New perspectives in assisted reproductive technologies in cattle (Nuevas perspectivas en tecnologías de reproducción asistida en bovinos) [+]

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[+]Nota del Editor: Se respetó la versión original de la conferencia en idioma inglés.

Abstract

The use of assisted reproductive technologies in cattle is been increasing in the past few years. Fixed-timed artificial insemination (FTAI) procedures are responsible for 85% of all artificial insemination (AI) done in Brazil. Despite the numbers of *in vivo* derived (IVD) embryos tended to decrease in the last 10 years, *in vitro* production (IVP) of embryos has increased significantly, with more than 57% of all IVP embryos in the world produced in Brazil. These biotechnologies have 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. Inside this picture, FTAI remains growing for both, dairy and beef cattle, although, there are new possibilities to get even better efficiency. The use of GnRH and new approaches to help this efficiency were reported recently. In addition, there is much more room for improvement in other reproductive technologies, since there are several reports of high pregnancy loss and high peripartum loss, when IVP embryos are used, and even more dramatic for cloning. This review aims to update the readers with new data regarding assisted reproductive technologies.

Keywords: artificial insemination, bovine, embryo, *in vitro* production, superovulation, cloning, somatic cell nuclear transfer.

Resumen

El uso de tecnologías de reproducción asistida en el ganado ha aumentado en los últimos años. Los procedimientos de inseminación artificial de tiempo fijo (FTAI) son responsables del 85% de toda la inseminación artificial (AI) realizada en Brasil. A pesar de que el número de embriones derivados in vivo (IVD) tendió a disminuir en los últimos 10 años, la producción in vitro (IVP) de embriones ha aumentado significativamente, con más del 57% de todos los embriones de PIV en el mundo producidos en Brasil. Estas biotecnologías han ayudado a mejorar sustancialmente la eficiencia reproductiva del ganado vacuno y lechero. En relación con la transferencia de embriones, la producción de embriones derivados in vivo (IVD) se mantuvo relativamente estable, con una producción promedio de 30 a 40,000 embriones por año, mientras que la producción in vitro (PIV) de embriones tuvo un aumento sustancial, de aproximadamente 12,500 embriones de PIV en 2000 a más de 300,000 embriones de PIV después de 2010. Dentro de esta imagen, el ALCA sigue creciendo tanto para el ganado lechero como para el ganado de carne, aunque hay nuevas posibilidades para obtener una eficiencia aún mayor. El uso de GnRH y los nuevos enfoques para ayudar a esta eficiencia se informaron recientemente. Además, hay mucho más margen de mejora en otras tecnologías reproductivas, ya que existen varios informes de pérdida de embarazo alta y pérdida de periparto alta, cuando se utilizan embriones de PIV, e incluso más dramáticos para la clonación. Esta revisión tiene como objetivo actualizar a los lectores con nuevos datos sobre tecnologías de reproducción asistida. Palabras clave: inseminación artificial, bovino, embrión, producción in vitro, superovulación, clonación, transferencia nuclear de células somáticas.

Introduction

Beef and dairy productivity, also, its demand in Brazil, are increasing with the years, which is directly related to technological advances in animal breeding, such as greater use of artificial insemination (AI) and embryo transfer (ET). The number of calves produced within a year is the most important measurement of efficiency and cost-effectiveness in beef cattle, which represents shortened calving intervals, earlier births during calving season, increased calf uniformity, more concentrated calving season and heavier calves at weaning time. Calves produced can be destined to meat production or herd replacement (Baruselli et al., 2017). On the other hand, dairy farms, productivity generally depends upon reproductive performance,

due to its direct impact especially on the average milk production per cow per day, number of replacements produced, and rates of voluntary and involuntary culling (Britt, 1985).

There are many reproductive biotechnologies currently available to be used in order to improve reproductive efficiency and enhance the genetic merit on bovine herds. The most common technology is the fixed time artificial insemination (FTAI). 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 2017 was responsible for 85% of all artificial insemination (AI) done in Brazil (number based on the number of protocols commercialized) in a total of 14 million of AI (number based on doses of semen commercialized; (Baruselli et al., 2018b).

