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Update and overview on assisted reproductive technologies (ARTs) in Brazil

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Abstract

The impressive increase in the use of assisted reproductive technologies (ARTs), especially in cattle, during the last few years in Brazil is well known worldwide. In 2015, there were over 13.7 million artificial inseminations (AI), of which, about 77% were carried out using fixed-time AI (FTAI). This technology has helped to substantially improve reproductive efficiency in beef and dairy cattle. In relation to embryo transfer, production of *in vivo* derived (IVD) embryos remained relatively stable, with average production of 30-40,000 embryos per year, whereas *in vitro* production (IVP) of embryos had a substantial increase, from about 12,500 IVP embryos in 2000 to more than 300,000 IVP embryos after 2010. The increasing availability and use of sex-sorted sperm was one of the factors responsible for a recent shift from the predominance of IVP embryos from beef breeds to dairy breeds in Brazil. Moreover, there was also an increase from 13% in 2014 to 29% in 2015 in the percentage of vitrified/frozen embryos. Moreover, the successful use of protocols for fixed-time ET (FTET) due to their high efficiency and ease of implementation, has facilitated the dissemination of ET programs all over Brazil. However, there is room for improvement, since there are several reports of high pregnancy loss and high peripartum loss, when IVP embryos are used. The production of healthy cattle by somatic cell nuclear transfer has also increased in the last few years in Brazil, but despite substantial progress in reducing postnatal losses, no drastic increase in cloning efficiency up to parturition has occurred.

Keywords: artificial insemination, bovine, embryo, *in vitro* production, superovulation.

Introduction

Currently, Brazilian cattle industry has one of the largest commercial herds in the world, about 208.3 million head (Associação Brasileira das Indústrias Exportadoras de Carne - ABIEC, 2014). Brazil produced 10.7 million tons of beef in 2014 (ABIEC, 2014), being second place in the world ranking of meat production. Moreover, the dairy herd in Brazil ranks in the fifth position worldwide (Food Agriculture Organization of United Nations - FAO, 2012). Despite the magnitude of the herd, the annual Brazilian production of milk in 2014 was 24.741 billion liters, with a productivity of only 1,380 L of milk/cow/year (Instituto Brasileiro de Geografia e Estatística - IBGE, 2014). This is obviously very low production if compared, for example, with data from the USA herd (10,096 L of milk/cow/year), currently the largest producer of milk in the world (United States Department of Agriculture - USDA, 2014). However, both Brazilian beef and dairy productivity is increasing, which is directly related to technological advances in animal breeding, such as greater use of artificial insemination (AI) and embryo transfer (ET).

To have an idea on the evolution of these biotechnologies, in 2002 only 5-6% of heifers and cows were artificially inseminated in Brazil, about 7 million AIs, with only 1% of inseminations being through fixed-time artificial insemination (FTAI). In contrast, in 2015, about 13 million AIs were performed corresponding to 10-12% of females of reproductive age and 77% of these inseminations were performed by FTAI (Pietro Baruselli, 2016; School of Veterinary Medicine and Animal Science, USP, São Paulo, SP, Brazil; unpublished).

In relation to embryo production in cattle, there are two different scenarios. While production of *in vivo* derived (IVD) embryos remained relatively stable over the last 15 year, with average production of 30-40,000 embryos per year, the *in vitro* production (IVP) of embryos had a substantial increase from about 12,500 IVP embryos in 2000, to over 348,000 IVP embryos in 2014, representing almost 60% of the world embryo production.

Sex-sorted sperm has been widely and increasingly used in Brazil, especially for AI or IVP. Unfortunately, epidemiological data on the use of sex-sorted sperm in Brazil are not available. Regarding IVP, data from the last 3 years from one of the main labs in Brazil confirm other data from the literature that there is a reduction in embryo production per cultured oocyte if sex-sorted sperm is used for *in vitro* fertilization when compared with conventional unsorted sperm (23.6% [311,788/1,323,541] *vs*. 28.5% [242,259/848,939]; $P < 0.01$).

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For cloning, data from the Brazilian Association of Zebu Breeders (ABCZ) show a gradual increase in registered *Bos indicus* calves (predominantly of Nelore and Gir breeds) produced by somatic cell nuclear transfer (SCNT) during the years 2010 (n = 5), 2011 (n = 23), 2012 (n = 22), and 2013 (n = 41). Unofficial data indicate a continuous increase in number of healthy calves produced by SCNT from 2014 to 2016.

This manuscript aims to present an update and overview of the assisted reproduction technologies (ARTs) in Brazil focused on AI and ET in cattle and to describe reports on how these technologies have positively influenced the reproductive efficiency of dairy and beef herds.

Artificial insemination

As mentioned above, Brazil has one of the largest cattle herds in the world; however the use of AI is still low. In 2015, there were over 13.7 million inseminations, which correspond to 10-12% of cows and heifers of reproductive age (Fig. 1). Out of this total AIs, about 4.7 million were performed in dairy cows, with a decrease of 12.4% compared with the previous year. In beef cattle, 9 million inseminations were performed, with an increase of 16.2% in relation to 2014. In 2015, more than 10.5 million FTAIs were performed, with an increase of 11.2% compared to 2014, and FTAI now represents ~77% of all AIs carried out in Brazil (Fig. 1). These data demonstrate that FTAI is increasing the use of AI across Brazil with a doubling in the overall use of AI during the last decade, but over a 10-fold increase in the use of FTAI from ~1 million protocols in 2005 (11% of all AIs) to 10.5 million protocols in 2015 (77% of all AIs).

