heifers submitted to natural breeding with greater DAWG and diameter of the largest follicle, and smaller hWIT had greater probability to become pregnant. Further, pregnancy rates to TAI and natural breeding were greater on heifers with greater USC (Martins *et al*., 2017).

Finally, the risk factors influencing P/AI of young Nelore heifers (aging 14.4 ± 0.92 months old; n = 631) subjected to three consecutive TAI using Resynch 14 and Doppler ultrasonography was evaluated (Colli *et al*., 2017). Weight, BCS, age and diameter of the largest follicle at the end of the synchronization program were evaluated. A positive correlation was found between P/AI 30 days after TAI and weight (r^2 = 0.09; P = 0.03), age (r^2 = 0.07; P = 0.06), BCS (r^2 = 0.07; P = 0.09), diameter of the largest follicle on D0 (r^2 = 0.20; *P* <0.0001) and diameter of the largest follicle at P4 device removal (r^2 = 0.11; P = 0.007). Also, a negative correlation was observed between the incidence of false positives (heifers diagnosed as pregnant at Doppler evaluation 22 days after AI and then as nonpregnant at pregnancy confirmation 30 days after AI) and weight (r^2 = -0.15; P = 0.009), age (r^2 = -0.10; P = 0.07), largest follicle at device removal (r^2 = -0.10; P = 0.10) and largest follicle at AI (r^2 = -0.15; P = 0.01).

Thus, it is possible to obtain pregnancy rates > 70% in Nelore heifers with 14 months old after three TAI (Colli *et al*., 2017; Fig. 5) and that there is a positive correlation between weight, age, BCS, SCFT, and diameter of the largest follicle at the end of the synchronization protocol and P/AI 30 days after TAI.

Conclusions and future directions

The reduced reproductive efficiency of Brazilian bovine herd is still a limiting factor for the sustained growth of beef and dairy chains. We are well below the capacity of production of calves per cow per year (~68.5% weaning rate) and the quality of produced calves is far short of the ideal (~12% of females are inseminated). Besides, our heifers start reproductive life still very belatedly (around three to four years old), and reproductive efficiency is still impaired. In dairy, Brazil is not self-sufficient to supply the national market and the low productivity per cow (less than 2,000 L of milk per lactation) evidences the low genetic selection. Thus, our production system requires the development of complementary strategies to hasten and maximize the use of AI in dairy and beef herds, with easy and direct application and high reproductive efficiency to improve productivity.

In this context, several synchronization protocols (i.e. with different duration of P4 source treatment and acceleration of luteolysis) for dairy and beef cows and heifers were well studied and are shown herein. Also, the use of Resynch programs can reduce the interval between services and allow the massive use of AI in farms, resulting in the production of greater quantity and quality of calves. Finally, heifers' reproductive age can be hasten by correctly managing their mothers so they give birth in the most adequate season of the year (greater weaning weight), associated with calves' and heifers' correct nutrition and the use of adequate characteristics of evaluation as age, weight, BCS, USC, DAWG, dRIB and SCFT to select the heifers that are more suitable for early reproduction.

The dissemination of the use of these strategies isolated or in combination enables the production of greater quantity (reduction of the calving interval and hastening of reproductive age of heifers) and quality (maximization of the use of AI) of beef calves, and the production of dairy females with greater milk production per lactation (reduction of the calving interval associated to genetic gain). These technologies can contribute to improve the national production of kilograms of meat and liters of milk per hectare (what is a reflection of production efficiency), and consequently improve livestock profitability.

References

Alves BRC, Cardoso RC, Prezotto LD, Thorson JF, Bedenbaugh M, Sharpton SM, Caraty A, Keisler DH, Tedeschi LO, Williams GL. 2015. Elevated body weight gain during the juvenile period alters neuropeptide y-gonadotropin-releasing hormone circuitry in prepubertal heifers. *Biol Reprod*, 92:46, 1-10. doi: 10.1095/biolreprod.114.124636.

Abreu FM, Cruppe LH, Maquivar M, Utt MD, Madsen CA, Vasconcelos JLM, Mussard ML, Day ML, Geary TW. 2014a. Effect of follicle age on conception rate in beef heifers. *J Anim Sci*, 92:1022-1028.

Abreu FM, Geary TW, Cruppe LH, Madsen CA, Jinks EM, Pohler KG, Vasconcelos JLM, Day ML. 2014b. The effect of follicle age on pregnancy rate in beef cows. *J Anim Sci*, 92:1015-1021.