Brazil first reported the production of more than 10,000 IVP embryos in 2000 (Viana et al., 2018), corroborating the growth in commercial IVP boosted in South American during this same period. The South America accounted for 58.4% (48,670 of 83,329) of the IVP embryos transferred worldwide in 2002, only 2 years later (Thibier and Stringfellow, 2003). Besides to this increase in numbers of produced embryos, the *in vitro* fertilization/culture has almost fully replaced the traditional *in vivo* superovulation & uterine flushing (MOET) as the technique of choice for embryo production in Brazil (Viana et al., 2017).

Production of *in vivo* derived (IVD) embryos remained relatively stable over the last 15 years, 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 300,000 IVP embryos since 2011, representing 60 to 70% of the world embryo production (Sartori et al., 2016). However, these trends are not necessarily followed by other countries, since the IETS records showed that in 2016 production of *in vitro* embryos (IVP) were approximately 632,000 as compared to 665,000 *in vivo* derived (IVD) bovine embryos been produced world-wide (Perry, 2018).

Recent data from approximately 25,000 pregnancies in cattle, have shown there is around 12% pregnancy loss between 32 and 60 days of pregnancy (Wiltbank et al., 2016). Although the IVP has been improving significantly in technology and efficiency along the years, we still face big

differences between conception rates (P/AI or P/ET) compared to FTAI (34.0% - 195/573 vs 50.3% – 174/346). In addition, by evaluating pregnancy loss from 30 to 60 days, the difference becomes even bigger (15.9% vs 5.2%) getting worst when healthy births per protocol of synchronization are evaluated (17,3% vs 39,6%, (Sartori et al., 2016). However, regarding the somatic cell nuclear transfer (SCNT) technique efficiency, the numbers are even more impressive. Besides having similar conception rate (44.7%), there is only 12% (36/300) of birth rate, with approximately 72.3% of pregnancy loss from 30 d to birth (Sartori et al., 2016). This suggests that those biotechnologies have greater failure in conceive and maintain pregnancy during the period from 30 to 60 days of pregnancy than AI, hence, a lot to be studied and reported.

The use of fixed time-artificial insemination in dairy cattle

The main objective of reproductive management strategies in dairy farms is to increase the number of cows pregnant early in lactation in order to achieve a profitable calving interval for the herd (Santos et al., 2004a, Giordano et al., 2011) and due to the simplicity of technique the use of AI is favorite in largest farms. When AI is employed, the main question is whether cows should be bred to estrus or FTAI especially because the studies that properly compared insemination to estrus vs. insemination to a FTAI protocol are controversial. Some authors have described lower (Strickland et al., 2010), similar (Rabiee et al., 2005, Nascimento et al., 2013a), or greater (Nascimento et al., 2013b) pregnancies per AI (P/AI) when cows are bred to estrus.

Lactating cows have shortened period of estrus (Lopez et al., 2004) and the average estrous cycle length of 23 d (Sartori et al., 2004a) which offer a limited period for detection of estrus and insemination leading to a considerable possibility of missing the estrus. Another problem is the delayed cyclicity in 20-30% of the cows even after the voluntary waiting period (Wiltbank et al., 2002, Walsh et al., 2007, Chebel et al., 2010). It can be caused by many factors, such as postpartum diseases (Santos et al., 2009) and BCS loss during the transition period (Barletta et al., 2017).

The use of synchronization of ovulation protocols can allow for fixed-time artificial insemination (FTAI) and eliminate the need for detection of estrus, thereby potentially improving reproductive efficiency and profitability of dairy herds (Norman et al., 2009). Use of FTAI programs for first

AI can increase the service rate in dairy herds by allowing prompt AI after the voluntary waiting period (Pursley et al., 1997).

A recent publication from Sartori et al (2016) described the impact of intensifying the use of FTAI on reproductive efficiency in a dairy herd in Brazil. They analyzed approximately 4,500 AIs performed between 2009 and 2014. These data were from a high producing dairy farm, managed in a free stall system with average milk yield of 10,700 kg/year during the period. From 2009 to 2011, using the less intensive program, cows received two treatments with prostaglandin F2α (PGF2α) at ~40 and ~54 d in milk (DIM) and were bred if detected in estrus (pedometer a checking for standing estrous twice a day) from 40 to 72 DIM. By ~70 DIM non-bred cows were submitted to FTAI. After 2012, the PGF2α was given at ~40 DIM and estrus detection until ~54 DIM, non-bred cows until that were submitted to FTAI GnRH-EC based as previously described by 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. Concluding there 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 d, with no change in pregnancy loss (Sartori et al., 2016). 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., 2014b).