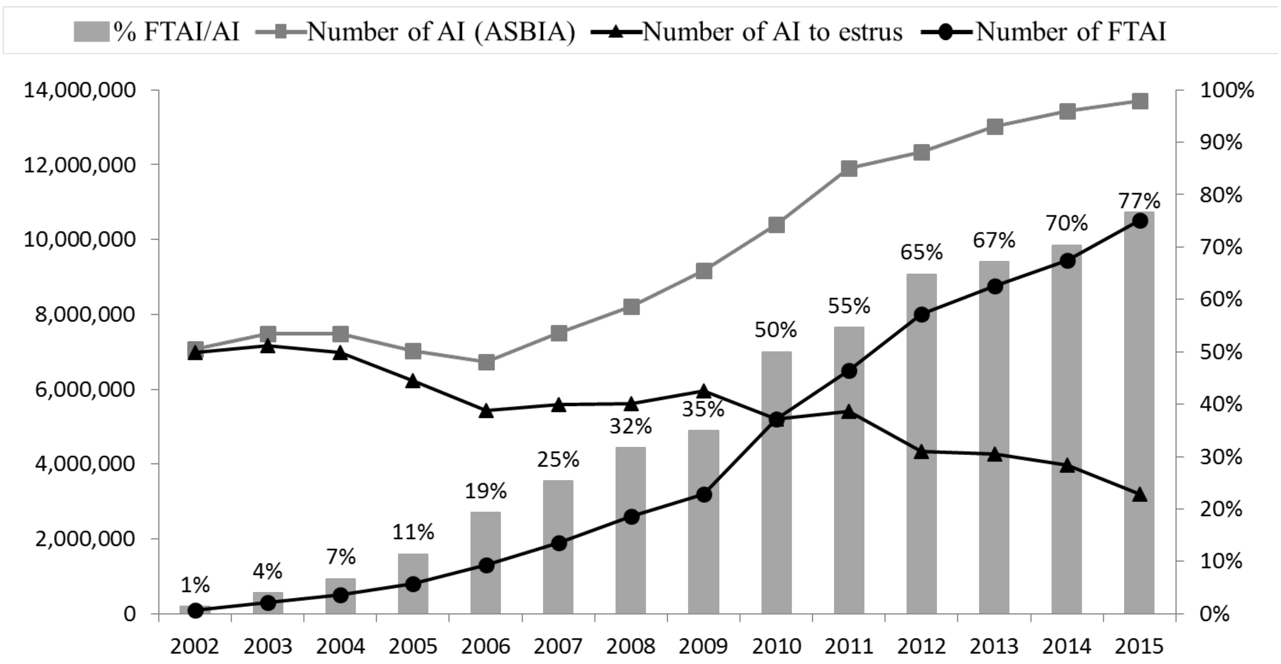

Figure 1. Data of artificial insemination (AI) based on cows and heifers bred to estrus or to fixed-time AI (FTAI) systems in Brazil during the period of 2002 to 2015. Numbers of FTAI were estimated based on hormones/products sold for each FTAI protocol.

Use of FTAI in dairy cattle

Although most dairy cows and heifers are bred by bulls in Brazil, AI is the preferred ART for most progressive dairy farms. When AI is employed, the question practitioners and producers ask is whether they should breed cows to estrus or FTAI. In fact, this doubt is understandable because studies that properly compared insemination to estrus *vs*. insemination to a FTAI protocol have described lower (Strickland *et al*., 2010; Carvalho and Fricke, 2016; University of Wisconsin-Madison; unpublished), similar (Rabiee *et al*., 2005; Nascimento *et al*., 2013b), or greater (Nascimento *et al*., 2013a) pregnancies per AI (P/AI) when cows are bred to estrus. However, suboptimal estrus detection rates in cycling cows (Lopez *et al*., 2004; Fricke *et al*., 2014) and a substantial percentage (~24%) of cows that are not cycling (Wiltbank *et al*., 2002; Santos *et al*., 2009), produces the problem of low service rates (SR) and, in general, lower 21-days pregnancy rates (21-day PR = P/AI x SR, every 21 days after the voluntary waiting period; VWP) for cows bred to estrus than cows bred to FTAI (Nascimento *et al*., 2013a; Wiltbank and Pursley, 2014).

In order to evaluate the impact of intensifying the use of FTAI on reproductive efficiency in a dairy herd in Brazil, an analysis of 4,512 AIs (1,688 in primiparous and 2,824 in multiparous cows) was performed between 2009 and 2014. These data were from a dairy farm, managed in a free stall system with a yearly rolling herd average milk yield of 10,700 kg during the period. Based on changes in the reproductive management strategy, data were compared between the times before (year 2009-2011) and after (year 2012-2013) intensifying the use of FTAI. Before the more intensive reproductive management program, cows received two treatments with prostaglandin F2α (PGF2α) at ~40 and ~54 days in milk (DIM) and were bred if detected in estrus from 40 to 72 DIM. During this time cows were visually checked for standing estrus twice a day combined with use of pedometers as an estrus detection aid. Cows not bred by ~73 DIM were then enrolled in a FTAI protocol. Pregnancy diagnosis was conducted every 14 days. In 2012 and 2013, cows received one PGF2α treatment at ~40 DIM and were bred to any detected estrus until ~54 DIM, when cows that were not inseminated were then enrolled in a FTAI protocol. Pregnancy diagnosis was conducted every 7 days. In both situations, even after AI to estrus or to FTAI, cows observed in estrus were inseminated. The main FTAI protocol used during the period of the study was the following. Day-10: Progesterone insert + 2 mg estradiol benzoate (EB) or 100 µg GnRH, D-3: 500 µg cloprostenol sodium; Day-2: P4 insert removal + 500 µg cloprostenol sodium + 1.0 mg estradiol cypionate (ECP), D0: FTAI (Melo *et al*., 2016).

When reproductive management was intensified, the proportion of cows inseminated by FTAI increased (P < 0.01) from 29.1% (559/1920) to 56.9% (1474/2592), and cows were inseminated earlier (Fig. 2).

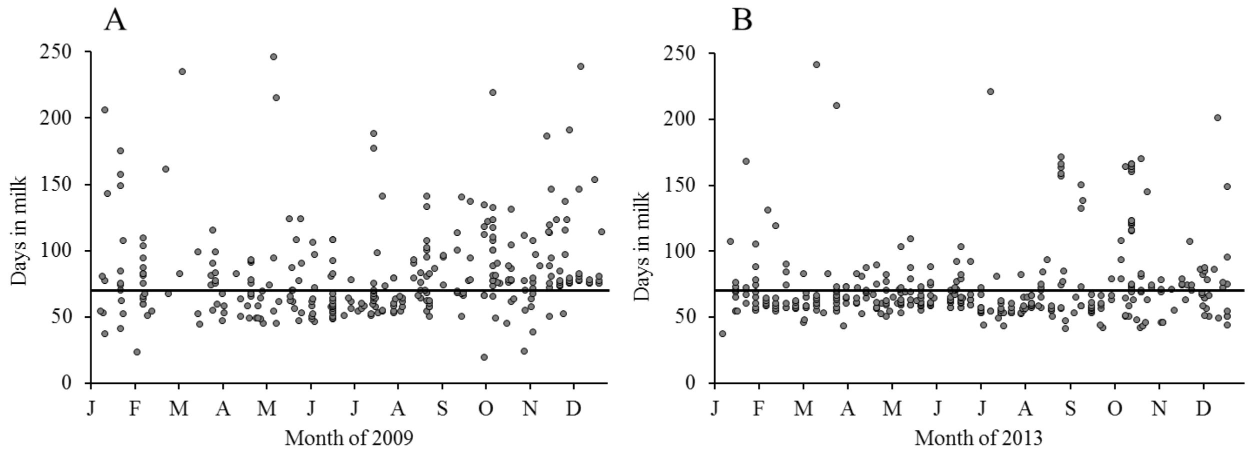

Figure 2. Distribution of first postpartum AI according to days in milk (DIM) in lactating dairy cows receiving reproductive management strategies before (year 2009); A), or after (year 2013); B) intensifying the use of FTAI. Horizontal lines represent 70 DIM.

Data from a survival analysis show that after intensifying the use of FTAI, cows were inseminated for the first time earlier (P < 0.01; Fig. 3A) and became pregnant sooner (P < 0.01; Fig. 3B).