Araújo RR, Ginther OJ, Ferreira JC, Palha MM, Beg MA, Wiltbank MC. 2009. Role of follicular estradiol-17beta in timing of luteolysis in heifers. *Biol Reprod*, 81:426-437.

Associação Brasileira de Inseminação Artificial (ASBIA). 2017. [WWW Document]. Available on: http://www.asbia.org.br/novo/relatorios/.

Barbuio JP, Girotto RW, Marques MO, Penteado L, Rezende RG, Mingoti RD, Vieira LM, Colli MHA, Baruselli PS. 2016. Pregnancy rate of lactating Nelore cows synchronized with progesterone devices or new progestogen implants or used for eight or nine days. *Anim Reprod*, 13:419. (abstract).

Baruselli PS. 2016. IATF supera dez milhões de procedimentos e amplia o mercado de trabalho. *Rev CFMV*, 69:57-60.

Bó GA, de la Mata JJ, Baruselli PS, Menchaca A. 2016. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. *Theriogenology*, 86:388-396.

Bridges GA, Mussard ML, Hesler LA, Day ML. 2014. Comparison of follicular dynamics and hormone concentrations between the 7-day and 5-day CO-Synch + CIDR program in primiparous beef cows. *Theriogenology*, 81:632-638.

Caccia M, Bó GA. 1998. Follicle wave emergence following treatment of CIDR-B implanted beef cows with estradiol benzoate and progesterone. *Theriogenology*, 49:341 (abstract).

Carvalhaes RPA, Colli MHA, Almeida TT, Mingoti RD, Vieira LM, Santos MH, Sales JNS, Baruselli PS. 2016. Pregnancy rate following FTA of nelore (*Bos indicus*) cows submitted to protocols of 3 or 4 cattle handling with administration of PGF in different moments. *Anim Reprod*, 13:432. (abstract).

Carvalho JBP, Carvalho NAT, Reis EL, Nichi M, Souza AH, Baruselli PS. 2008. Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology*, 69:167–175.

Cerri RL, Rutigliano HM, Chebel RC, Santos JEP. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. *Reproduction*, 137:813-823.

Colli MHA, Carvalhaes RPA, Mingoti RD, Vieira LM, Rezende RG, Santos MH, Sales JNS, Baruselli PS. 2016. Pregnancy rate to FTAI of Crossbred heifers (B. indicus x B. taurus) submitted to protocols of 3 or 4 handling cattle. *Anim Reprod*, 13:425. (abstract).

Colli MHA, Rezende RG, Elliff FM, Zanatta GM, Mingoti RD, Gonçales Junior WA, Ferreira RM, Baruselli P.S. 2017. Factors influencing the pregnancy rate of 14 month old Nelore heifers submitted to 3 consecutive TAI. *Anim Reprod*, 14. (abstract).

Crepaldi GA, Freitas BG, MIngoti RD, Colli MHA, Gonçales Junior WA, Ferreira RM, Baruselli PS. 2017. Reproductive efficiency of Nelore cows submitted to three different reproductive strategies in a 64 days breeding season. *Anim Reprod*, 14. (abstract).

Cutaia L, Tribulo R, Tegli J, Moreno D, Bó GA. 2002. The use of estradiol and progesterone inserts during mid-diestrus to synchronize return to estrus in beef cows and heifers. *Theriogenology*, 58:373. (abstract).

Elliff FM, Rezende RG, Carneiro TO, Barreto AENP, Reis EL, Consentini, CEC, Mingoti RD, Belli RS, Baruselli PS. 2017. Effect of time of permanence (7 vs. 8) of different intravaginal progesterone devices (PRIMER® Multidose or Monodose) on conception rate of dairy cows. *Anim Reprod*, 14. (abstract).

El-Zarkouny SZ, Stevenson JS. 2004. Resynchronizing estrus with progesterone or progesterone plus estrogen in cows of unknown pregnancy status. *J Dairy Sci*, 87:3306-3321.

Freitas BG. 2015. Influência do desenvolvimento corporal na resposta aos programas de sincronização para inseminação artificial em tempo fixo em novilhas Nelore de 14 meses de idade. São Paulo, SP: Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo. Dissertation.

Garcia MR, Amstalden M, Williams SW, Stanko RL, Morrison CD, Keisler DH, Nizielski SE, Williams GL. 2002. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. *J Anim Sci*, 80;2158-2167.

Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, Roberts AJ, Waterman RC, Alexander LJ, Geary TW. 2013. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. *J Anim Sci*, 91:1176-1185.

Lonergan P. 2011. Influence of progesterone on oocyte quality and embryo development in cows. *Theriogenology*, 76:1594-1601.

Mantovani AP, Reis EL, Gacek F, Bó GA, Binelli M, Baruselli PS. 2005. Prolonged use of a progesterone-releasing intravaginal device (CIDR®) for induction of persistent follicles in bovine embryo recipients. *Anim Reprod*, 2:272-277.

Mantovani AP, Nichi M, Sá Filho MF, Ayres H, Vettorato LF, Bó GA, Baruselli PS. 2010. Follicular growth and plasma progesterone patterns in *Bos indicus* x *Bos taurus* heifers submitted to different PGF2 α/progesterone-based synchronization protocols. *Anim Reprod*, 7:91-96.

Marques MO, Ribeiro Júnior M, Silva RCP, Sá Filho MF, Vieira LM, Baruselli PS. 2012. Ressincronização em bovinos de corte. *In*: 5º Simpósio Internacional de Reprodução Animal Aplicada (SIRAA), 2012, Londrina, PR, Brazil. Londrina: UEL. pp. 82-92

Marques MDO, Morotti F, Silva CB, Ribeiro Júnior M, Silva RCP, Baruselli PS, Seneda MM. 2015. Influence of category - heifers, primiparous and multiparous lactating cows − in a large-scale resynchronization fixed-time artificial insemination program. *J Vet Sci*, 16:367-371.

Martins CM, Elliff FM, Ferreira RM, Mingoti RD, Reis PO, Baruselli PS. 2017. Factors that affect pregnancy rate to TAI and to natural breeding of 14 months old Nelore heifers . *Anim Reprod*, 14. (abstract).

Mendanha MF, Sala RV, Reis TANPS, Carvalho FJ, Guimarães LHC, Macedo GG, Sá Filho MF, Baruselli PS. 2012. Momento da administração de prostaglandina e número de utilizações do dispositivo de progesterona na eficiência da IATF em novilhas girolando. *In*: Anais da XXVI Reunião Anual da Sociedade Brasileira de Tecnologia de Embriões (SBTE), Fóz do Iguaçu, PR, Brazil.Foz do Iguaçu: SBTE. pp. 370. (abstract).

Meneghetti M, Sá Filho OG, Peres RFG, Lamb GC, Vasconcelos JLM. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows I: basis for development of protocols. *Theriogenology*, 72:179-189.

Mihm M, Curran N, Hyttel P, Knight PG, Boland MP, Roche JF. 1999. Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in heifers. *J Reprod Fertil*, 116:293-304.

Mingoti RD, Freitas BG, Ramos RS, Bastos MR, Teixeira AA, Rezende MLG, Castro MW, Faquim A, Sá Filho MF, Baruselli PS. 2016. Pregnancy rate to TAI Nelore (*Bos indicus*) protocol submitted to 3 or 4 managements using Sincrogest or CIDR New and Reused. *Anim Reprod*, 13:415. (abstract).

Motta JCL, Colli MHA, Penteado L, Bayeux BM, Mingoti RD, Bó GA, Lugo LC, Rezende RG, Baruselli PS. 2016. Pregnancy rate to FTAI in Nelore and crossbreed heifers submitted to J-Synch protocol (6 days). *Anim Reprod*, 13:401. (abstract).

Munro RK, Moore NW. 1985. Effects of progesterone, oestradiol benzoate and cloprostenol on luteal function in the heifer. *J Reprod Fertil*, 73:353–359.

Nogueira GP. 2004. Puberty in South American *Bos indicus* (Zebu) cattle. *Anim Reprod Sci*, 82/83:361-372.

Penteado L, Rezende RG, Mingoti RD, Colli MHA, Sá Filho MF, Santos FB, Lemos Motta JC, Bayeux BM, Vieira LM, Baruselli PS. 2016. Pregnancy rate of Nelore cows submitted to resynchronization starting 14 or 22 days after prior FTAI. *Anim Reprod*, 13:450. (abstract).

Pereira MH, Sanches CP, Guida TG, Rodrigues AD, Aragon FL, Veras MB, Borges PT, Wiltbank MC, Vasconcelos JL. 2013. Timing of prostaglandin F2α treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. *J Dairy Sci*, 96:2837-2846.