North American dairy herds generally use FTAI protocols based on GnRH, such as Ovsynch (Pursley et al., 1995, Wiltbank and Pursley, 2014). In addition, many dairy herds use presynchronization prior to the Ovsynch FTAI program in order to increase fertility (Moreira et al., 2001, Souza et al., 2008b, Stevenson, 2016). The objective is to maximize the number of cows in early diestrus (day 6-7 of the estrous cycle), with follicles responsive to GnRH, assuring high synchronization and adequate circulating P4 during development of the preovulatory follicle in most of the cows. The FTAI programs that combine presynchronization with optimized Ovsynch programs are the basis for the fertility programs that not only increase service rate but also can increase P/AI (Wiltbank and Pursley, 2014, Carvalho et al., 2018).

However, there is a lack of studies regarding the use of presynchronization protocols in dairy cattle. Concerned with this problem, a recent study, evaluated the P/AI using three protocols only differing on the strategy to induce ovulation at the end of the protocol: using EC or GnRH or both with all cows enrolled, previously submitted to a presynchronization strategy. Overall ovulation rate to the GnRH administered on D0 of the protocol (GnRH1) was 63.3% (229/362), and did not differ (P = 0.34) between experimental groups. The ovulation rate of the present study was similar to other studies that reported rates around 60-70% (Souza et al., 2008a, Giordano et al., 2013, Giordano et al., 2016).

It should be mentioned that the 16.8 µg dose of buserelin acetate used on D0 of our study represents 68% more in relation to the recommended dose of 10.0 µg (Monteiro et al., 2015). This dose was increased based on data that have shown a decreased ovulation rate and lower GnRH-induced LH surge when cows are treated with a recommended dose of gonadorelin (100 µg) in the presence of a CL (Giordano et al., 2012, Melo et al., 2016). Also, in data from Consentini (2019), there were no differences between experimental treatments on P/AI on d30 with an overall P/AI of 40.4% (367/909). Pregnancy loss and P/AI on d60 did not differ between treatments. The authors concluded that reproductive program have potential to promote high fertility and the 3 strategies to induce the final synchronized ovulation produced similar fertility but further research is needed to optimize the presynchronization strategy.

The use of fixed time-artificial insemination in beef cattle

Breeding records compiled in 2016 clearly shows that Brazilian beef and dairy herds still heavily utilize natural service as their main breeding system (approximately 88 to 90% of the cows are bred by bulls; (Baruselli, 2016). Meanwhile, it has been found that when FTAI is used early in the breeding season for beef herds, it can clearly increase reproductive performance compared to natural service (Baruselli et al., 2018a).

In beef cattle operations, FTAI protocols have been personalized to use just the hormones that are allowed for use in the specific country and that match the management style of the operation and the physiology of the animals that are being bred. Most of beef cattle herds in the tropics, especially in Brazil, are composed of *B. indicus* which have a longer postpartum anoestrus and

low BCS when kept at pasture (Bó et al., 2003). This results in economic losses because of the increased calving to conception interval, and reduced pregnancy rates (Bó et al., 2007). Synchronization protocols that result in FTAI have become an important part of strategies for improving management of reproduction in cattle operations in many parts of the world including the USA and Brazil (Sartori et al., 2016, Stevenson and Britt, 2017, Baruselli et al., 2018b). These strategies allow controlled breeding seasons, increased reproductive efficiency, and improved genetic progress (Bó et al., 2018) .

Programs for FTAI in *Bos indicus* have generally utilized estradiol (E2) products, such as E2 benzoate (EB), although many countries with *Bos indicus* do not currently have approval for use of E2 products in FTAI protocols. It is generally assumed that E2 protocols are more efficient in *Bos indicus* beef cattle than protocols that are initiated with gonadotropin-releasing hormone (GnRH), although direct comparison of these protocols are needed.