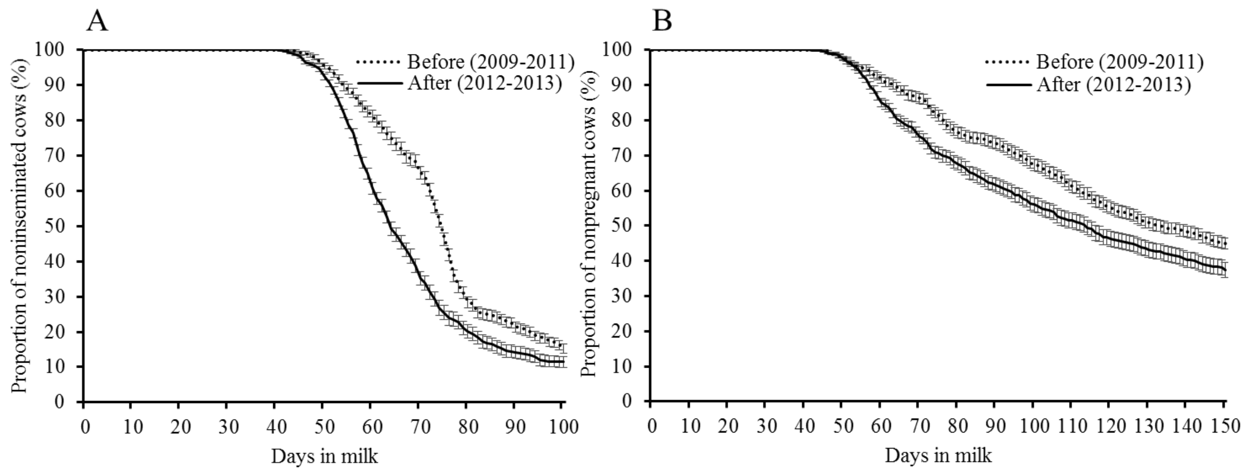

Figure 3. Survival curve by days in milk for proportion of noninseminated (A; P < 0.01) and nonpregnant (B; P < 0.01) dairy cows receiving reproductive management strategies before (year 2009-2011), or after (year 2012-2013) intensifying the use of FTAI.

The results related to the reproductive performance of cows are shown in Table 1. There was a significant decrease in the proportion of cows not inseminated by 70 DIM after the intensification of FTAI, resulting in more cows pregnant by 103 DIM. Moreover, with the more intensive use of FTAI during 2012 and 2013, overall fertility also increased, as seen by greater P/AI at 30 and 60 days, with no change in pregnancy loss (Table 1). This improved P/AI may be resulting from several factors, such as better cow comfort, health and nutrition, but especially due to improvements in the FTAI protocol (Binelli *et al*., 2014).

Table 1. Proportion of noninseminated cows at 70 days in milk (DIM) and proportion of pregnant cows at 103 DIM, pregnancy/AI, and pregnancy loss in dairy cows receiving reproductive management strategies before (year 2009-2011), or after (year 2012-2013) intensifying the use of FTAI.

	Before (2009-2011)	After (2012-2013)	P value
Noninseminated cows at 70 DIM, % (n/n)	64.8% (374/577)	35.0% (314/898)	<0.01
Cows pregnant at 103 DIM[a], % (n/n)	34.2% (184/538)	45.4% (408/899)	<0.01
Pregnancy/AI, % (n/n)			
31 days	27.9% (539/1,920)	37.1% (903/2,592)	<0.01
59 days	23.8% (463/1,920)	32.4% (777/2,592)	<0.01
Pregnancy loss between 31 and 59 days, % (n/n)	14.1% (76/539)	14.0% (126/903)	0.99

[a]Equivalent to three estrous cycles after the voluntary waiting period.

There was a major effect of intensification of FTAI on 21-days PR (Fig. 4), which increased linearly throughout the evaluated years, resulting in a decrease of approximately 35 days (from 180 days in 2009 to 145 days in 2013) on days open, or time from calving to conception. This improved 21-days PR was a result of greater SR associated with increased P/AI as reproductive management was progressively intensified (Table 1).

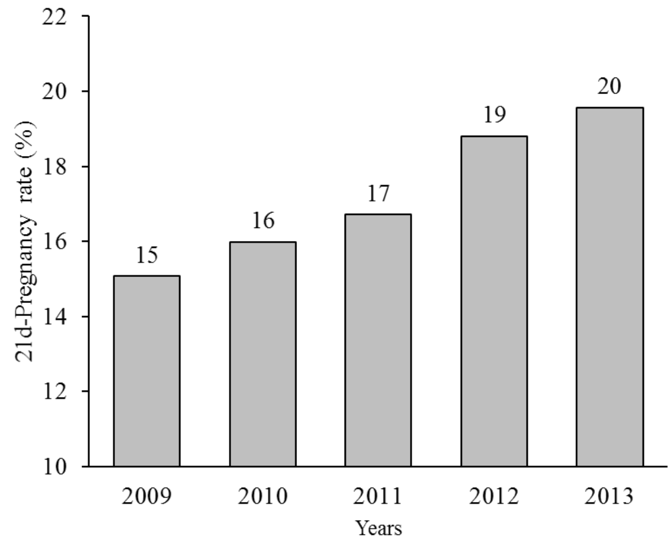

Figure 4. Results of 21-days pregnancy rate throughout the years in dairy cows receiving reproductive management strategies before (year 2009-2011), or after (year 2012-2013) intensifying the use of FTAI.

Thus, the intensive use of FTAI improved reproductive efficiency on this farm and this appears to be the best current alternative for other dairy farms in Brazil. We have also analyzed a large database of AI and FTAI from eight Brazilian dairy farms that were using a typical reproductive management strategy for Brazilian dairy herds and the results were very similar to those observed on the example farm above, prior to intensification of the reproductive management strategy [i.e., P/AI at 30 days = 29.0% (10029/34472), P/AI at 60 days = 24.9% (4076/16315), and pregnancy loss between 30 and 60 days = 14.7% (706/4782)]. Moreover, high pregnancy loss between 30 days and calving [28.2% (2832/10029)] and low birth rates [20.8% (7197/34472)] are of major concern, which may justify, even more, the intensification of reproductive management.

Use of FTAI in beef cattle

Most of beef cattle herds in Brazil are composed of *Bos indicus* and it is noteworthy that zebu cattle have longer postpartum anestrus and low body condition score (BCS) when kept on pasture (Bó *et al.*, 2003), resulting in economic losses because of the increased interval from calving to conception and reduced P/AI (Bó *et al.*, 2007). In a pasture-based cow-calf production system, the use of reproductive programs, such as synchronization of ovulation for FTAI (synchronization protocols based on P4 and E2), is essential to produce high pregnancy rates (PR) in the breeding season and it has been increasingly incorporated in cow-calf operations (Pessoa *et al.*, 2016).

Data generated by the GERAR group (Specialized Group in Applied Reproduction to the Herd; created by a partnership between the School of Veterinary Medicine and Animal Science, São Paulo State University in Botucatu, and Zoetis, São Paulo) that is composed of more than 250 Brazilian technicians which discuss innovations and results for FTAI, show the evolution of P/AI from 2007 to 2015 in millions of heifers and cows submitted to FTAI (Fig. 5; Table 2). The main FTAI protocol used during the period of the study was the following. Day-11: Progesterone insert + 2 mg EB, D-4: 12.5 mg dinoprost tromethamine, Day-2: P4 insert removal + 0.6 mg ECP + 300 IU equine chorionic gonadotropin (eCG) or calf removal for 48 h, Day-0: FTAI (Meneghetti *et al.*, 2009; Peres *et al.*, 2009; Sá Filho *et al.*, 2009).