Peres RFG, Claro Junior I, Sá Filho OG, Nogueira GP, Vasconcelos JLM. 2009. Strategies to improve fertility in *Bos indicus* postpubertal heifers and nonlactating cows submitted to fixed-time artificial insemination. *Theriogenology*, 72:681-689.

Pessoa GA, Martini AP, Chaiben MFC, Vieira LM, Girotto RW, Pugliesi G, Santin T, Rubin MIB, Baruselli PS, Sá Filho MF. 2015. Adjustment of the estradiol benzoate dose in the resynchronization protocol with unknown pregnancy status in suckled beef cows. *Anim Reprod*, 12:, 610. (abstract).

Pugliesi G, Miagawa BT, Paiva YN, França MR, Silva LA, Binelli M. 2014. Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: how early can we detect pregnancy? *Biol Reprod*, 91:95, 1-12.

Pugliesi G, Rezende RG, Silva JCB, Lopes E, Nishimura TK, Baruselli PS, Madureira EH, Binelli M. 2017. Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. *Rev Bras Reprod Anim*, 41:140-150.

Rezende RG, Freitas BG, Mingoti RD, Colli MHA, Carvalho JBP, Sá Filho MF, Motta JCL, Macedo GG, Baruselli PS. 2016. Follicular dynamics of Nelore cows submitted to resynchronization 14 days after TAI using injectable P4 for synchronization of follicular wave. *Anim Reprod*, 13:375. (abstract).

Roche JF, Austin EJ, Ryan M, O'Rourke M, Mihm M, Diskin MG. 1999. Regulation of follicle waves to maximize fertility in cattle. *J Reprod Fertil Suppl*, 54:61-71.

Sá Filho MF, Ayres H, Ferreira RM, Marques MO, Reis EL, Silva RCP, Rodrigues CA, Madureira EH, Bó GA, Baruselli PS. 2010. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (*Bos indicus*) cows. *Theriogenology*, 73, 651-658.

Sá Filho MF, Penteado L, Reis EL, Reis TANPS, Galvão KN, Baruselli PS. 2013. Timed artificial insemination early in the breeding season improves the reproductive performance of suckled beef cows. *Theriogenology*, 79:625-632.

Sá Filho MF, Marques MO, Girotto R, Santos FA, Sala RV, Barbuio JP, Baruselli PS. 2014. Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle. *Theriogenology*, 81:284-290.

Santos, MH. 2016. Desenvolvimento de protocolos para IATF com 7 dias de permanência do CIDR® em fêmeas Nelore. 80f. Dissertation (Masters in Science) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Pirassununga, 2016.

Siqueira LG, Areas VS, Ghetti AM, Fonseca JF, Palhao MP, Fernandes CA, Viana JH. 2013. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. *J Dairy Sci*, 96:6461-6472.

Stevenson JS, Cartmill JA, Hensley BA, El-Zarkouny SZ. 2003. Conception rates of dairy cows following early not-pregnant diagnosis by ultrasonography and subsequent treatments with shortened Ovsynch protocol. *Theriogenology*, 60:475-483.

Thatcher WW, Bazer FW, Sharp DC, Roberts RM. 1986. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pigs and horses. *J Anim Sci*, 62:25-46.

Vasconcellos GSFM, Vieira LM, Guerreiro BM, Freitas BG, Kametani MK, Miranda MS, Moreira RC, Vianna FP, Baruselli PS. 2014. Evaluation of the resynchronization efficiency using GnRH or estradiol benzoate at the beginning of the protocol in lactating Holstein cows. *Anim Reprod*, 11:361. (abstract).

Vieira LM, Sá Filho MF, Pugliesi G, Guerreiro BM, Cristaldo MA, Batista EOS, Freitas BG, CarvalhoFJ, Guimaraes LHC, Baruselli PS. 2014. Resynchronization in dairy cows 13 days after TAI followed by pregnancy diagnosis based on corpus luteum vascularization by color doppler. *Anim Reprod*, 11:378. (abstract).

Vieira LM, Guerreiro BM, Batista EOS, Freitas BG, Sá Filho MF, Vasconcellos GSFM, Soares JG, Bragança LGM, Plá SS, Baruselli PS. 2015. Estradiol, GnRH or their association at the beginning of the resynchronization protocol results in similar reproductive efficiency in dairy cows. *Anim Reprod*, 12:635. (abstract).