Recent physiological experiments using daily ovarian ultrasonography have demonstrated that protocols initiated with EB produce synchrony in emergence of the new follicular wave resulting in subsequent ovulation of a single dominant follicle at the end of the protocol in *Bos indicus* heifers and cows (Madureira, 2019), results that were previously reported for EB treatments (Meneghetti et al., 2009, Wiltbank and Pursley, 2014, Santos et al., 2018). Use of GnRH at the initiation of the protocol caused ovulation in ~55% of *Bos indicus* heifers and cows and had synchronized emergence of a new follicular wave (from D0 to D5 of the protocol) in a high percentage of cows, similar to EB treatment [92.4% (61/66) for GnRH and 91.4% (66/72) for EB., the number of CL that were present at the time of prostaglandin $F2\alpha$ (PGF) treatment was much greater in animals treated with GnRH than EB due to GnRH-induced ovulation at protocol initiation (Madureira, 2019) and EB-induced regression of CL during the protocol (Dias et al., 2009, Binelli et al., 2014a).

Nevertheless, initiation of the protocol with EB or GnRH produced a high percentage of heifers or cows that ovulated at the end of the protocol (~90%). In addition, the presence of younger CL at the time of P4 implant removal of the GnRH-initiated protocol require the use of two PGF treatments to assure CL regression to the protocol (Sa et al., 2011, Jinks et al., 2013, Madsen et al., 2015). This data demonstrated the potential of the use GnRH in beef cattle in Brazil,

contesting the general assumption that E2 protocols are more efficient in *Bos indicus* beef cattle than protocols that are initiated with GnRH.

Embryo transfer

As mentioned before, 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, especially when beef cows, crossbreds, or heifers are used as embryo recipients (Sartori et al., 2016).

The physiological differences between the most representative breeds in Brazil for beef and dairy production, respectively, *B. indicus* and *B. taurus*, need also to be considered when managing females for *in vivo*-derived (IVD) embryos by multiple ovulation and embryo transfer (MOET), or *in vitro* embryo production (IVP). 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. Indeed, IVP is increasing compared to standard *in vivo* embryo production mainly because of its greater efficiency in terms of embryo production numbers from the same donor cow (Viana et al., 2017, Viana et al., 2018). Overall, the *in vivo* technology (MOET) produces 5 embryos per procedure per donor at every 45 days. In contrast, the *in vitro* (IVP) can produce 3 embryos per procedure per donor at every 15 days. After one year of embryo production, MOET produces 40 embryos while PIVE produces 72 embryos (Baruselli et al., 2018b).

Regarding the particularities of this both breeds (*B. indicus* and *B. Taurus*), one of the protocols, called P-36, most frequently used in *B. indicus* cows and heifers for MOET was developed by Barros and Nogueira (2005) and had its effectiviness confirmed by Baruselli et al (2006) and Nogueira et al (2007). Nevertheless, it required different approach for *B. Taurus* cows. The protocol is based in an intravaginal P4 implant for 36 hours after PGF2α administration, and induction of ovulation with exogenous LH, administered 12 hours after the P4 device removal (48 hours after PGF2α administration). The FTAI is usually performed 12 and 24 hours later, since ovulation occurs between 24 and 36 hours after LH administration.

The use of the traditional P-36 protocol in *B. taurus* breeds, however, has resulted in decreased numbers of viable embryos in comparison with conventional protocols using oestrus detection. In Holstein (Baruselli et al., 2006) and Angus (Bo et al., 2006) donors. Viable embryo production was increased with the P-36 protocol, when the ovulation induction treatment (LH or GnRH) was administered at 60 hours (P-36/LH60), rather than 48 hours (P-36/LH48) after PGF2α administration. On the other hand, the opposite occurred when used in *B. indicus* breeds. The P-36/LH60 protocol caused a decrease in embryo production when compared to P-36/LH48 protocol (Baruselli et al., 2006).

Therefore, it can be inferred that ovulation in superstimulation protocols must be induced earlier in *B. indicus* donors; whereas, in *B. taurus* donors, it seems necessary to delay treatment with an ovulation inducer, thereby allowing an increase in follicle size, and acquisition of LH receptors. *Bos indicus* breeds have a reduced capacity for LH secretion and a greater sensitivity to exogenous gonadotropins than *B. taurus* (Randel, 1984).