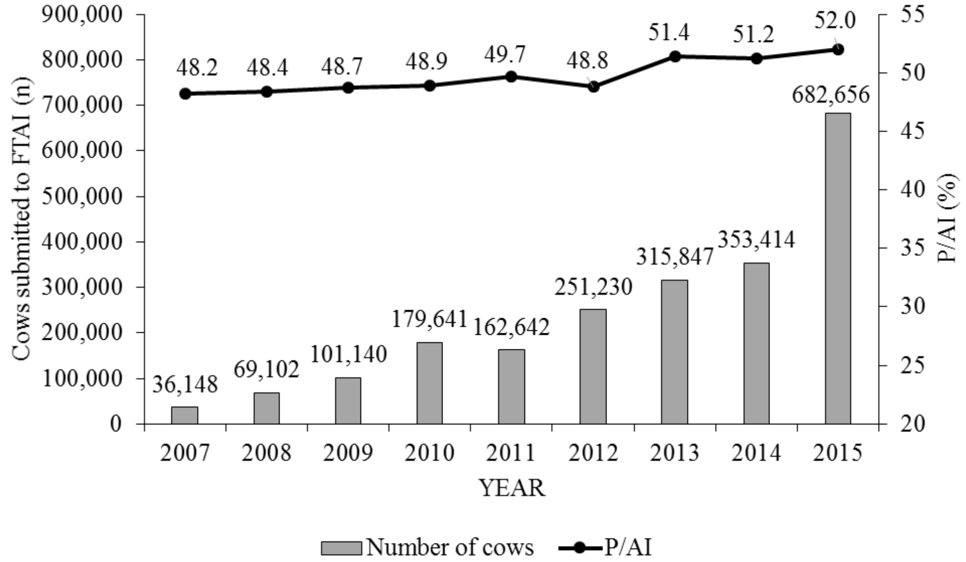

Figure 5. Number of beef heifers and cows submitted to FTAI and P/AI between 2007 and 2015.

Table 2. Pregnancy per AI of heifers, primiparous, multiparous and non-lactating beef cows submitted to FTAI between 2007 and 2015.

Year	Heifers %, (n)	Primiparous%, (n)	Multiparous %, (n)	Non-lactating, % (n)
2007	39.6% (3,037)	44.5% (5,249)	49.7% (22,519)	45.1% (1,510)
2008	44.8% (4,944)	42.6% (9,763)	50.9% (44,628)	45.6% (5,354)
2009	50.5% (8,347)	43.4% (15,476)	49.9% (70,308)	46.5% (5,526)
2010	39.7% (24,372)	48.5% (18,819)	50.7% (123,380)	49.4% (9,566)
2011	49.3% (21,810)	41.6% (22,453)	51.2% (105,440)	52.0% (11,076)
2012	47.1% (42,030)	44.1% (32,345)	50.2% (130,236)	52.1% (10,252)
2013	49.0% (58,032)	47.8% (42,467)	53.1% (189,726)	50.1% (24,432)
2014	46.8% (56,026)	48.0% (47,882)	53.0% (200,082)	50.8% (26,091)
2015	48.5% (124,687)	47.1% (80,690)	54.1% (392,511)	51.5% (69,734)

As shown in Table 2, the fertility in all types of beef cattle has been relatively constant (~50%) during the last 3 years. Nevertheless, there is likely to be room for improvement in many of the herds since some herds (~24%) had average P/AI greater or equal to 60% (Fig. 6). These herds are likely to have more intensive reproductive programs, better nutrition with fewer cows with low BCS (Fig. 7), and may use cattle with better fertility traits, such as Nelore X Angus crossbreds (Fig. 8).

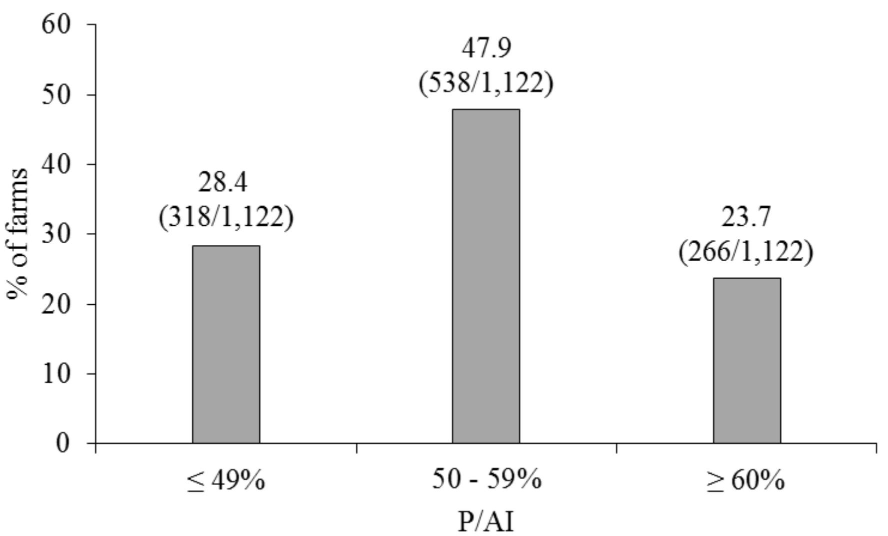

Figure 6. Distribution of farms according to P/AI of beef cows submitted to FTAI in 2015.

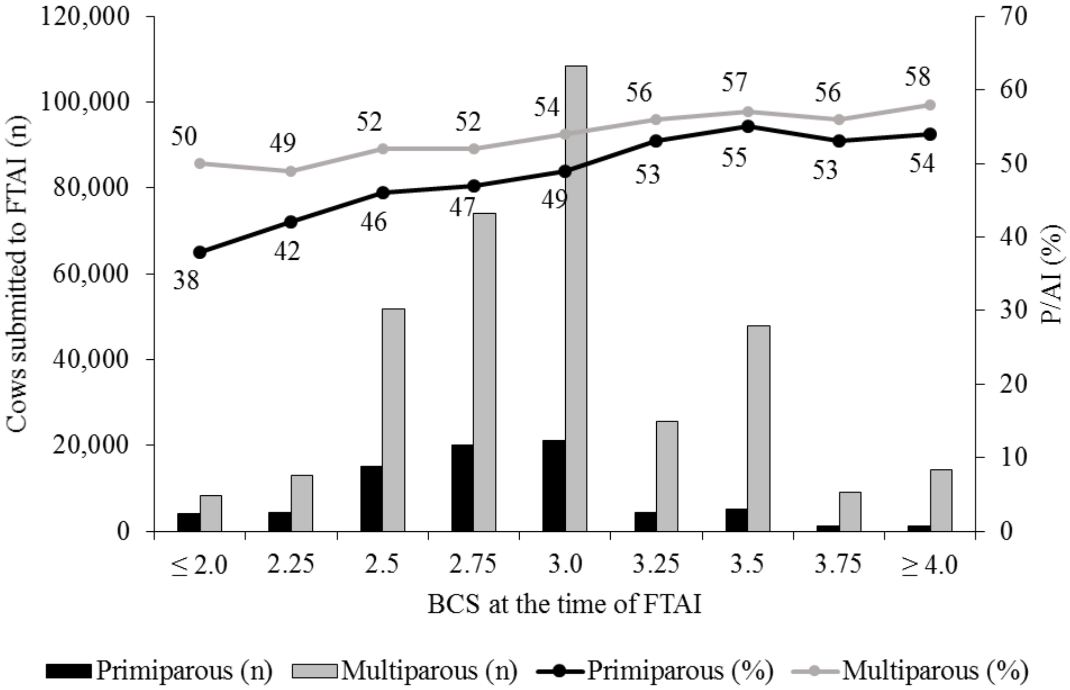

Figure 7. Number and P/AI of primiparous and multiparous beef cows submitted to FTAI according to BCS.

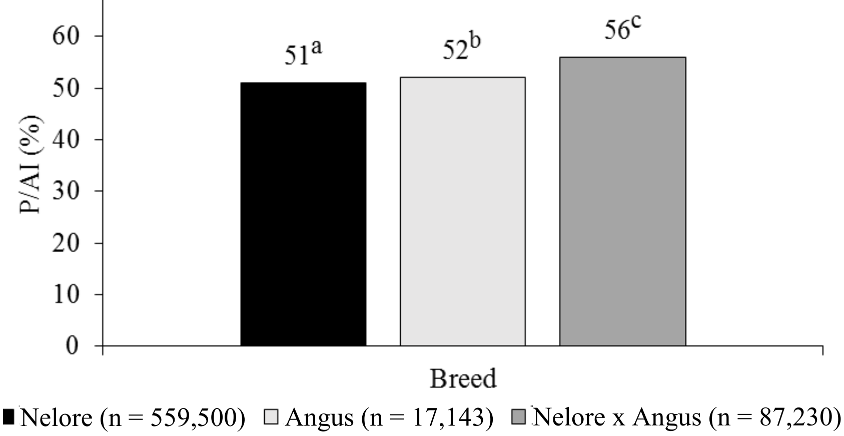

Figure 8. Breed effect on P/AI of cows submitted to FTAI. [a,b,c]$P < 0.01$.

Embryo transfer

Evolution of the embryo industry in Brazil from 1995 to 2014

One of the most remarkable aspects of the use of ARTs in Brazil was the evolution of the cattle embryo industry during the last 15 years, particularly the emergence and later widespread use of IVP. In the early 90's, the Brazilian embryo industry was already substantial, and the country was the largest embryo producer, outside Europe and North America. However, the adoption of IVP after the year 2000 boosted the embryo industry, and since 2005, Brazil accounts for more than 20% of the world embryo production. In 2014, Brazil produced 348,468 embryos *in vitro*, which corresponds to 59.0% of the total world IVP (Perry, 2015).

The success of IVP in Brazil was due to a complex interplay of technical and economic factors that likely explain why it initially diverged from the trends elsewhere (Faber *et al*., 2003). Initially, in the period from the emergence of the first commercial IVP companies in 1999 to 2003, there was a relatively high cost and low efficiency of IVP (Hasler, 2000), but this was balanced by the high commercial value of the donors used. Thus, during this initial growth phase (first phase) IVP expanded mainly within the market of high genetic merit cows and the number of both IVD and IVP embryos increased similarly (Fig. 9).

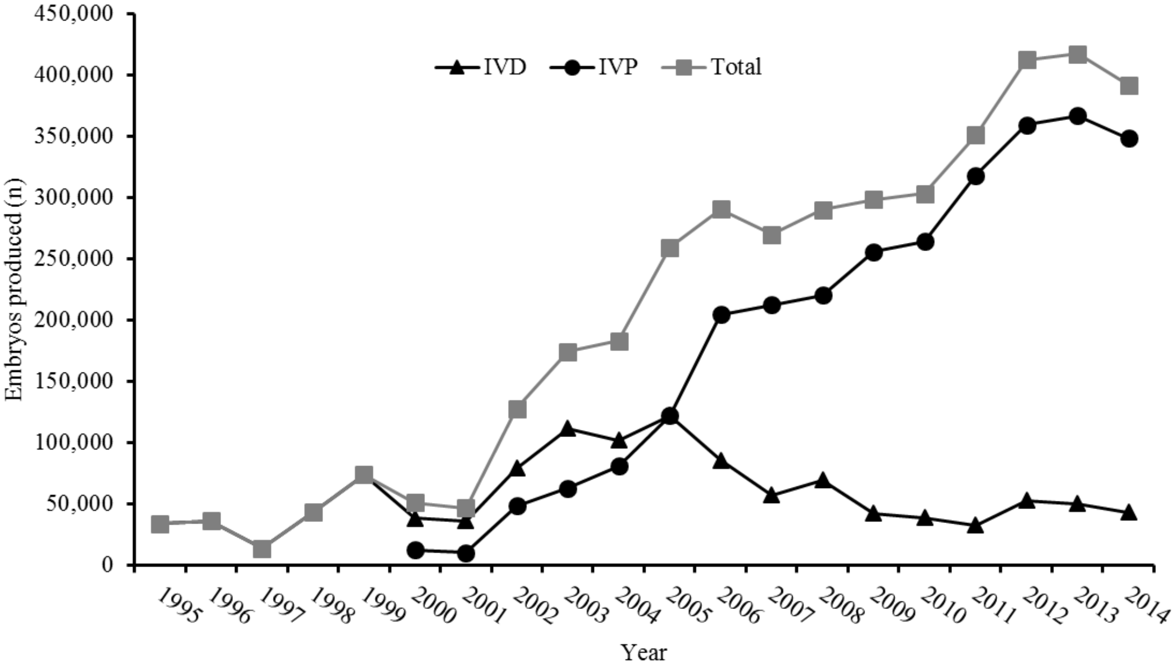

Figure 9. Production of bovine embryos in Brazil, according to the technique employed, during the period of 1995 to 2014. IVD: embryos produced by superovulation (*in vivo*); IVP: embryos produced *in vitro*.

A second phase of growth in the use of IVP embryos occurred between 2003 and 2010, driven largely by a shift from the production of embryos from high genetic merit animals to the production of replacement bulls. Prior to this second phase, the large size of beef cattle population in Brazil and the relatively low use of AI at that time (~6%; Baruselli *et al*., 2012) resulted in a repressed demand for such animals (bulls), especially in the Nelore breed. However at the peak of this second growth phase, in 2005, embryo production in Nelore (214,500) accounted for 82.7% of all embryos produced in the country, and for 90.0% of the embryos from beef breeds (Viana *et al*., 2012). Meanwhile, embryo prices began to decrease in Brazil due to many factors including: increasing efficiency of embryo production protocols, increased recovery of cumulus-oocyte complexes (COC) and greater blastocyst rates obtained in *Bos indicus* breeds (Pontes *et al*., 2009; Viana *et al*., 2012), and an increase in the scale of embryo production in commercial embryo production companies in Brazil. The IVP industry became more competitive, and eventually replaced multiple ovulation and embryo transfer (MOET) as the technique of choice for embryo production. Total embryo production increased rapidly, reaching numbers over 250,000 for the years after 2005.