Also because of the greater AFC and better oocyte quality in *B. indicus* in comparison to *B. taurus*, IVP is much more successful in *B. indicus*. Using Gir (*B. indicus*), Holstein and crossbreds (1/4 Holstein x 3/4 Gir or 1/2 Holstein-Gir) and comparing for total and viable oocyte yield, and IVP of embryos (Pontes et al., 2010). The number of total and viable oocytes, and embryos produced were greater in Gir than in Holstein cattle (17.1 vs. 11.4; 12.1 vs. 8.0; 3.2 vs. 2.2, respectively). Moreover, embryo production (5.5 blastocysts) was even greater in Holstein-Gir crossbreds compared to the other breeds.

Another study was conducted to compare IVP between Nelore and Holstein heifers (Gimenes et al., 2015). More oocytes were recovered (37.1 vs. 15.4), more embryos were produced (7.3 vs. 1.1), and a greater blastocyst rate was obtained (28.3 vs. 14.1%) from Nelore than Holstein heifers. In another study (Sales et al., 2015), Gir cows had a greater number of oocytes recovered by ovum pickup (OPU; 23.4 vs. 14.9), better quality of oocytes as demonstrated by greater cleavage rates (73.6 vs. 40.8%), greater number of blastocysts (3.8 vs. 0.7) and better blastocyst rates (36.7 vs. 12.1%) than Holstein donors.

Besides all the variation inherent to the technique, still is possible to improve reproductive efficiency and profitability using the IVP embryos. One example described by Sartori et al. (2016) is a farm with 1,500 lactating crossbred cows (Girolando [5/8 Holstein x 3/8 Gir] breed). In this case, cows receive IVP embryos from sex-sorted semen, to improve the replacement or sale. In 2015, using more than 6,500 embryos were transferred, with acceptable P/ET at 30 d (43%) and 21-d PR (~20%).

However, high incidence of pregnancy loss between 30 and 65 d (15%) and between 30 d 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-d PR (Sartori et al., 2016).

In the same study, another farm was described, with 1,100 lactating cows (Holstein and Girolando breeds), which uses AI or transfer of IVP embryos, despite having greater pregnancy losses (39.3 for AI and 43.6% for IVP), 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 (43.1% vs 23.0), and greater birth rates for ET vs. AI (24. vs 13.9%)(Sartori et al., 2016). In addition, the use of sex-sorted sperm for IVF allowed an increased number of heifers born with IVF and greater genetic improvement.

Pregnancy losses for the farms described above are much greater than those described before (Wiltbank et al., 2018), probably due to the use of different embryo recipients, as well as quality of IVP embryos selected for transfer. For the farms described above, lactating cows were primarily used as recipients, while other reports used data from all kinds of 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 d [42.9% (7204/16771)], however, pregnancy loss between 30 and 60 d [15.9% (820/5147)], and pregnancy loss between 30 d 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., 2004b, Sartori et al., 2004b).

Besides all this factors, ET still faster genetic progress, also have being described as having potential to increase fertility in dairy cows experiencing heat stress (Putney *et al.*, 1989; Ambrose *et al.*, 1999; Baruselli *et al.*, 2010; Rodrigues *et al.*, 2004, 2007, 2011) and those diagnosed as repeat-breeders (Dochi *et al.*, 2008; Rodrigues *et al.*, 2011). Transferring embryos, skip the problems associated with fertilization failure and disruption of the oocyte quality in dairy cows also related to heat stress (Ferreira et al., 2011) 2016). Utilizing data from a large commercial herd in Brazil, some authors (Baruselli et al., 2011, Baruselli et al., 2018b) showed that conception results can be increased in about ~8% (cooler months) to 20% (warmer months) percentage points with the use of ET when compared to AI. The scenario for repeat-breeder cows in a comparison between AI and ET is more dramatic, where conception results found by Baruselli *et al.* 2011 increased in about 15 to 20% points.

Nevertheless, in addition to fertility, several other factors need to be considered to determine if the use of embryo technologies is economically comprehensive. The cost of an embryo, labor, recipient utilization, genetic progress, and fertility to AI programs are all additional important factors that cannot be ignored (Ribeiro et al., 2012). Calculating and comparing the costs of five breeding programs for lactating dairy cows including embryo transfer (ET) from superovulated (SOV) cows; in vitro produced embryos from ovum pick up (OPU) or from oocytes of dairy cows from slaughterhouse; timed AI; and timed AI combined with insemination after detection of estrus (timed AI + DE).