We are currently in the midst of the third growth phase with increasing use of sex-sorted sperm in IVP, which occurred mainly in dairy breeds. In dairy breeds, production of a high percentage of female calves has many economic advantages and use of in IVP allows the production of approximately 90% of the embryos with the desired sex (Morotti *et al*., 2014). Thus, this third growth phase of the Brazilian embryo industry after 2010 has been marked by a clear shift from the predominance of beef breeds to dairy breeds. For example, in 2014, embryo production in dairy breeds increased by 46.5% and the total numbers of embryos produced from dairy breeds exceeded, for the first time, the number of embryos from beef breeds, (270,367 of 391,805, or 69.0% of total embryos). The expansion in the dairy sector also highlighted a new trend in the Brazilian embryo industry, the use of large-scale IVP to produce crossbred calves (Pontes *et al*., 2010). Producers and veterinarians explored the possibilities of obtaining the gains due to heterosis while maintaining herds with specific crossbred values (F1, ¾, etc.). For example, 79.3% of embryos produced in dairy breeds in 2014 were from Gir x Holstein crosses.

The inherent characteristics of dairy production, such as smaller herds and lack of a set breeding season, limits the availability of recipients, and thus required the development and use of cryopreservation alternatives. In 2015, the three main commercial laboratories in Brazil produced more than 276,000 embryos from ~50,000 donors with a blastocyst rate ~30% (more than 1 million oocytes used for IVF). Of those embryos, 111,000 were conventional embryos and 165,000 were produced using sex-sorted sperm and 29% (80,000) of these embryos were vitrified or frozen. In addition, these laboratories reported an increase in embryo production of more than 30% compared to 2014 (211,000 IVP embryos) and the percentage of vitrified/frozen embryos in these laboratories increased from 13% in 2014 to 29% in 2015. The continuing development and use of the direct transfer technique (over 9,000 embryos in 2015) is likely to lead to further increases in the use of cryopreserved IVP embryos. Moreover, the successful use of protocols for fixed-time ET (FTET), due to their high efficiency and ease of implementation, has facilitated the dissemination of ET programs across Brazil.

Use of IVP embryos for reproductive management in dairy cattle

As seen above, the use of IVP embryos in dairy herds has increased in recent years. In 2015, the two largest laboratories that produce embryos from dairy breeds transferred more than 27,000 embryos, obtaining reasonable pregnancies per ET (P/ET), and acceptable pregnancy losses (Table 3), especially when beef cows, crossbreds, or heifers are used as embryo recipients. Moreover, the best IVP embryos are usually selected for vitrification, which may explain the observation of similar pregnancy losses for fresh and vitrified embryos, as presented in Table 3.

Table 3. Pregnancy per ET (P/ET) at 30 and 60 days and pregnancy loss between 30 and 60 days for fresh and vitrified IVP embryos from different dairy breeds in Brazil.

	30 days P/ET %, (n/n)	60 days P/ET %, (n/n)	Pregnancy loss % (n/n)
Gir			
Fresh	46.5 (4322/9294)	42.3 (3933/9294)	9.0 (389/4322)
Vitrified	34.1 (726/2128)	31.3 (667/2128)	8.1 (59/726)
Girolando (5/8 Holstein x 3/8 Gir)			
Fresh	45.1 (2214/4909)	43.1 (2116/4909)	4.4 (98/2214)
Vitrified	32.0 (340/1063)	30.7 (326/1063)	4.1 (14/340)
Holstein			
Fresh	38.2 (2409/6302)	34.4 (2170/6302)	13.3 (320/2409)
Vitrified	37.8 (1033/2735)	34.6 (947/2735)	8.3 (86/1033)
Jersey			
Fresh	35.4 (118/333)	33.6 (112/333)	5.1 (6/118)
Vitrified	40.3 (133/330)	37.8 (125/330)	6.0 (8/133)

However, results can vary from farm to farm, and rigorous evaluation and monitoring are necessary for this technology to be used on a large scale as a substitute for AI or FTAI. The following data describe two cases in which the use of IVP embryos enhanced reproductive efficiency and/or profitability.

The first dairy farm has 1,500 crossbred lactating cows (Girolando [5/8 Holstein x 3/8 Gir] breed) producing more than 25,000 kg of milk per day. The farm uses an intensive ET program, in which all cows receive IVP embryos using sex-sorted sperm in order to increase numbers of genetically-superior calves to be used as replacement heifers or for sale. Fig. 10 shows the number of embryos transferred from 2004 to 2015 in this farm. Between 2004 and 2010, there was a minor increase in embryos transferred, however, after that, there was a continuous increase in the use of ET. Over the past 3 years, more than 85% of calves that were born on this farm were females. Currently, only high-genetic merit cows (top 10%) are used as donors, providing embryos for the entire herd.

In 2015, more than 6,500 embryos were transferred, with acceptable P/ET at 30 days (43%) and 21-days PR (~20%). However, high incidence of pregnancy loss between 30 and 65 days (15%) and between 30 days and birth (30%) is an important issue. In addition, other factors such as low BCS, absence of CL at the beginning of the protocols for fixed-time ET (FTET), and subclinical mastitis affected ($P < 0.05$) P/ET and 21-days PR (Pereira and Coelho, 2016).

In addition, this farm also uses fresh, vitrified, and frozen embryos, and a study was done to compare P/ET among these treatments (Fleury *et al*., 2015). Grade I blastocysts or expanded blastocysts (Stringfellow and Seidel, 1998) were transferred to previously synchronized recipients. The P/ET were 51.4% (133/259) for embryos transferred fresh, 35.9% (84/234) for vitrified, and 42.1% (96/228) for direct transfer embryos. The P/ET obtained from IVP embryos vitrified or frozen were not different between each other, but they were lower than the P/ET obtained when IVP embryos were transferred fresh ($P < 0.05$). Therefore, these results highlighted the aspects of cryopreservation of IVP embryos with the convenience of direct transfer as compared with vitrification.

The second farm has 1,100 lactating cows (Holstein and Girolando breeds) with average milk production of 30 kg/day. The reproductive management consists of use of AI or transfer of IVP embryos. As shown in Table 4, despite having greater pregnancy losses, the IVP technique was chosen as a better reproductive management strategy for this dairy farm, as compared to AI, due to greater P/ET *vs*. P/AI, and greater birth rates for ET *vs*. AI. In addition, the use of sex-sorted sperm for IVF allowed an increased number of heifers born with IVP and greater genetic improvement.

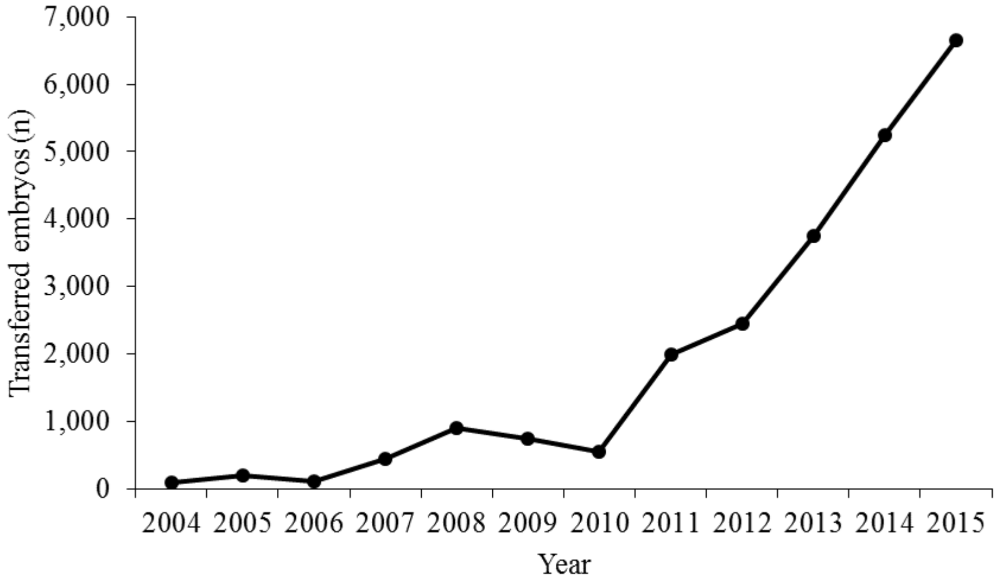

Figure 10. Number of embryos transferred from 2004 to 2015 in one dairy farm. From Pereira and Coelho (2016).

Table 4. Pregnancy per AI or P/ET at 30 days, birth rate, and pregnancy loss between 30 days and calving for Holstein and Girolando (5/8 Holstein x 3/8 Gir) lactating cows submitted to AI or ET on the same dairy farm.

	P/AI or P/ET at 30 days, % (n/n)	Pregnancy loss, % (n/n)	Birth rate, % (n/n)
Holstein			
AI	23.0[a] (895/3899)	39.3[A] (352/895)	13.9[a] (543/3899)
ET	43.1[b] (1026/2382)	43.6[B] (447/1026)	24.3[b] (579/2382)
Girolando			
AI	30.9[a] (1053/3413)	26.1[a] (275/1053)	22.8[a] (778/3413)
ET	45.4[b] (926/2038)	33.6[b] (311/926)	30.2[b] (615/2038)

[a,b]P < 0.01 within column and within breed. [A,B]P < 0.10 within column and within breed.

Pregnancy losses for the farms described above are much greater than those shown in Table 3, probably due to the use of different embryo recipients, as well as quality of IVP embryos selected for transfer. For the farms described above (Table 4), lactating cows were primarily used as recipients, whereas data presented in Table 3 are mainly from non-lactating embryo recipients. In fact, data of other dairy farms (n = 7) in which IVP embryos were transferred to lactating cows show acceptable P/ET at 30 days [42.9% (7204/16771)], however, pregnancy loss between 30 and 60 days [15.9% (820/5147)], and pregnancy loss between 30 days and calving [33.4% (2323/6956)] are high, resulting in low birth rates [28.8% (4663/16170)]. Greater pregnancy loss in lactating dairy cows as compared to heifers or non-lactating cows has been well-described elsewhere (Santos *et al*., 2004; Sartori, 2004).

Use of IVP embryos in beef cattle

Similar to what was reported for dairy cattle, data for beef cattle from the same IVF labs in Brazil, demonstrate acceptable P/ET at 30 days, especially when fresh embryos were transferred (Table 5). Pregnancy losses between 30 and 60 days may also be considered acceptable, and are similar for fresh or vitrified embryos (Table 5). However, as discussed in the next section of this manuscript, results are still not ideal, if compared with other ARTs.

Table 5. Pregnancy per ET (P/ET) at 30 and 60 days and pregnancy loss between 30 and 60 days for IVP embryos from beef breeds in Brazil.

	P/ET at 30 days, % (n/n)	P/ET at 60 days, % (n/n)	Pregnancy loss, % (n/n)
Nelore			
Fresh	44.4 (5,311/11,964)	40.4 (4,838/11,964)	9.1 (483/5,311)
Vitrified	34.8 (3,181/9,143)	31.8 (2,905/9,143)	8.6 (276/3,181)
Senepol			
Fresh	43.3 (3,408/7,874)	38.0 (2,996/7,874)	12.3 (421/3,408)
Vitrified	37.7 (2,967/7,873)	34.2 (2,694/7,873)	9.2 (273/2,967)

Reproductive efficiency of FTAI vs. FTET in beef cattle

Despite the many advances in the use of ARTs in Brazil, there is still substantial room for improvement, especially regarding cryopreservation/vitrification of IVP embryos. Below, we describe results of a study that evaluated reproductive efficiency in beef cows submitted to FTAI, or receiving the transfer of vitrified IVD or IVP embryos by FTET (Sartori *et al*., 2013).

Nelore (*Bos indicus*) cows (with a calf or not) were synchronized with the same protocol within a 3-months period (Fig. 11). For FTAI, 346 cows were bred on day 0 using frozen/thawed semen of five bulls. For ET, cattle received IVD (n = 274) or IVP (n = 573) vitrified embryos (produced with semen from seven bulls, of which, three were the same bulls used for FTAI) on days 6, 7, or 8 of the protocol after confirming the presence of a CL. The same groups of cows were used for all treatments. Transfers of IVD and IVP embryos, but not FTAI were concurrent, and there were two time-periods for AI or ET for each treatment group. Pregnancy was diagnosed by transrectal ultrasonography on day 30 after ovulation. Presence of an amniotic vesicle with an embryo was used as indicator of pregnancy. Pregnant cows were re-examined 30 days later, on day 60 of expected gestation.

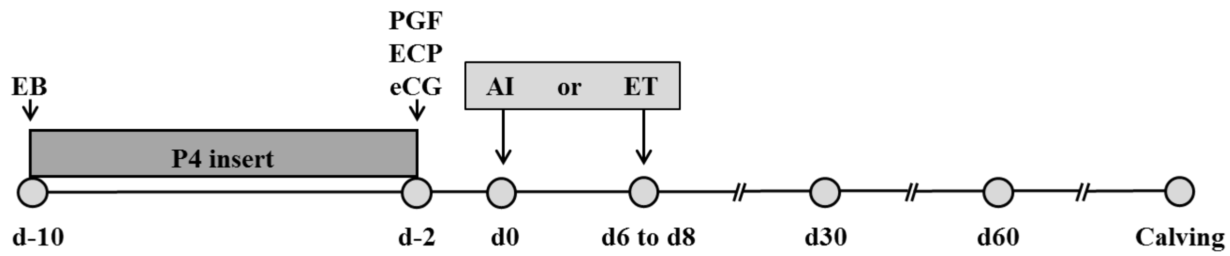

Figure 11. Schematic illustration of the protocol for FTAI or FTET in embryo recipient cows. Day-10: placement of an intravaginal insert of progesterone and 2 mg of estradiol benzoate (EB) i.m. Day-2: insert was removed and cows received i.m. treatments of 0.150 mg sodium cloprostenol (PGF2α), 300 IU equine chorionic gonadotropin (eCG) and 0.6 mg estradiol cypionate (ECP). Day 0: FTAI. Day 6 to Day 8: Embryo transfer. Day 30: Ultrasonography for pregnancy diagnosis. Day 60: Ultrasonography to confirm pregnancy.