The cost per breeding was highest ($258.9) for ET when the embryo donor was a nonlactating cow inseminated with sexed semen and lowest ($16.3) when timed AI + detecting estrous using conventional semen was used. As expected, using sexed semen increased cost per breeding, but the increment was proportionally greater when used for insemination than for production of embryos. This is because semen represents a larger portion of the breeding costs with AI than embryo production and transfer. (Ribeiro et al., 2012). These data reinforce the concept that for embryo technologies to be economically attractive to replace AI programs, they have to offer major increments in fertility.

The use of somatic cell nuclear transfer (SCNT)

Somatic cell nuclear transfer (SCNT) has been successfully used in a number of mammals for applications in animal breeding (Dang and Zhang, 2019). The network of laboratories performing IVP is a platform for the development of other technologies, particularly those that require substantial investments in laboratory equipment (e.g. micromanipulation, intracytoplasmic sperm injection [ICSI], somatic cell nuclear transfer [SCNT], etc.) but have limited commercial use *per se* (Viana et al., 2018). 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., 2017). Some improvements in survival rate can be expected by using specific and intensive management and clinical procedures during the perinatal and postnatal periods.

It appears that SCNT embryos have similar developmental potential to the blastocyst stage compared to that of in vitro fertilized (IVF) embryos (Akagi et al., 2014) .In addition, in the last years the results described in Brazil are very similar to those reported in the literature. The 30-d P/ET is similar to results with IVP embryos (~40%), however the pregnancy loss is still very high (Sartori et al., 2016) 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 (Sartori et al., 2016). 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.

Most embryos losses probably occur during the peri-implantation stage leading to pregnancy failure, although, fetal losses are also observed until late gestation (Akagi et al., 2014) leading to lower this birth rate after embryo transfer than IVP. However, an excess of aberrant phenotypes occur in the fetus and placenta for embryos generated by assisted reproduction, such as large offspring syndrome (Liu et al., 2013, Chen et al., 2015).

These abnormalities in phenotypes may be due to the abnormal epigenetic profile of the transferred somatic donor cell, when it is reprogramed from a differentiated status to a totipotent state [(Kang et al., 2001, Santos et al., 2003, Akagi et al., 2014). Altered global DNA methylation

has been observed in bovine SCNT embryos compared to IVF embryos and in vivo produced during preimplantation stages of development (Dean et al., 2001, Kang et al., 2001, Santos et al., 2003). Developmental events that are heavily regulated by epigenetic mechanisms including X chromosome inactivation and genomic imprinting are also impaired in bovine SCNT embryos (Xue et al., 2002).

Despite the low efficiency of embryo reconstruction and very high rates of embryonic loss, abortion, and stillbirths (Chavatte-Palmer et al., 2012), SCNT numbers have increased in the past few years, demonstrating that the demand for the use of the technology has overcome the technical difficulties and, consequently, the high production cost. As expected, most clones were from Nelore (62.5%) and Gir (26.9%), which are important beef and dairy zebu breeds in Brazil, respectively. Nonetheless, it is remarkable that the majority of calves (142 of 160, 89.0%) were females and this was true for all breeds (Viana et al., 2018).

Therefore, since 2005, cloning services have been provided by commercial laboratories in Brazil for propagation of valuable genetics, whether 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, mostly in the Cerrado savanna; however, no cloned animal has been produced from these samples (Sartori et al., 2016).

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)(Sartori et al., 2016). There are evidences that SCNT technology have being increasing since then, especially because the companies that installed this technique are still working and growing, although, no other studies have been published in order to improve the efficiency or to describe the market worldwide.

Conclusion

Despite all the enhancements and different managements described above, there is much more room for improvement for biotechnologies. The FTAI technology is a great bet for improving animal breed programs, due the faster application of a bull with desired genetic merit in a large group of animals. However, these other technologies such as IVP and SCNT, as long as remain being studied and improved, can be an excellent tool to help the market to accelerate generations or help to elucidate physiological processes such as pregnancy loss.

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