All data regarding pregnancy diagnosis, pregnancy losses, and reproductive responses are shown in Table 6. The FTAI group had better results for almost all variables that were analyzed. Cows that received FTAI had greater P/AI at 30 and 60 days than cows receiving IVD or IVP embryos. However, when comparing cows that received ET, there was no detectable difference for P/ET at 30 days. Nevertheless, at 60 days, cows receiving IVP embryos had lower P/ET than cows receiving IVD embryos. Pregnancy loss between 30 and 60 days was lower for cows receiving FTAI, intermediate and not different from the other groups for cows receiving IVD embryos, and greater for cows receiving IVP embryos. For unknown reasons, FTAI cows had relatively high and similar rates of later pregnancy loss as IVP cows. Fewer cows receiving IVD embryos had later pregnancy losses, as compared with cows from the two other groups (Table 6). Moreover, gestation length was shorter for FTAI cows than for cows receiving IVD or IVP embryos (293.4 ± 5.3^a [275 to 303], 296.7 ± 6.3^b [270 to 315], and 296.8 ± 7.1^b [277 to 319] days, respectively; mean ± SD [range]; $P < 0.001$). Another important aspect to be considered was that for all calculations mentioned above, for the FTAI group, 100% of cows submitted to the protocol were considered in the analyses, however for the ET groups, only data from cows that had a CL at the time of transfer (~80%) were analyzed. When this variable was used for analysis, more healthy calves were born per cow submitted to a synchronization protocol for the FTAI group and less for the IVP group (Table 6).

Table 6. Pregnancy per AI or P/ET, pregnancy loss, abortion, and peripartum loss in Nelore cows that received fixed-time AI (FTAI) or vitrified *in vitro* produced (IVP) or *in vivo* derived (IVD) embryos.

	FTAI	IVD	IVP
30 days pregnancy, % (n/n)	50.3^a (174/346)	39.4^b (108/274)	34.0^b (195/573)
60 days pregnancy, % (n/n)	47.7^a (165/346)	35.4^b (97/274)	28.6^c (164/573)
Embryo/fetal loss (30 to 60 days), % (n/n)	5.2^b (9/174)	10.2^{ab} (11/108)	15.9^a (31/195)
Later pregnancy loss (60 days to calving), % (n/n)	15.2^a (25/165)	6.3^b (6/96)	16.5^a (27/164)
Peripartum loss, % (n/n)	2.1^b (3/140)	4.4^{ab} (4/90)	9.5^a (13/137)
Total loss, % (n/n)	21.3^b (37/174)	19.4^{ab} (21/108)	36.4^a (71/195)
Healthy calf born per synchronization protocol, % (n/n)	39.6^a (137/346)	25.4^b (87/342)	17.3^c (124/716)

$^{a,b,c}P < 0.05$.

Cloning

The birth of Vitória in 2001, a Simmental calf clone produced from embryonic cells, marked the beginning of the cloning era in Brazil. Subsequently, the production of cloned calves from fetal fibroblasts and from adult cell lines in 2002 was reported by different research groups. This was followed by production of many other cloned calves, demonstrating the potential of using SCNT commercially, in cattle and possibly other species. Private companies and producers were interested in applying this technology in animal production, especially for high genetic value animals. A technical committee was subsequently formed by researchers from several universities and research centers in 2007 to set the criteria for creating the Genealogical Register of Zebu breeds for the Ministry of Agriculture. However, the registration of cloned animals was released by the Ministry only after May 2009. By that time, about 70 cloned cattle had already been born and commercialized in Brazil. The registration of these cloned cattle by the breed associations, although not representing a complete dataset, at least provides information about how SCNT is being used in Brazil. Therefore, since 2005, cloning services have been provided by commercial laboratories in Brazil for propagation of valuable genetics, either for animal production purposes or for preservation of rare genotypes. With respect to endangered livestock, not much has been done in Brazil, other than the production of two cloned heifers of the Junqueira breed in 2005. Nevertheless, in 2012, the Brazilian Agricultural Research Corporation and the Brasilia Zoological Garden began collecting and freezing blood and umbilical cord cells from wild animals that had died (Scientific American, March 11, 2013. http://www.scientificamerican.com/article/cloning-endangered-animals), mostly in the Cerrado savanna; however, no cloned animal has been produced from these samples.

In contrast, for animal production the situation is quite different. Data from the Brazilian Association of Zebu Breeders (ABCZ) show a gradual increase in registered *Bos indicus* calves (predominantly of Nelore and Gir breeds) produced by SCNT during the years 2010 (n = 5), 2011 (n = 23), 2012 (n = 22), and 2013 (n = 41). Unofficial data indicate a continuous increase in number of healthy calves produced by SCNT from 2014 to 2016.

It is important to point out, however, that somatic bovine cloning is still besieged by low efficiency (number of live calves as a proportion of embryos transferred). The epigenetic modifications that are established during cellular differentiation are likely to be a major factor producing this low efficiency since they may act as barriers to the proper reprogramming of somatic nuclei. The 30 days P/ET is similar for cloned embryos and IVP embryos, however the overall efficiency is low due to the large proportion of pregnancies that are lost during gestation (Gerger *et al*., 2016) and in neonatal and postnatal periods (Chavatte-Palmer *et al*., 2004; Panarace *et al*., 2007).

After many years of research, no dramatic increase in cloning efficiency has been observed, with the rate of survival of cloned embryos still varying from 0 to 12% (De Bem *et al*., 2011; Sangalli *et al*., 2014; Gerger *et al*., 2016). Some improvements in survival rate can be expected by using specific and intensive management and clinical procedures during the perinatal and postnatal periods (Meirelles *et al*., 2010).

In the last 3 years the results described in Brazil (Table 7) are very similar to those reported in the literature. The 30-days P/ET is similar to results with IVP embryos (~40%), however the pregnancy loss is still very high, as shown in Table 7, and is similar to the losses described by Panarace *et al*. (2007). Nevertheless, post-partum death appears to be decreasing (78% survival in 2016) due to a better understanding on how to care for newborn calves. This gives some hope that this technology may be of practical use in the future, although the problems of nuclear reprogramming and exceedingly high pregnancy losses still need to be unraveled.

Table 7. Pregnancy per ET (P/ET) at 30, 60 and 90 days, birth rate, pregnancy loss and postpartum loss of bovine embryos produced by somatic cell nuclear transfer.

	P/ET at 30 days, % (n/n)	P/ET at 60 days % (n/n)	P/ET at 90 days % (n/n)	Birth rate %, (n/n)	Pregnancy loss 30 days to birth % (n/n)	Postpartum survival %, (n/n)
2014	42.0 (126/300)	26.5 (80/300)	25.0 (75/300)	12.0 (36/300)	71.4 (90/126)	58.3 (21/36)
2015	34.6 (128/370)	15.7 (58/370)	12.4 (46/370)	10.0 (37/370)	71.1 (91/128)	59.4 (22/37)
2016	44.7 (83/186)	27.4 (51/186)	26.0 (48/186)	12.4 (23/186)	72.3 (60/83)	78.2 (18/23)

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